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Potential vectors of *Xylella fastidiosa* in Portuguese olive orchards: survey in Alentejo region and control measures

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2017

Agradecimentos/ Acknowledgments

Quando vemos um cartaz de um filme, apenas aparecem os nomes do elenco e quando assistimos a esse mesmo filme, só vemos os atores, mas não são só os atores que fazem o filme. É por isso que quando vejo um filme, faço questão de assistir aos créditos que passam no fim. É a minha forma de reconhecer e homenagear o trabalho de uma equipa mais vasta que o próprio elenco. Cada um daqueles nomes que ficam no ecrã por apenas um ou dois segundos depois de a maior parte da audiência ter abandonado a sala representa uma pessoa especialista no que faz que dedicou grande parte do seu tempo para tornar aquele filme no melhor que ele podia ser. Isto serve para dizer que ninguém chega sozinho a lado nenhum e que apesar de esta tese ser minha, não resulta apenas do meu esforço e dedicação. Há um grupo de pessoas que colaborou comigo e que me ajudou nesta viagem e este pequeno espaço que lhes dedico pode não ser suficiente para reconhecer a sua importância, mas espero que sirva para lembrar as personagens que mais impacto tiveram na história por detrás desta tese e para lhes agradecer mais uma vez pelo seu contributo.

Como não poderia deixar de ser, tenho de começar por agradecer à professora Teresa e ao professor Fernando pela confiança que depositaram em mim e por me terem orientado ao longo deste ano.

Quero agradecer à professora Teresa por me ter acompanhado de perto, por arranjar sempre tempo para esclarecer as minhas dúvidas (algumas mais existenciais do que outras), pelas revisões da tese, pelas sugestões e conselhos que melhoraram a qualidade do meu trabalho, mas em especial obrigada pela sua preocupação em fazer com que tudo corresse bem, pela grande paciência para uma constante insatisfeita, pelo seu constante incentivo e pela sua visão positiva da vida. Só tenho a acrescentar que quando for grande quero ter metade do seu otimismo! Um terço se calhar já chegava!

Ao professor Fernando quero agradecer a oportunidade de participar neste trabalho. Sabe-se lá onde estaria eu nesta altura se não fosse este projeto! Obrigada por todo o esforço na recolha das amostras, pela simpatia que teve sempre quando trouxe as amostras para a FCUL, pelas suas revisões e sugestões que contribuíram bastante para melhorar a qualidade da tese, mas acima de tudo pela disponibilidade para esclarecer qualquer dúvida ou qualquer outro aspeto que fosse necessário, apesar da distância.

Agradeço também à professora Filomena pela oportunidade de frequentar este mestrado, por querer sempre saber da nossa opinião e por tentar constantmente tornar o mestrado na melhor experiência possível. Obrigada por arranjar sempre um tempinho no meio da azáfama diária para tirar qualquer dúvida, por acompanhar o nosso trabalho, por encontrar sempre o lado positivo em qualquer situação e por torcer sempre para que as coisas corram bem.

Quero deixar o meu obrigado aos colegas que partilharam o laboratório comigo ao longo do ano, em particular à Jéssica pela sua boa disposição persistente e à Sara por tornar a triagem mais divertida sempre que nos cruzámos.

Obrigada também à Célia Mateus do INIAV pelo empréstimo de vários livros que impulsionaram o início das minhas identificações numa altura em que achava que não iria ser capaz de identificar alguma espécie, mas em particular pela simpatia e sorriso que teve sempre nas ocasiões em que me recebeu.

Quero ainda agradecer aos meus amigos, mas em particular à Inês. Obrigada por me emprestares a chave da sala da microscopia sempre que estava fechada, obrigada por leres a minha tese e pelas sugestões, obrigada pela companhia durante algumas sessões fotográficas e por teres clicado no botão para guardar as fotos umas duas ou três vezes em alturas críticas em que os modelos fotográficos não queriam colaborar comigo! Acho que é isto… Já chega, não é? Não! Oito letras não chegam para explicar a gratidão que sinto pela tua amizade e pelo teu apoio especialmente este ano, mas como apenas oito letras formam a palavra “obrigada”, não há muito mais que possa dizer. Obrigada pela companhia ao almoço, pelos regressos a casa mais animados, enfim, pelas pequenas coisas do dia-a-dia que ajudam a apagar a força do tempo. Obrigada pelo apoio e pela paciência. Obrigada por estares presente!

Por último, mas não menos importante, quero agradecer à minha família, em particular à minha Mãe e ao meu Pai, aos meus Avós e às minhas irmãs Sofia e Diana por tudo o que deram para eu chegar aqui, por terem interesse em perceber o que eu faço, mas acima de tudo pela paciência, compreensão, carinho e apoio incondicional que me dão. Não há palavras que cheguem para vos agradecer, por isso fica apenas um obrigado do tamanho do mundo.

Ele é iletrado, mas quero agradecer ao Dénis, o meu fiel gatito, por nunca me deixar sozinha durante a fase mais crítica que foi a escrita deste trabalho. Fica aqui um "miau!" para ti!

De resto, deixo um obrigada a todos os que não tendo sido mencionados tiveram algum tipo de influência no meu trabalho e aos amigos e colegas que, não acompanhando a minha jornada diariamente, foram perguntando como iam correndo as coisas e dando uma força sempre que me viam. Obrigada a todos!

Abstract

The recent emergence of *Xylella fastidiosa* Wells *et al*., 1987 in several countries around Europe and its involvement in the Olive Quick Decline Syndrome in Italy represents a high risk for susceptible crops in other areas where favourable climatic conditions and insect vectors are present. Olive culture is the most important permanent culture in Portugal, particularly in Alentejo Region, where most national olive production occurs. So far, *X. fastidiosa* has not been detected in the country, but the identification and monitorization of the potential vectors is essential as a preventive measure.

Knowing this, one of the goals of this study was the identification of xylem-feeding Auchenorrhyncha occurring on olive trees and weeds associated to olive groves in Alentejo Region. A survey was carried in 126 locations around Alentejo from late-October to mid-November of 2016. The artropodofauna associated with olive trees and weeds was vacuum-sampled and brought to lab for identification. Auchenorrhyncha were identified to the lowest taxonomic level based on morphology and when species could not be determined, morphospecies were used.

Forty-four Auchenorrhyncha species belonging to six different families were collected. The only potential vectors identified were spittlebugs: *Philaenus* sp. (five specimens) and *Neophilaenus campestris* (Fallén, 1805) (twenty specimens). The potential vectors were collected from weeds and olive canopy, but mostly from weeds, showing the importance of these species as alternative hosts.

As an important suppression force of pests, the presence of natural enemies in the olive groves that may be helpful in the control of potential *X. fastidiosa* vectors was also evaluated and some considerations about management measures were made. Seven predatory groups (Aranea, Coccinellidae, Formicidae, Mantodea, Neuroptera, Opiliones, Pseudoscorpiones) and five parasitoid wasp superfamilies (Chalcidoidea, Chrysidoidea, Cynipoidea, Ichneumonoidea and Platygastroidea) were collected. Spiders and ants were the most common groups within the considered predators and Chalcidoidea, from which several families are known to parasitize spittlebugs, and Ichneumonoidea were the dominant parasitoid superfamilies. Weeds were associated with higher abundance of both types of natural enemies by comparison with olive trees.

The association of *Philaenus* sp. and *N. campestris* to olive trees, a susceptible host, provides evidence that, if *X. fastidiosa* introduction occurs, there are potential vectors in Alentejo olive groves capable of spreading the bacterium, contributing to its establishment. This highlights the importance of taking actions aimed at introduction prevention and at a precocious detection like: special care in plant trade; continued monitoring of potential vectors and identification of susceptible hosts; education and involvement of farmers and other stakeholders into monitorization; investigation of resistant olive tree varieties; and agricultural practices compatible with the conservation of natural enemies.

Key-words: epidemiology; *Neophilaenus campestris*; pest management; *Philaenus*; plant disease.

Resumo alargado

Os Hemiptera são uma ordem de insetos caracterizada pela estrutura única da sua armadura bucal do tipo picador-sugador que lhes permite ingerir vários tipos de fluídos. A maioria dos Hemiptera é fitófaga alimentando-se de células do mesófilo, de seiva floémica ou xilémica. O seu modo especializado de alimentação permite que contactem com outros microrganismos que colonizam o sistema vascular das plantas, podendo ingeri-los e funcionar como vetores de bactérias, fungos e vírus, responsáveis por importantes doenças em plantas.

Xylella fastidiosa Wells et al. 1987 é uma bactéria transmitida por insetos picadores-sugadores do xilema com mais de 350 plantas hospedeiras identificadas. Esta bactéria é responsável por várias doenças economicamente importantes como a Doença de Pierce na vinha, a Clorose Variegada dos Citros em citrinos, o "Almond Leaf Scorch" em amendoeira, a "Peach Phony Disease" em pessegueiro ou o recente Declínio Súbito do Olival em oliveira, entre muitas outras. O mecanismo que leva ao desenvolvimento da doença não está completamente esclarecido e os sintomas provocados pela infeção por *X. fastidiosa* dependem da planta hospedeira. A infeção pela bactéria não resulta necessariamente no desenvolvimento de doença, dependendo da combinação entre a planta hospedeira e a subespécie de *X. fastidiosa* envolvida.

A transmissão de *X. fastidiosa* implica três passos: aquisição, retenção e inoculação. Ao alimentar-se de uma planta infetada, o inseto vetor pode ingerir bactérias que se fixam à superfície externa da cutícula do estomódeo. As bactérias multiplicam-se e produzem um biofilme que as mantém agregadas. Ao alimentar-se de uma planta saudável, algumas das bactérias retidas pelo vetor podem ser inoculadas na planta, onde se estabelecem e multiplicam, gerando uma nova infeção. Os vetores adultos têm uma capacidade de transmissão persistente, mas não as ninfas que durante a ecdise entre instares libertam a sua cutícula. Uma das principais particularidades do modo de transmissão de *X. fastidiosa* é a ausência de um período de latência.

A eficiência na transmissão de *X. fastidiosa* depende de vários fatores como o período de aquisição, o período de inoculação, as preferências alimentares dos vetores entre plantas e na própria planta, a discriminação de plantas infetadas por parte dos vetores, o número de vetores a alimentar-se na própria planta e a concentração da bactéria na planta hospedeira, entre outros.

Apesar do largo número de vetores potenciais, só algumas espécies é que têm um papel significativo na propagação de *X. fastidiosa* numa cultura particular de uma região específica. A significância de um vetor na dispersão de *X. fasidiosa* depende não só da sua competência na transmissão, mas das interações ecológicas com a planta hospedeira e o ambiente. A eficiência na transmissão é relevante, mas a importância dos vetores na dispersão depende maioritariamente do habitat, da seleção do hospedeiro, da densidade e mobilidade dos vetores e da sua distribuição espacial e temporal.

Doenças relacionadas com *X. fastidiosa* afetam o continente americano há mais de um século, mas só recentemente é que este fitopatogéneo foi reportado noutras regiões, como é o caso de Taiwan, do Irão e de alguns países na Europa. O primeiro registo confirmado de *X. fastidiosa* na Europa deu-se em 2013 na Região de Apúlia, no Sul de Itália, onde a bactéria está associada ao Declínio Rápido do Olival e o principal vetor envolvido na dispersão da doença é a cigarrinha-da-espuma *Philaenus spumarius* Linnaeus, 1758. Desde a primeira deteção, novos focos associados a mais de uma subespécie da bactéria foram reportados em França, Espanha e na Alemanha. A Autoridade Europeia de Segurança Alimentar (EFSA) identificou Aphrophoridae, Cercopidae, Cicadellinae, Cicadidae e Tibicinidae como os grupos de potenciais vetores de *X. fastidiosa* na Europa.

Em Portugal, *X. fastidiosa* ainda não foi detetada, mas a existência de um clima favorável, plantas hospedeiras suscetíveis e de vetores potenciais associada à posição do país no comércio e turismo mundial, constituem condições propícias à sua introdução e dispersão. O olival tem sido a cultura mais negativamente afetada na Europa desde que *X. fastidiosa* foi detetada no continente. Portugal é um dos dez maiores produtores de azeite e de azeitona a nível mundial, sendo a olivicultura a cultura permanente mais relevante a nível nacional, especialmente no Alentejo.

Sendo a existência de vetores capazes essencial para o estabelecimento da bactéria numa dada região, dado que a monitorização dos vetores potenciais é essencial para o desenvolvimento de estratégias de contenção e não havendo ainda estudos publicados direcionados à identificação dos vetores potenciais de *X. fastidiosa* em Portugal, este trabalho procurou fazer o levantamento e identificação dos vetores potenciais presentes no olival alentejano. Para tal, selecionaram-se 126 locais de amostragem que foram amostrados entre 25 de outubro de 2016 e 15 de novembro de 2016. Em cada local, procedeu-se à colheita com aspirador da fauna associada à copa de cinco oliveiras e à vegetação espontânea, quando presente.

Foram triadas 113 amostras de copa de oliveira e 43 amostras de vegetação espontânea, tendo sido contabilizados 22149 exemplares pertencentes a 21 ordens. Os Auchenorrhyncha foram separados e identificados até ao nível taxonómico mais baixo possível. No caso dos adultos, quando não foi possível a determinação da espécie, foram consideradas morfoespécies. Os adultos recolhidos foram fotografados e este trabalho inclui 29 arranjos gráficos de imagens com características somáticas e genitais de 22 espécies de Auchenorrhyncha.

Apesar de terem sido identificadas 44 espécies e morfoespécies de Auchenorrhyncha adultos pertencentes a 6 famílias distintas, apenas *Philaenus* sp. (5 indivíduos) e *Neophilaenus campestris* (Fallén, 1805) (20 indivíduos), são especialistas da seiva xilémica, sendo as únicas apontadas como potenciais vetores nos olivais da área de estudo. Capturaram-se 172 cicadelídeos adultos, mas nenhum Cicadellinae foi encontrado. Contudo, como as ninfas de Auchenorrhyncha só foram identificadas até à família, não se excluí a possibilidade da ocorrência de Cicadellinae em olival. Considerando o limitado carácter temporal da prospeção realizada e a variabilidade na riqueza específica e abundância ao longo do ano e entre anos, outras espécies de vetores potenciais poderão ocorrer nos olivais alentejanos, questão que deverá ser estudada no futuro. Os vetores potenciais foram recolhidos em maior abundância em vegetação espontânea, pelo que olivais onde ocorre vegetação espontânea deverão estar mais suscetíveis ao estabelecimento de *X. fastidiosa*, em caso de introdução.

Não sendo apontado como um potencial vetor de *X. fastidiosa* por se alimentar preferencialmente da seiva floémica, no decorrer deste trabalho foi identificado um indivíduo pertencente à espécie *Orosius albicinctus* Distant, 1918, colhido em vegetação espontânea. Esta espécie é um importante vetor de fitoplasmas noutros países e este é possivelmente o seu primeiro registo em Portugal continental.

A conservação de populações de inimigos naturais, que incluem agentes patogénicos, predadores e parasitoides, é uma medida de luta indireta que ajuda a regulação das populações de inimigos das culturas. Assim, o segundo objetivo deste trabalho foi a avaliação da presença da artropodofauna auxiliar nos olivais estudados com eventual utilidade no controlo biológico dos vetores potenciais de *X. fastidiosa*. Foram consideradas duas guildes de artrópodes auxiliares: predadores e parasitóides. Dentro do grupo dos parasitóides foram identificadas cinco superfamílias de vespas parasitóides (Chalcidoidea, Chrysidoidea, Cynipoidea, Ichneumonoidea e Platygastroidea). Chalcidoidea e Ichneumonoidea foram as superfamílias mais representadas constituindo 90.85% dos parasitoides capturados. Chalcidoidea e Chrysidoidea incluem espécies parasitóides de ovos, ninfas e adultos de Auchenorrhyncha. Neste trabalho, foram encontrados quatro Auchenorrhyncha parasitados: três Delphacidae por Dryinidae e um *N. campestris* por um Hymenoptera não determinado. O número médio de parasitóides capturados por

amostra foi sempre superior em amostras de vegetação espontânea, independentemente da superfamília, mostrando que a vegetação espontânea é importante para comunidade de parasitóides. Nenhum Pipunculidae (Diptera) foi capturado nas amostras recolhidas, mas esta família parasita exclusivamente Cercopoidea e pode ser importante na regulação das populações dos vetores potenciais identificados, pelo que numa monitorização mais alargada no tempo se deverá dar especial atenção a este grupo. Dentro dos predadores foram identificados sete grupos (Aranea, Coccinellidae, Formicidae, Mantodea, Neuroptera, Opiliones e Pseudoscorpiones). As aranhas e formigas foram os grupos mais abundantes, constituindo 94.44% dos predadores capturados. O número médio de aranhas capturadas foi semelhante em amostras de oliveira e de vegetação espontânea, mas as formigas foram cerca de 3 vezes mais abundantes nas amostras de vegetação espontânea. Pseudoscorpiones e Mantodea foram exclusivamente encontrados em vegetação espontânea, enquanto os Opiliones apenas foram colhidos em oliveira.

Adicionalmente, com base nos resultados obtidos e na bibliografia existente sobre os vetores potenciais e inimigos naturais identificados, foram discutidas algumas medidas de gestão, entre as quais: a monitorização continuada dos vetores potenciais e a identificação de hospedeiros alternativos utilizados pelos vetores potenciais com a realização de testes para a deteção da bactéria em vetores potenciais e plantas suscetíveis independentemente do seu estado de infeção; a educação e o envolvimento dos agricultores e outros *stakeholders* na monitorização do olival para que haja uma deteção precoce, em caso de introdução; a investigação de variedades cultivares de oliveira resistentes ou tolerantes a *X. fastidiosa*; e a utilização de práticas culturais compatíveis com a conservação e potenciação dos inimigos naturais.

Palavras-chave: epidemiologia; fitopatologia; gestão de pragas; *Neophilaenus campestris*; *Philaenus*.

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List of abbreviations and acronyms

AAP – Acquisition Access Period

AD – Alfalfa Dwarf

ALS – Almond Leaf Scorch

CLS – Coffee Leaf Scorch

CoDiRO – Complesso del Disseccamento Rapido dell'Olivo

CVC – Citrus Variegated Chlorosis

CYDV – Cereal Yellow Dwarf Virus

EFSA – European Safety Food Auhority

EPPO – European and Mediterranean Plant Protection Organization

EU – European Union

IAP – Inoculation Access Period

OLS – Oleander Leaf Scorch

OQDS – Olive Quick Decline Syndrome

PC – Principal Component

PCA – Principal Component Analysis

PD – Pierce's Disease

PLS – Plum Leaf Scald

PPD – Phony Peach Disease

TYLCV – Tomato Yellow Leaf Curl Virus

USA – United States of America

ZYMV – Zucchini Yellow Mosaic Virus

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Introductory note

The work presented in this thesis was developed under the project “A Protecção Integrada do olival alentejano. Contributos para a sua inovação e melhoria contra os seus inimigos-chave” (ALT20-03-0145-FEDER-000029).

1. Introduction

1.1. Hemiptera as vectors of plant disease

1.1.1. Hemiptera classification

Hemiptera is the fifth largest group of insects after Coleoptera, Diptera, Hymenoptera and Lepidoptera, having about 82000 described species (Forero 2008; Cryan & Urban 2012). Historically, higher-level classification of this group has suffered many changes due to advances in knowledge about the phylogenetic relationships between different clades which are extensively reviewed by Forero (2008).

Nowadays, hemipterans are typically classified into four different sub-orders: Heteroptera, Coleorrhyncha, Stenorrhyncha and Auchenorrhyncha (Forero 2008). Heteroptera (true bugs) contain seven infra-orders: Enicocephalomorpha, Dipsocoromorpha, Leptodomorpha, Gerromorpha, Nepomorpha, Cimicomorpha and Pentatomorpha. Coleorrhyncha (moss bugs) are a small group with only one extant family (Peloridiidae). Stenorrhyncha include four superfamilies: Aleyrodoidea (whiteflies); Aphidoidea (aphids or plant lice); Coccoidea (scale insects); and Psylloidea (psyllids). Auchenorrhyncha are divided into two infraorders: Fulgoromorpha (planthoppers), which only have one superfamily (Fulgoroidea); and Cicadomorpha with three recognized superfamilies – Cercopoidea (spittlebugs or froghoppers), Cicadoidea (cicadas) and Membracoidea (leafhoppers and treehoppers).

Traditionally, Hemiptera has been divided into Heteroptera and "Homoptera" (comprising Coleorrhyncha, Stenorrhyncha and Auchenorrhyncha). This division has been perpetuated by North American entomology for a long time (Forero 2008), so it is not uncommon to encounter references to "Homoptera" in the literature even today, as a simple internet research would show.

The phylogenetic relationships in Hemiptera are not fully resolved. For instance, there is a relative consensus about the monophyly of Heteroptera, Coleorrhyncha and Stenorrhyncha, but there is still disagreement about Auchenorrhyncha being a monophyletic group. For this reason, some authors consider Cicadomorpha and Fulgoromorpha as sub-orders, together with Heteroptera, Coleorrhyncha and Stenorrhyncha (Quartau, personal communication). This controversy around Auchenorrhyncha classification is further explained by Cryan & Urban (2012) in a detailed review about morphological and molecular evidence for and against the monophyly of Auchenorrhyncha.

Despite the existence of conflict in classification within the order, Hemiptera can be easily recognised by the unique structure of the mouthparts, a unifying character that supports hemipterans as a monophyletic group (Forero 2008). The characteristic structure of hemipteran mouthparts is schematically represented in **Figure 1.1**. The mandibles and maxillae are modified into two pairs of concentric piercing stylets. The mandibular stylets surround the maxillary ones and form two channels: the food channel (also known as alimentary channel), and the salivary channel. The flexible multi-segmented labium covers the mandibular and maxillary stylets, but never enters the pierced tissue while feeding. Sometimes, the stylets are much longer than the labium and, when not in use, they may be coiled within an integumental fold called crumena. Maxillary and labial palps are always absent (Gillot 2005; Forero 2008).

The specialized mouthparts of hemipterans allow them to penetrate several types of tissues for feeding (Gillot 2005). Some hemipterans (part of Heteroptera) are predators, feeding on body fluids of other arthropods and vertebrates, but most are phytophagous (Stenorrhyncha, Coleorrhyncha, Auchenorrhyncha and part of Heteroptera).

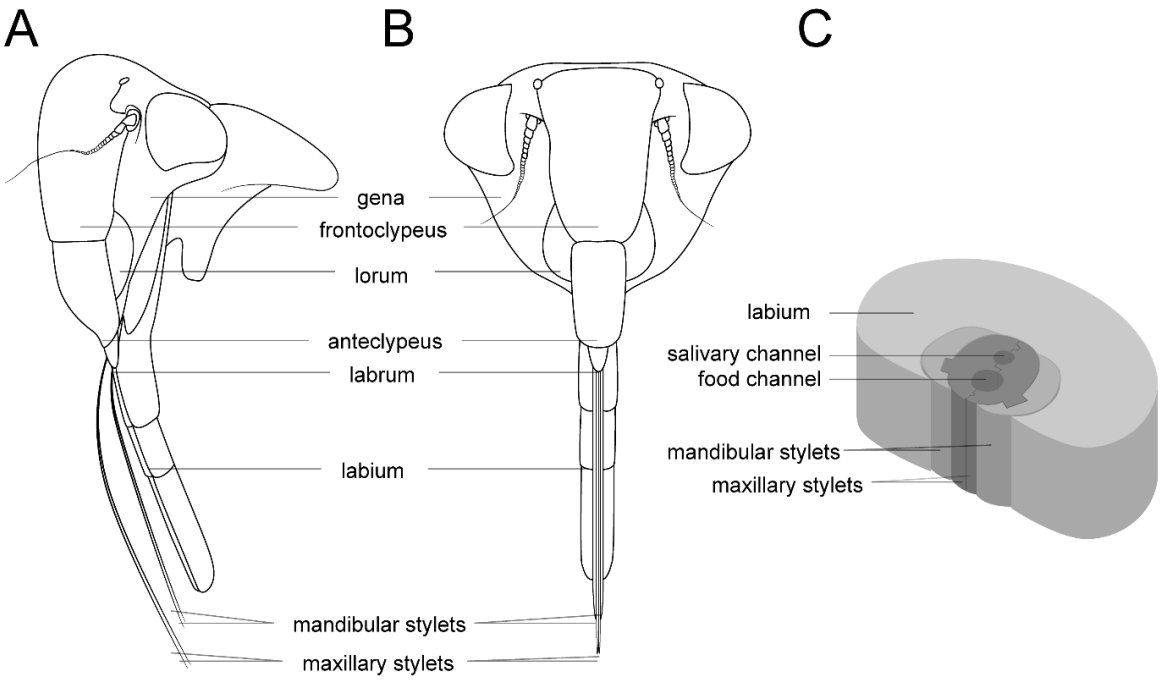

Figure 1.1. Representation of hemipteran mouthparts. **A** – Lateral view. **B** – Frontal view. **C** – Section of rostrum showing the salivary and alimentary channels formed by the maxillary stylets. **Author's original.**

1.1.2. Feeding habits

Phytophagous Hemiptera probe on three distinct feeding sites: mesophyll cells, xylem sap and phloem sap (Tonkyn & Whitcomb 1987; Perilla-Henao & Casteel 2016). Each of these plant tissues provides different challenges in terms of location, food quality and quantity which require adapted feeding modes, so typically different groups of phytophagous hemipterans specialize on probing a specific plant tissue, although exceptions exist. Tonkyn & Whitcomb (1987) made an excellent review about the constraints associated to probing in these different plant tissues. In a nutshell, xylem vessels are larger than phloem vessels so, xylem-feeders typically have larger stylets to acquire more food, faster. Xylem sap has a lower nutritional content and its composition does not vary significantly between plants, in opposition to phloem sap which is very rich in sugars, so phloem-feeders tend to be host-specific (or to have narrow host ranges) while xylem-feeders tend to be highly polyphagous. Xylem sap is under tension, but phloem sap is under pressure so, while phloem-feeders ingest food passively, xylem-feeders must pump xylem sap and usually have an enlarged clypeal region to support large cybarial muscles involved in suction.

Mesophyll feeders include, in Heteroptera, stink bugs (Pentatomorpha), plant bugs (Miridae) and lace bugs (Tingidae). Some scale insects (Coccoidea), aphids (Adelgidae) and leafhoppers of the subfamily Typhlocybinae also are typical mesophyll feeders. Phloem feeding is the most common feeding habit within Stenorrhyncha, but it is also typical in planthoppers (Fulgoroidea), treehoppers (Membracidae) and most leafhoppers (Cicadellidae). Xylem feeding is the predominant pattern for most spittlebugs or froghoppers (Cercopoidea), all cicadas (Cicadidae), all sharpshooters (Cicadellinae) and probably for leafhopper subfamilies Evacanthinae and Mileewinae as well (Tonkyn & Whitcomb 1987).

Among phytophagous hemipterans there are two usual modes of feeding: the stylet-sheath feeding, typical of all Stenorrhyncha, Coleorrhyncha and Auchenorrhyncha and some Pentatomorpha; and the lacerate-and-flush feeding, characteristic of other phytophagous Heteroptera (Miles 1972). The groups which probe by stylet-sheath feeding secrete two types of saliva with distinct composition, consistency and function. A watery saliva is involved in moistening food and mixing it with hydrolytic enzymes and a solidifying saliva plays a role in mechanical penetration of plant tissue.

In the stylet-sheath feeding mode, the behaviour of the insect can generally be described into four phases. The process starts with surface exploration, where the insect touches the plant surface repeatedly with the tip of the labium in order to identify the appropriate probing site (Miles 1972). The tapping allows the differentiation between the smooth epidermis and the rough vascular bundles, essential to probing site selection. Chemical cues detected by chemoreceptors on the mouthparts can also play a role in this choice (Cook & Denno 1994).

When an adequate site is selected, the insect secretes a drop of solidifying saliva (**Figure 1.2A**), externally called flange, and tissue penetration starts (**Figure 1.2B**). The insect firmly presses the labium against the plant surface and inserts the stylets through the flange into plant tissue while secreting solidifying saliva that moulds to the stylets forming a tubular structure enclosing them called stylet sheath (**Figure 1.2C**). The stylets penetration occurs in a series of backward and forward movements. The stylets move forward into the plant tissue and then are partially retracted. A tiny drop of solidifying saliva is secreted and watery saliva is discharged to aid penetration. The stylets push forward, moulding the stylet sheath and piercing the plant tissue a little further, and partially retract again. The process is repeated until the stylets reach the preferred tissue (mesophyll, phloem or xylem) and ingestion begins (Miles 1972).

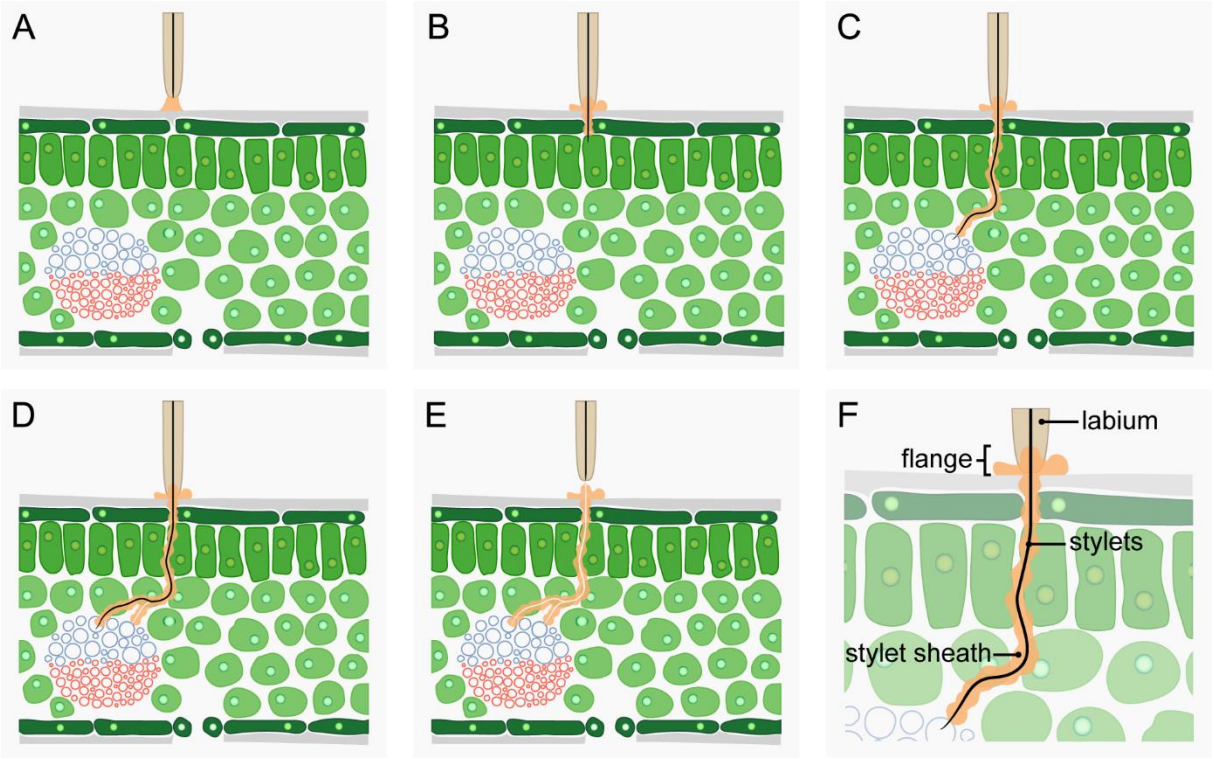

Figure 1.2. Schematic representation of the stylet-sheath feeding mode. **A** – Secretion of a drop of solidifying saliva after probing site selection. **B** – Beginning of stylets penetration into the plant tissue and formation of the stylet sheath. **C** – Deep penetration of the stylets into the plant tissue and food ingestion (xylem sap in this representation). **D** – Partial withdrawal of the stylets, formation of a branch in the stylet sheath and more food ingestion. **E** – Total withdrawal of the stylets. **F** – Graphic legend. **Author's original.**

Species-dependent mechanisms are involved in food ingestion. As previously said, typically, phloem-feeders ingest food passively while xylem- and mesophyll-feeders must pump or suck it (Tonkyn & Whitcomb 1987). When ingestion is finished, the insect withdraws the stylets through the stylet sheath sealing it with watery saliva. Withdrawal of the stylets can be complete (**Figure 1.2E**), if the insect is done feeding, or partial (**Figure 1.2D**). In the latter case, the stylets are partially withdrawn and start penetrating the wall of the stylet sheath producing a branch in the stylet sheath (Miles 1972).

The specialized feeding mode of phytophagous hemipterans can cause direct damage to plants. The lacerate-and-flush feeding has immediate damage, resulting in necrosis, whereas the stylet-sheath feeding results in growth disturbances in a wider time scale (Miles 1972). Indirect damage is related with opening wounds in plants that make them more susceptible to plant pathogens such as viruses and fungi (Gillot 2005). Besides the damage that these insects can cause to crops due to their feeding habits, they are in contact with microbes colonizing the vascular system of plants and can act as vectors of phytopathogens such as viruses and bacteria responsible for major plant diseases (Gillot 2005; Perilla-Henao & Casteel 2016). Interestingly, the transmission ability of bacterial or viral phytopathogens seems to be variable among hemipteran groups (**Figure 1.3**). Some groups are known vectors of both pathogen types (true bugs, leafhoppers, treehoppers and planthoppers), part only seem to transmit viruses (whiteflies, scale insects and aphids) while others appear to exclusively vector bacteria (psyllids, cicadas and spittlebugs).

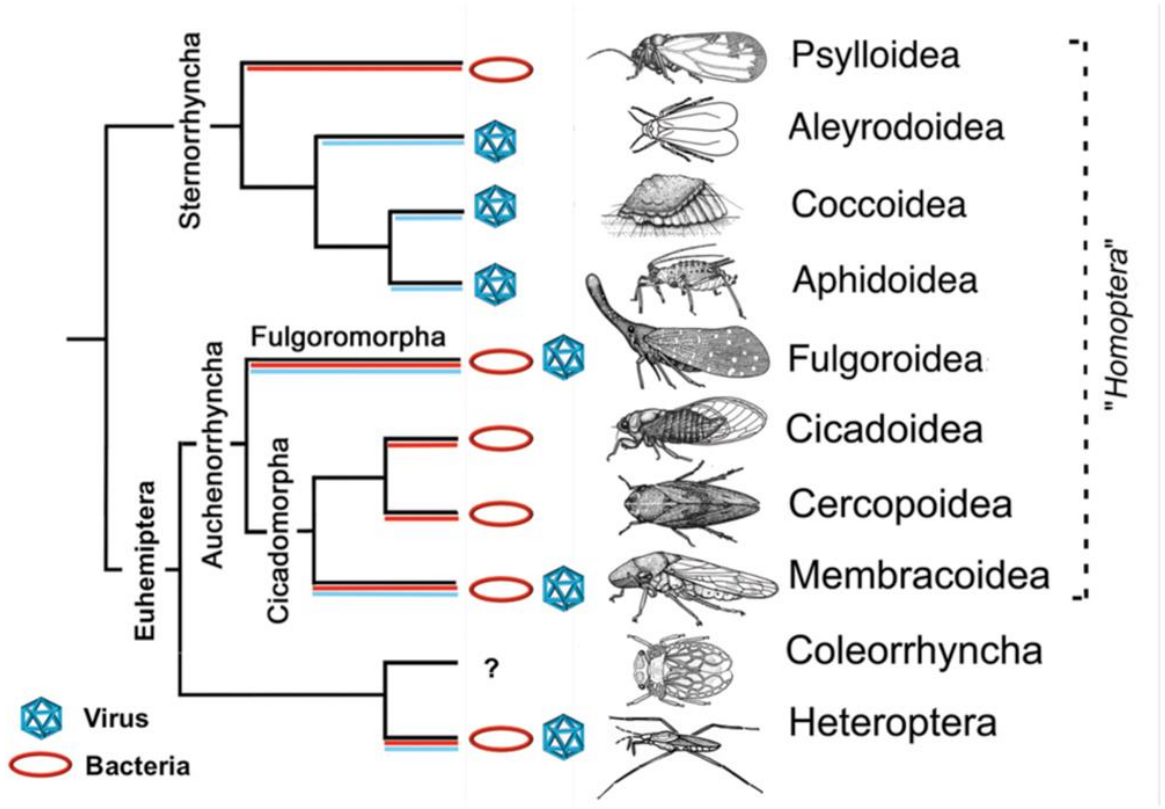

Figure 1.3. Hemipteran groups reported as vectors of viral and/ or bacterial plant pathogens. **From: Perilla-Henao & Casteel 2016.**

1.2. Xylella fastidiosa

1.2.1. Plant diseases caused by *Xylella fastidiosa*

Sharpshooters and spittlebugs are the main vectors of *Xylella fastidiosa* Wells et al. 1987, a gram-negative, xylem-limited bacterium. All xylem-feeding hemipterans may act as vectors (Redak et al. 2004) even that transmission efficiency varies. This vector-borne bacterium is considered one of the most important phytopathogens (Mansfield et al. 2012) due to its scientific importance, since it was the first bacterial plant pathogen to have its genome fully sequenced, but also due to its economic impacts since it causes disease in numerous agricultural crops, ornamental and wild plants (Hopkins & Purcell 2002). At this time, more than 350 plant hosts have been identified (EFSA 2015).

Xylella fastidiosa first caused the "California vine disease" during the 1880s in the Los Angeles area, California, United States of America (USA) (Purcell 2013). At the time, it could not be identified the causal agent of such disease, later known as Pierce's Disease (PD) in honour of Newton Pierce, the plant pathologist that first described in detail the disease symptoms. For a long time, nearly to 80 years, it was thought that the causal agent of the disease was a virus.

Decades later, a second outbreak of PD hit the region and new investigation efforts were made to understand the disease. These efforts lead to the discovery that xylem sap sucking insects belonging to Cercopoidea and Cicadellidae were vectors of the plant pathogen (Severin 1949, 1950). Other advances in investigation lead to uncovering clues that the phytopathogen could be a bacterial agent that was not culturable. The eventual success in the development of a culture medium (Davis et al. 1978) allowed the confirmation of a bacterium as the causal agent that was described and named *Xylella fastidiosa* by Wells et al. (1987).

Since the first report of a *X. fastidiosa* caused disease, the phytopathogen has been recognized as the causal agent of different diseases. Such diseases include: 1) Pierce's Disease (PD) in *Vitis* spp.; 2) Phony Peach Disease (PPD) in *Prunus persica* (L.) Batsch; 3) Alfalfa Dwarf (AD) in *Medicago sativa* L.; 4) Citrus Variegated Chlorosis (CVC) in *Citrus* spp.; 5) Almond Leaf Scorch (ALS) in *Prunus amygdalus* (Mill.) D. A. Webb; 6) Plum Leaf Scald (PLS) in *Prunus domestica* L.; 7) Oleander Leaf Scorch (OLS) in *Nerium oleander* L.; 8) Coffee Leaf Scorch (CLS) in *Coffea arabica* L.; 9) Olive Quick Decline Syndrome (OQDS) in *Olea europaea* L.; among many others (Hopkins & Purcell 2002; Janse & Obradovic 2010).

1.2.2. *Xylella fastidiosa* diversity

A few different subspecies of *X. fastidiosa* have been reported and include: 1) *X. fastidiosa* subsp. *fastidiosa*; 2) *X. fastidiosa* subsp. *multiplex*; 3) *X. fastidiosa* subsp. *pauca*; 4) *X. fastidiosa* subsp. *sandyi*; and 5) *X. fastidiosa* subsp. *tashke* (Schaad et al. 2004; Janse & Obradovic 2010; Almeida & Nunney 2015). A sixth subspecies (*X. fastidiosa* subsp. *morus*) has been proposed by Nunney et al. (2014). The known genetic diversity of *X. fastidiosa* is mostly associated to isolated strains from cultivated crops of economic relevance, but other unknown subspecies may inhabit unstudied hosts (Baldi & La Porta 2017).

Xylella fastidiosa has been detected in an increasing number of plant hosts, either symptomatic or asymptomatic. Symptoms are host plant-dependent but can include: leaf scorch, typically beginning from the margins and spreading to the entire leaf; chlorotic foliar lesions; premature fall of leaves or fruits; stunted growth; undersized leaves or fruits; and dieback of individual branches (Hopkins & Purcell 2002). Initially, "*X. fastidiosa* was regarded as an extended group of bacteria capable of infecting a wide range of host plants" (Baldi & La Porta 2017) but different subspecies have been associated to disease in different plants. This means that, depending on the host-pathogen combination, a particular strain or subspecies of *X. fastidiosa* may or may not induce the development of disease symptoms in a certain host. For instance, *X. fastidiosa* subsp. *pauca* has been known to cause OQDS in olive trees (Cariddi et al. 2014; Martelli et al. 2016) but it does not cause disease in several mechanically inoculated varieties of grapevine and citrus (EFSA 2016). Furthermore, OQDS has been associated to *X. fastidiosa* subsp. *pauca* (Cariddi et al. 2014; Martelli et al. 2016) but olive tree infection by other subspecies has not been correlated with olive trees displaying leaf scorch and branch dieback symptoms (Krugner et al. 2014). With the recognition of different *X. fastidiosa* subspecies and strains associated to disease in different plants it has been hypothesized that different subspecies are pathogenic only to a restricted number of plant hosts (Almeida 2016).

1.3. *Xylella fastidiosa*-vector relationship

Due to the longstanding paradigm that vector-borne plant diseases were typically caused by viruses and the later discovery of vector-borne phytopathogenic bacteria (Purcell 2013), research about phytopathogenic vector-borne viruses is better developed than research about vector-borne bacteria and some terminology related to virus–vector interactions was adopted to phytopathogenic bacteria-vector interactions (Perilla-Henao & Casteel 2016).

The relationship between the vector and the virus/bacteria can be classified as non-persistent, semi-persistent or persistent depending on the required time for acquisition and how much time the vector can retain the virus/bacteria while remaining infective (Perilla-Henao & Casteel 2016). For non-persistent pathogens, transmission can occur only within seconds or minutes after acquisition and therefore the vector needs multiple encounters with infected hosts to maintain transmission ability. For semi-persistent pathogens, the vector requires feeding periods from hours to days for acquisition and transmission ability is maintained for days. In persistent pathogens, it is necessary a long feeding period from hours to days and the vector's ability to transmit the pathogen remains after a single encounter with an infected host until death (Fereres & Moreno 2009; Perilla-Henao & Casteel 2016).

In respect to the retention local in the vector, viruses/ bacteria are distinguished into circulative and non-circulative. Whereas non-circulative pathogens are retained in association with the cuticula of the food channel, cibarium or foregut region (Ng & Zhou 2015); circulative pathogens go beyond the foregut and enter the vector's body, invading the haemolymph and salivary glands of their vectors as part of the transmission process (Perilla-Henao & Casteel 2016). The term "cuticula-borne" is also used to refer to non-circulative pathogens and, depending if the pathogen is retained in the alimentary canal or the foregut walls, it can be also designated as "stylet-borne" or "foregut-borne", respectively. By analogy, "salivary gland-borne" is another form to refer to circulative pathogens (Fereres & Moreno 2009).

Finally, pathogens can be classified in terms of their ability to replicate or not within the vector. Non-propagative pathogens do not multiply within the insect vector, while propagative pathogens use the vector as an alternative host and multiply within the vector (Fereres & Moreno 2009; Perilla-Henao & Casteel 2016). All vector-borne phytopathogenic bacteria are considered to be propagative, and propagation can occur extracellularly (between host cells) or intracellularly (within host cells) (Perilla-Henao & Casteel 2016).

Sharpshooter adults remain infective for long periods of time (Severin 1949), which is evidence that *X. fastidiosa* and its vectors have a persistent relationship. Lack of transstadial transmission (Purcell & Finlay 1979) is evidence that *X. fastidiosa* is non-circulative and that it is attached to the vector's cuticula since nymphs shed their cuticula after each moult. Absence of transovarial transmission has been shown once (Freitag 1951) and also supports a non-circulative relationship between *X. fastidiosa* and its vectors. Also, microscopic studies showed that the bacterium is present in the foregut walls of vectors. Location of *X. fastidiosa* within the foregut of the vector seems to affect transmission efficiency. Almeida & Purcell (2006) showed a positive strong correlation between the presence of *X. fastidiosa* in the precibarium and transmission efficiency. To conclude, as all vector-borne phytopathogenic bacteria, *X. fastidiosa* multiplies within its vectors (Perilla-Henao & Casteel 2016) and its propagation occurs extracellularly in the foregut. Also, *X. fastidiosa* does not require a latent period (Purcell & Finlay 1979), which means that a few cells are required to successful transmission and that biofilm formation is not a requirement to transmission.

1.4. Transmission

Transmission of *X. fastidiosa* occurs in three basic steps: acquisition, retention and inoculation. First, the vector must feed on an infected host acquiring the pathogen (acquisition); then *X. fastidiosa* must

attach to the foregut cuticula where it multiplies (retention); and finally, while the vector is feeding on a healthy host, *X. fastidiosa* must detach from the vector's cuticula and enter the new host (inoculation) (Almeida et al. 2005a; Chatterjee et al. 2008). It is considered as a successful transmission if *X. fastidiosa* multiplies after inoculation, generating a new infection (Chatterjee et al. 2008).

It is not exactly known how disease symptoms occur. It is hypothesised that wilting and scorching symptoms develop due to blockage of water transport in xylem vessels by *X. fastidiosa* multiplication and biofilm production which is supported by *X. fastidiosa*-induced water stress (Daugherty et al. 2010a) and by studies showing that regions of the plant displaying more severe symptoms are associated with higher *X. fastidiosa* populations (Purcell & Hopkins 1996; Alves et al. 2004). Other studies have not showed a correlation between *X. fastidiosa* colonization and symptom development or severity which lead to proposal of other hypotheses like symptom development being related to *X. fastidiosa*-generated phytotoxins or to growth regulator imbalance (Hopkins 1989). A recent study showed a spatial association between secretion of lipase/esterase LesA by *X. fastidiosa* and PD symptom severity; LesA accumulates more abundantly near the leaf margins where leaf necrosis is worse and gradually decreases to the leaf centre where symptoms develop later and are less severe (Nascimento et al. 2016). These results lead to another hypothesis, suggesting that *X. fastidiosa* secretion of lipase/ esterase LesA can be responsible for symptom development in grapevines.

Phloem-feeders, such as *Euscelis lineolatus* Brullé, 1832, have been shown acquisition ability (Elbeaino et al. 2014), but transmission ability seems to require the vector to be a xylem-feeder specialist (Redak et al. 2004). Transmission experiments with several phloem-feeders such as *Macrosteles fascifrons* (Stäl, 1858) [mentioned as *Macrosteles divisus* (Uhler, 1877)] (Cicadellidae: Deltocephalinae); *Agalmatium bilobum* (Fieber, 1877) [mentioned as *Hysteropterum severini* (Caldwell and DeLong, 1948)] (Fulgoroidea: Issidae); or *Euscelidius variegatus* (Kirschbaum, 1858) [mentioned as *Euscelis maculipennis* DeLong and Davidson, 1934] (Cicadellidae: Deltocephalinae) have not been successful (Severin 1949; Purcell 1980).

The inoculation mechanism by vectors is not quite well understood. Hopkins (1989) hypothesised that the negative tension in xylem would be able to move bacterial cells from the alimentary canal into the plant, but successful transmission to dormant grapevines (Almeida et al. 2005b) and almond (Almeida & Purcell 2003a) that have positive root pressure suggests that the vector probing behaviour is actively related to inoculation. Since there are differences between probing behaviour in different tissue specialists (Tonkyn & Whitcomb 1987), this may be another reason for why phloem-feeders that may acquire the bacterium have not shown ability to transmit the pathogen.

1.4.1. Acquisition and inoculation access periods influence transmission

Several factors seem to modulate *X. fastidiosa* acquisition and inoculation efficiency by vectors such as bacterial populations in host plant, acquisition and inoculation periods, probing behaviour and vector preferences (Almeida et al. 2005a). Some literature indicates that transmission efficiency rises proportionally with increasing acquisition access period (AAP) due to increased ingestion of bacterial cells and increased opportunities to foregut attachment (Redak et al. 2004). For instance, Purcell & Finlay (1979) showed that when PD vector *Graphocephala atropunctata* (Signoret, 1854) individuals were given one-hour AAP, inoculation had 30% success while an AAP equal or larger than 24 hours resulted in near 90% inoculation success.

Other studies suggest that acquisition efficiency is not a good predictor for transmission success (Rashed et al. 2011). For example, in transmission experiments to grapevine with two possible vectors of PD in Taiwan, *Kolla paulula* (Walker, 1858) and *Bothrogonia ferruginea* (Fabricius, 1787), both

sharpshooters, had high acquisition rates (83.3 and 70.0%, respectively) but low transmission rates (12.3 and 6.7%, respectively) (Tuan et al. 2016).

Inoculation access period (IAP) also is important for transmission efficiency as higher IAPs increase the number of inoculated cells and the likelihood of *X. fastidiosa* establishment in xylem vessels (Almeida et al. 2005a; Cornara et al. 2016a). For instance, longer IAPs have been associated to higher transmission rates as Daugherty & Almeida (2009) showed for glassy-winged sharpshooter *Homalodisca vitripennis* (Germar, 1821) and blue-green sharpshooter *G. atropunctata* with grapevines.

1.4.2. Vector preferences influence transmission

Vector preference influences the density of insects and their residence time on plants. A longer AAP increases the probability of acquisition by vectors (Purcell & Finlay 1979) and a higher number of vectors feeding on the same plant increases transmission rates (Severin 1950; Daugherty & Almeida 2009; Krugner et al. 2012; Tuan et al. 2016). Besides, different hosts have been associated to different concentrations of *X. fastidiosa*. For example, Almeida & Purcell (2003b) showed that *X. fastidiosa* strains causing both PD and ALS develop concentrations ten-times higher in grapevine than almond; Prado et al. (2008) revealed that a *X. fastidiosa* strain causing CVC had lower concentrations in coffee than in citrus plants; and Almeida et al. (2001) reported that populations of *X. fastidiosa* in sweet orange leaves displaying CVC symptoms were 10^2 to 10^3 times lower than in grapevine leaves displaying PD symptoms. Knowing this, preference for plant hosts that typically develop denser *X. fastidiosa* populations also increases the probability of acquisition and a vector that prefers susceptible hosts will likely have a larger importance in disease spread.

1.4.3. Vector preferences regarding plant infection status

Vector preference and acceptance of symptomatic hosts is also important since discrimination against infected hosts, reduces exposure to the bacterium, limiting transmission and consequently disease spread. Marucci et al. (2005) studied differences in feeding preferences between citrus plants displaying CVC symptoms and healthy citrus plants for two known sharpshooter vectors of CVC: *Dilobopterus costalimai* Young, 1977 and *Oncometopia facialis* (Signoret, 1854). Both vectors showed a clear preference for healthy plants over plants with CVC symptoms showing that the vectors discriminated between symptomatic and healthy citrus plants. Investigation of *O. facialis* preference between *X. fastidiosa*-positive asymptomatic citrus plants and healthy citrus plants revealed no discrimination between healthy and symptomless-but-infected citrus (Marucci et al. 2005). Similar results were obtained by Daugherty et al. (2011) that evaluated distinction between heathy grapevines and infected-but-symptomless grapevines in *G. atropunctata* and *H. vitripennis*, known vectors of PD, with choice trials. Preference between symptomatic and asymptomatic *X. fastidiosa*-positive grapevines was also evaluated for both vectors and no significant differences were found between visits to symptomatic and asymptomatic grapevines although both vectors revealed a clear tendency to visit first asymptomatic plants (Daugherty et al. 2011). Sharpshooter discrimination against symptomatic hosts appears to be a general behaviour of the group but generalizations should not be made since avoidance of plants showing disease symptoms was only studied in a few *X. fastidiosa* vectors and plant hosts.

For *X. fastidiosa* vectors, visual clues seem to have a primary role in discrimination between plants displaying symptoms and infected symptomless plants (Daugherty et al. 2011; Rashed et al. 2011). In a choice experiment between symptomatic plants painted green to mimic healthy plants and asymptomatic plants painted red and orange to mimic disease symptoms, sharpshooter vectors were less likely to alight on plants that looked symptomatic (Daugherty et al. 2011).

Rashed et al. (2011) studied the background matching behaviour of green sharpshooter *Draeculacephala minerva* (Ball, 1927), *H. vitripennis* and *G. atropunctata*, three PD vectors, with green and brown

backgrounds. While green-coloured *D. minerva* and *G. atropunctata* revealed a tendency to choose first, spend more time and visit more frequently a green background; *H. vitripennis*, which is mainly brown, showed the opposite tendency, preferring a brown background. Besides serving as a protective trait against predators, this behaviour may affect exposure to the pathogen due to differential distribution of bacterial populations within the plant host (Rashed et al. 2011).

The literature on other vector-borne phytopathogens shows that, in some cases, vectors prefer plants displaying symptoms and that infected plants can improve the vector fitness. For example, the whitefly *Bemisia tabaci* (Gennadius, 1889), a vector of Tomato Yellow Leaf Curl Virus (TYLCV) in several Solanaceae, shows preference for TYLCV-infected *Datura stramonium* L.; and TYLCV infection is positively correlated with egg survival and fecundity as well with body size in whitefly females and males (Chen et al. 2013). Visual and olfactory cues seem to be the reason for this type of attraction. Several studies have shown that virus-induced changes in leaf colour, as yellowing, can attract aphid vectors (Döring & Chittka 2007) and that virus-induced alterations in plant volatile emissions (Jiménez-Martínez et al. 2004; Mauck et al. 2010) also can increase host attractiveness.

Despite most studies referring to beneficial effects of phytopathogens to respective vectors, there is also evidence of neutral and negative effects. For example, de Oliveira et al. (2013) investigated the attack rates by parasitoids in an aphid vector of Cereal Yellow Dwarf Virus (CYDV) and the results showed that CYDV-carrying aphids were more frequently stung by parasitoid wasps than CYDV-free aphids. Contrary to references about other phytopathogens manipulating host attractiveness to vectors, there is no evidence so far that *X. fastidiosa* manipulates positively either the attractiveness or nutritional quality of infected plants for sharpshooters, but further investigation is necessary.

Multiple interactions between different pathogens and vectors with a mutual host can also have importance. Sasu et al. (2009) noted that bacterial wilt disease caused by *Erwinia tracheiphila* (Smith, 1895) Bergey et al., 1923, which is transmitted by the striped cucumber beetle *Acalymma vittatum* (Fabricius, 1775), is greatly reduced among plants exhibiting symptoms of infection by Zucchini Yellow Mosaic Virus (ZYMV) which is transmitted by several aphids. Shapiro et al. (2012) studied how floral and foliar volatiles responses of wild gourd *Cucurbita texana* (Scheele) A. Gray in relation to infection by *E. tracheiphila* and ZYMV were altered and how they affected the attraction by *A. vittatum*. The results showed that 1) foliar volatiles are similar between healthy, ZYMV-infected and *E. tracheiphila*-infected-but-symptomless branches, while *E. trachiephila*-infected branches displaying wilt symptoms produce more volatiles; 2) ZYMV induces suppression of some floral volatiles; and 3) *A. vittatum* has a preference for leaves of *E. tracheiphila*-infected plants displaying symptoms and flowers of healthy plants. These results may explain why ZYMV-infected plants have lower incidence of bacterial wilt since ZYMV reduces the floral volatiles playing a role in attraction of the vector of bacterial wilt, making ZYMV-infected plants less attractive to the beetle and therefore minimizing the bacterial wilt transmission probability on ZYMV-infected plants (Shapiro et al. 2012). In a similar way, several susceptible hosts to *X. fastidiosa* may also be hosts to other phytopathogens that may affect plant attractiveness and transmission rates by different vectors, but, so far, this kind of interactions seem to have not been studied.

1.4.4. Vector preferences within plant

Xylella fastidiosa is irregularly distributed in plants (Hopkins 1981; Daugherty et al. 2010b) so within-plant feeding preference is another aspect that can contribute to disease spread. If vectors prefer to feed on plant parts that tend to develop denser populations of *X. fastidiosa*, then acquisition likelihood should increase. Daugherty et al. (2010b) studied the feeding site preference of *D. minerva* and *G. atropunctata* in alfalfa. *G. atropunctata* showed preference for feeding in the upper part of the plant, where *X. fastidiosa* density was lower, while the bottom part of the plant was the preferred feeding site for *D.*

minerva, where *X. fastidiosa* density was the highest. In a subsequent transmission experiment where both vectors were confined only to the top or the bottom part of infected alfalfa for acquisition, the results showed that 1) transmission efficiency to grapevine was higher when vectors were confined to the lower part of alfalfa; and 2) in that case, *D. minerva* was more efficient than *G. atropunctata* (Daugherty et al. 2010b). Rashed et al. (2011) studied if bacterial acquisition efficiency is linked to the plant site where vector feeding occurs. Despite significant differences between the two studied sharpshooters (*H. vitripennis* and *G. atropunctata*), no significant differences were shown in respect to feeding site (stem vs leaf) (Rashed et al. 2011). A study on the preferred permanency sites within young citrus trees by two CVC vector species, *D. costalimai* and *O. facialis*, showed a differential preference between both vectors (Marucci et al. 2004). While *D. costalimai* preferred to stay on the secondary leaf nervures, *O. facialis* did not show a clear preference for leaves or branches, since individuals revealed a change in permanence between leaves and branches with the time of day (Marucci et al. 2004). Variation of within plant preference with time of day was also observed by Miranda (2008) in *Bucephalogonia xanthophis* (Berg 1879), another CVC vector. This vector shows a clear preference for the higher parts of the plant and, in the superior part of the plant, it prefers the branches over the leaf blades and petioles (Miranda 2008). In another study of within-plant preference in grapevine, *G. atropunctata*, *H. vitripennis* and *Phera lacerta* Fowler, 1899 [mentioned as *Homalodisca lacerta* (Fowler, 1899)] clearly preferred the leaf blade over stems and petioles, but despite the difference in preference for feeding site, acquisition likelihood did not differ among species (Daugherty et al. 2011). Even with the several studies addressing vector preferences, only a few vector species mainly associated to PD in USA and CVC in Brazil have been used, so a broader range of species should be investigated before generalizations.

1.4.5. Other aspects of transmission efficiency

Vector age and gender impact in *X. fastidiosa* transmission has been little studied. Krugner et al. (2012) revealed similar acquisition and inoculation rates in grapevines independently of gender or age of *H. vitripennis*. Vector size may also have importance in transmission since larger vectors can acquire and retain larger numbers of cells. For instance, *H. vitripennis* with a head about two-times larger than *G. atropunctata* acquired significantly more bacterial cells compared to *G. atropunctata* (Rashed et al. 2011).

1.4.6. Host, pathogen and vector diversity in transmission

All xylem sap-feeding insects belonging to Auchenorrhyncha, which include sharpshooters (Cicadellinae), froghoppers (Cercopoidea) and cicadas (Cicadoidea), seem to be capable of *X. fastidiosa* transmission but not with the same efficiency (Redak et al. 2004). The diversity of pathogen strains, vector species and host plants and the different combinations between the three elements influences transmission efficiency and, with that, disease prevalence and spread (Lopes et al. 2009). Transmission efficiency of *X. fastidiosa* varies with the combination of vector species and host plants as shown by several studies (Severin 1949, 1950; Purcell 1980; Marucci et al. 2008; Lopes et al. 2009).

Marucci et al. (2008) evaluated the transmission efficiency of *X. fastidiosa* to citrus and coffee plants by four different sharpshooter vectors: *B. xanthophis*, *D. costalimai*; *O. facialis* and *Homalodisca ignorata* Melichar, 1924 and the results revealed that *H. ignorata* transmits more efficiently to citrus trees (30%) than to coffee plants (2.2%) while the other tested vectors had similar transmission efficiency for both hosts. While *B. xanthophis*, *D. costalimai* and *O. facialis* were significantly less efficient than *H. ignorata* in citrus, all tested vectors transmitted with similar efficiency to coffee plants (Marucci et al. 2008).

In a transmission experiment from grapevines and almond trees to grapevines and almond trees with three leafhopper species and one froghopper species, transmission efficiency was determined (Purcell 1980). The results showed that: 1) different source hosts did not affect transmission efficiency; 2) *Philaenus spumarius* Linnaeus, 1758 and *G. atropunctata* were the most efficient vectors to both hosts; and 3) although only *G. atropunctata* was significantly less efficient at transmission to almond than to grapevine, transmission rates to almond were generally lower than to grapevines among *P. spumarius* (97% to grapes, 75% to almond), *G. atropunctata* (92% to grapes, 48% to almond) and *D. minerva* (17% to grapes, 8% to almond) (Purcell 1980). Despite low rates of transmission to almond and grape by *D. minerva* (Purcell 1980), this species is an efficient vector to alfalfa (Lopes et al. 2009).

1.5. Disease spreading

Transmission efficiency of the present vectors is important for spread but the importance of vectors in natural disease spread is influenced mainly by ecological attributes such as habitat and host selection, vector density and mobility, and spatial and temporal distribution (Purcell 1980; Almeida et al. 2005a).

In California, several sharpshooter and froghopper species have been reported as PD vectors (Severin 1950; Purcell 1980), but only a few are important to disease spread (Redak et al. 2004). A low-efficient vector with high prevalence may be a key species to disease progression in a certain region comparing to a more efficient vector with a low density in the same area. Turner & Pollard (1959) tested the transmission efficiency of five vector species of PPD in south-eastern USA: *H. vitripennis* [mentioned as *Homalodisca coagulata* (Say, 1832)]; *Oncometopia orbona* (Fabricius, 1798) [mentioned as *Oncometopia undata* (Fabricius, 1794)], *Homalodisca insolita* (Walker, 1858), *Cuerna costalis* (Fabricius, 1803) and *Graphocephala versuta* (Say, 1830). Although *H. insolita* was the most efficient vector (47.8%) and *H. vitripennis*, the least efficient vector (24.4%), *H. vitripennis*, the most abundant species in peach orchards, together with *O. orbona* were the main vectors of PPD since they were the only species among the studied that fed regularly on peach trees (Turner & Pollard 1959). In Coastal California, *G. atropunctata* was the most important PD vector until the recent introduction of *H. vitripennis* (Janse & Obradovic 2010), even that *G. atropunctata* transmission efficiency to grape is a lot higher (>90%) (Purcell & Finlay 1979) than *H. vitripennis* transmission efficiency (15 to 20%) (Purcell & Saunders 1999). In Central Valley of California, *D. minerva* and red-headed sharpshooter *Xyphon fulgida* (Nottingham, 1932) [mentioned as *Carneocephala fulgida* Nottingham, 1932] are considered the main vectors of PD because of their higher relative abundance in relation to another xylem-feeders (Purcell & Franzier 1985; Janse & Obradovic 2010).

Disease spread by vectors is based on two factors: acquisition of pathogen from an infected plant host by the vector and successful inoculation by the infected vector to a new uninfected plant host. Spread is usually from wild, generally symptomless, hosts to cultivated hosts rather than between cultivated hosts, though the latter can occur. The first type has been referred in the literature as “primary spread” and, by contrast, transmission between cultivated hosts has been mentioned as “secondary spread” (Purcell 2013). Understanding the main disease spread mechanism in a certain pathosystem is of importance because different management and control measures can be applied.

In California, PD spread is mainly driven by primary spread since control of vectors within vineyards and removal of diseased grapevines did not decrease spreading of PD in California (Purcell & Franzier 1985; Redak et al. 2004). Furthermore, the spatial patterns of PD occurrence in Central Valley California revealed a higher PD incidence in vineyards near riparian vegetation or alfalfa fields affected by AD which decreased with distance to those nearby habitats, suggesting that the disease spread occurred through vector dispersal from habitats outside of the vineyard (Redak et al. 2004; Janse & Obradovic 2010; Purcell 2013). This pattern is easily explained by *G. atropunctata* use of riparian plants for breeding and overwintering and by the high abundance of *D. minerva* and *X. fulgida* in bermudagrass,

watergrass, and perennial forages in irrigated pastures (Delong & Severin 1949; Purcell & Franzier 1985). In the two PD outbreaks in southern California related with the introduction and spread of *H. vitripennis*, the vineyards were closely associated to nearby citrus groves were the insect overwinters (Hopkins & Purcell 2002).

In Brazil, *X. fastidiosa* is the etiological agent of CVC, producing the most damage in citrus orchards. More than twenty species of sharpshooters have been described in Brazilian citrus orchards and at least thirteen species have been confirmed CVC vectors. Some of those species are very abundant on weeds whereas others are mainly found on citrus trees. Roberto et al. (2002) evaluated the CVC spatial dynamics in a sweet orange orchard located in northern São Paulo, Brazil. Initially, when CVC incidence was low (11%), CVC-affected citrus trees were distributed randomly in the orchard, but, after a few months, symptomatic citrus trees formed clusters and, in a period of about two years, the disease affected 82% of the trees in the study region (Roberto et al. 2002). This spread pattern is very different from the gradual pattern observed for the PD affected vineyards in California and suggests that secondary spread is the main mechanism of CVC propagation in that region (Redak et al. 2004). The effectiveness in slowing CVC spread by removing diseased trees and by pruning branches with early symptoms in mature trees also provides important evidence that infected trees are the primary source of inoculum for vector transmission (Redak et al. 2004; Janse & Obradovic 2010). Being the citrus trees the main source of inoculum, vector species with high prevalence on citrus trees, such as *Acrogonia citrina* Marucci & Cavichioli, 2002, *B. xanthophis*, *D. costalimai* and *O. facialis* are the main vectors of CVC, instead of species common on weeds.

1.6. *Xylella fastidiosa* distribution

Xylella fastidiosa is primarily a species of the Americas. It is hypothesised that *X. fastidiosa* is originally from South and Central America since, so far, no susceptible native plant hosts from that region have been identified, suggesting a long period of co-evolution. The opposite is not true for North America where multiple native hosts such as *Ulmus americana* L., *Platanus occidentallis* L. or *Quercus* spp. are susceptible to the phytopathogen (Almeida & Nunney 2015).

Xylella fastidiosa-related diseases have been affecting the Americas for more than a century (Hopkins & Purcell 2002; Janse & Obradovic 2010; Purcell 2013) and although several introductions seem to have occurred in the American continent, the bacterium has only been reported much more recently in other regions. Since the Americas have been dealing with *X. fastidiosa* diseases for the longest period, most investigation work is from there and, due to the economic impact of *X. fastidiosa*-caused diseases in crops, most knowledge about disease dynamics is limited to agricultural systems (Almeida 2016).

Most *X. fastidiosa*-related diseases occur in North America, being more incident in tropical and subtropical regions which have less severe climatic conditions. Some diseases like PD, PLS and ALS have a widespread distribution in the American continent while other diseases like CVC and CLS seem to be more restricted to South America (Almeida & Nunney 2015).

Vector transmission is relevant but only in short-distance spread (Redak et al. 2004). This bacterium can only invade a new region by long-distance dispersal which is driven by human intervention. Transport of infected plants to places with adequate environmental conditions for pathogen survival, susceptible plant hosts and native xylem-feeders can initiate disease spread in a new region. Alternatively, infected vectors can also be carried in plant transport and initiate disease spread if they feed on susceptible hosts. As xylem-feeders tend to be tissue specific but not host specific due to the low variation of xylem composition among different plants (Tonkyn & Whitcomb 1987), it should not be hard for this to happen, given that the environmental conditions are favourable to the introduced vector.

Outside the Americas, there are confirmed reports of *X. fastidiosa* in several European countries on various host plants (Saponari et al. 2013; EPPO 2016a; EPPO 2016b; Denancé et al. 2017) and of ALS and PD in Iranian almond orchards and vineyards (Amanifar et al. 2014). Asian pear leaf scorch has been reported in Taiwan (Leu & Su 1993) but the isolated strains are very different from all the previously known and a new species of the same genus (*Xylella taiwanensis*) has been proposed (Su et al. 2016). PD has also been detected in grapevines in Taiwan and phylogenetic analysis of PD-associated strains showed a relation to the American strains of *X. fastidiosa* (Su et al. 2013). *X. fastidiosa*-positive individuals belonging to two Cicadellinae species, *K. paulula* and *B. ferruginea*, have been collected mainly on weeds nearby diseased vineyards and are possibly involved in PD spread in Taiwan (Su et al. 2013) since transmission tests showed transmission ability (Tuan et al. 2016).

Furthermore, there is a report of ALS in Turkey (Güldür et al. 2005) which has never been validated by PCR tests or subsequent prospections with visual inspections and testing (EPPO 2016c). The same happened with a report of OLS in Lebanon (Temsah et al. 2015) that was later contradicted (Habib et al. 2016) and with a report of ALS in India (Gupta & Sharma 1998) which has never been confirmed.

1.7. *Xylella fastidiosa* in Europe

1.7.1. Italy, the first detection in Europe

In 2013, *X. fastidiosa* was detected for the first time in Europe in symptomatic olive trees, oleander and almond trees in Province of Lecce, Puglia Region, Italy (Saponari et al. 2013). Despite the existence of a previous report from Kosovo of *X. fastidiosa* in grapevines and another report from France based only on symptom observation (Janse & Obradovic 2010), this was the first confirmed report of the phytopathogen in Europe. The detected pathogen belongs to *Xylella fastidiosa* subsp. *pauca* strain ST53, also named CoDiRO strain (from the Italian name for the olive-affecting disease "Complesso del Disseccamento Rapido dell'Olivo") (Cariddi et al. 2014), and it is genotypically similar to a strain present in Costa Rica, which is the probable source of introduction (Loconsole et al. 2016). Other infected hosts like *Prunus avium* L., *Polygala myrtifolia* L. and *Westringia fruticosa* (Willd.) Druce have also been detected in Puglia Region (Saponari et al. 2014a). The distribution of the bacterium in the Italian territory remains, so far, restricted to Puglia Region, although it has expanded northwards from Province of Lecce to Provinces of Taranto and Brindisi (Martelli 2016).

The disease started decimating Italian olive groves in the late 2000's. When detection occurred, *X. fastidiosa* had already destroyed about 8000 ha of olive groves in the region and in 2016 near 23000 ha have been destroyed (Frisullo et al. 2014; Martelli et al. 2016). The rapid spread and progression of disease symptoms lead to naming it as Olive Quick Decline Syndrome (OQDS). OQDS symptoms consist in appearance of leaf scorching and desiccation in small peripheral branches randomly distributed on the canopy that rapidly extend to the rest of it, culminating in tree death (Carlucci et al. 2013; Frisullo et al. 2014; Martelli et al. 2016). Older trees with poor management in the region showed more severe symptoms related to extensive galleries of the leopard moth larvae (*Zeuzera pyrina* Linnaeus, 1761), fungal colonization by several species of two fungi genera (*Phaeoacremonium* and *Phaemoniella*) (Nigro et al. 2013) and *X. fastidiosa* colonization (Saponari et al. 2013). *X. fastidiosa* was identified as the OQDS causal agent since *X. fastidiosa*-infected olive trees had a strong correlation with OQDS symptoms and *X. fastidiosa*-positive trees distribution was completely superimposed with olive trees displaying OQDS symptoms which was not verified for olive trees with moth galleries or fungi (Frisullo et al .2014).

After detection, Elbeaino et al. (2014) conducted a study on the potential vectors of OQDS in olive orchards of Puglia Region. The authors collected the insects with yellow sticky traps and by net sweeping and identified three species of potential vectors: two spittlebugs (Cercopoidea:

Aphrophoridae) – *P. spumarius* and *Neophilaenus campestris* (Fallén, 1805) – and one leafhopper (Cidadellidae: Deltocephalinae) – *E. lineolatus*. The potential vectors were tested for *X. fastidiosa* and some individuals from the three species were *X. fastidiosa*-positive: eight *P. spumarius*, fourteen *N. campestris* and sixteen *E. lineolatus* (Elbeaino et al. 2014). Other xylem-feeders such as *Cicada orni* Linnaeus, 1758 (Cicadidae) and *Cercopis sanguinolenta* (Scopoli, 1763) (Cercopoidea: Cercopidae) have been found in Puglia Region, but all tested individuals of these species have been *X. fastidiosa*-negative (Cornara et al. 2016b). In the first transmission tests to olive with *P. spumarius*, no successful transmissions occurred but a small number of individuals and plants was used (Saponari et al. 2014b). Recently, *P. spumarius* OQDS transmission ability to olive trees has been shown for naturally infected individuals (Cornara et al. 2017) and also in transmission experiments between olive trees under field conditions (Cornara et al. 2016b). *N. campestris* has not yet revealed transmission ability (Cornara et al. 2016b), however, a reduced number of individuals was used in the trials and demonstrated transmission ability should be more a question of testing a sufficient number of individuals as it was with Cicadellinae species in Americas (Redak et al. 2004). The relevance of *N. campestris* as vector of OQDS in Puglia olive groves should not be high since the populations seem to be little abundant (Cornara et al. 2016b) but it may be important to other hosts or in regions where higher populations of this species thrive.

Cornara et al. (2016b) assessed changes in the relative abundance and infectivity of adult *P. spumarius* on weeds and olive trees throughout the year and reported that this species was the most abundant in Italian olive orchards on both hosts composing 98.56% of the total spittlebug composition on olive trees. It seems that *P. spumarius* moves from weeds to olive trees during the dry period (from May to June) and returns to weeds at the end of this period since the relative abundance on olive canopy is higher during that period, while the reverse is observed for weeds (Cornara et al. 2016b). The same authors reported that before the shift from weeds to olive trees all collected individuals were tested negative for *X. fastidiosa* presence; the first *X. fastidiosa*-positive *P. spumarius* were collected from olive canopy on May and the proportion of infected individuals gradually increased during the dry season which suggests that olive trees are probably the main source of inoculum for transmission and is preliminary evidence that disease spread in Italian olive groves by *P. spumarius* occurs mainly by secondary spread (from olive tree to olive tree).

1.7.2. Other detections

In July 2015, *X. fastidiosa* subsp. *multiplex* was detected in a few ornamental plants displaying leaf scorch symptoms (*P. myrtifolia*) in Corsica, France. Since then other *X. fastidiosa*-positive plants as *Spartium junceum* L., *Lavandula stoechas* L. or *Myrtus communis* L. were also detected, but *P. myrtifolia* is the most affected plant in Corsica (EPPO 2015a). The different subspecies of the bacterium lead to the conclusion that *X. fastidiosa* introduction in France has a different origin from the one affecting Italian olive groves (EPPO 2015b). Later, in October 2015, the same subspecies of the pathogen was also found in *P. myrtifolia* in mainland France, specifically in Provence-Alpes-Côte d'Azur Region (PACA Region) (EPPO 2015c). Even more recently, in September 2016, an isolated finding of *X. fastidiosa* subsp. *pauca* was reported for *P. myrtifolia* in Menton town, PACA Region, near the Italian border (EPPO 2016d; Denancé et al. 2017). Denancé et al. (2017) studied the diversity of *X. fastidiosa* strains and subspecies present in France in an attempt to determine possible routes of introduction. Several strains from three *X. fastidiosa* subspecies (subsp. *multiplex*; subsp. *pauca*; subsp. *sandyi*) have been identified, although with different frequencies (about 300 samples of subsp. *multiplex*; 6 samples of subsp. *sandyi*; and 10 samples of subsp. *pauca*) suggesting that the emergence of *X. fastidiosa* in France is associated to several introduction events (Denancé et al. 2017). So far, no studies on the vectors involved in *X. fastidiosa* spread in France were published, but Germain (2016) has

identified 47 potential vector species in mainland France and 12 in Corsica based on bibliographic research.

In November 2016, the Spanish Authorities notified the presence of *X. fastidiosa* subsp. *fastidiosa* in three cherry trees of a Garden Centre in Mallorca, Balearic Islands (DGAV 2016a; EPPO 2016a). Since then, different plant species have been found in numerous locations around the Balearic Islands: 172 plants in Mallorca, 73 in Ibiza and 36 in Menorca (DGAV 2017a; EPPO 2017a). At least three subspecies of *X. fastidiosa* are involved: subsp. *fastidiosa* (in Mallorca), subsp. *multiplex* (in Mallorca and Menorca) and subsp. *pauca* (in Ibiza) (DGAV 2017b). Positive cases in Spain include olive trees, plum trees, almond trees, lavender, oleander, polygala, *Acacia saligna* (Labill.) H.L. Wendl and *Fraxinus angustifolia* Vahl. For the first time in Europe, the bacterium has been detected in a grapevine plant in Mallorca (EPPO 2017b). This is potentially worrying since in the end of nineteenth century the grape phylloxera affected vineyards all over the world; European varieties were particularly susceptible to phylloxera and the solution to control the outbreaks in Europe was to graft native varieties onto imported phylloxera-resistant North American rootstocks (Janse & Obradovic 2010) which are known to be susceptible to *X. fastidiosa* in the Americas. In June 2017, the Spanish Authorities have confirmed the first detection of *X. fastidiosa* subsp. *fastidiosa* in Spanish mainland (DGAV 2017b; EPPO 2017a) which occurred in almond trees in Autonomous Region of Valencia.

Even before detection, a first investigation on the potential vectors in Spain has been made (Lopes et al. 2014). The surveys occurred during autumn of 2004 in three regions of Spain (Andalucía, Murcia and Madrid) involving vineyards, citrus groves, olive orchards, riparian vegetation and weeds. The specimens were only identified to family or subfamily, except for abundant species. The only potential vectors were spittlebugs (Cercopoidea), including *Neophilaenus* sp., but a reduced number of individuals were found (Lopes et al. 2014), possibly due to the short sampling period. So far, there are no other published studies on the vectors of *X. fastidiosa* in Spain.

In June 2016, the German Authorities notified an isolated finding of *Xylella fastidiosa* subsp. *fastidiosa* in a potted plant of oleander located in a greenhouse of a small nursery of Saxony. In total, four plants of different genera have been found infected in the nursery, namely *Nerium*, *Rosmarinus*, *Streptocarpus* hybrid and *Erysimum* hybrid (EPPO 2016b). The rest of the German territory remains free from the bacterium based on official surveys.

Apart from these reports of *X. fastidiosa* in Italy, France, Spain and Germany, several interceptions of *X. fastidiosa*-carrying plants have been reported around Europe in Italy (Loconsole et al. 2016), France (Denancé et al. 2017), Netherlands (Bergsma-Vlami et al. 2015); Czec Republic (EPPO 2017c) and Switzerland (EPPO 2015d); mainly coffee plants imported from Honduras and Costa Rica, but also from Mexico and Ecuador (EFSA 2015).

1.8. Management measures

The apparent absence of *X. fastidiosa* in Europe for a long time and the risk of its emergence has been discussed by several authors in the past. Large scale importations of resistant grapevine rootstocks from North America to Europe as a "solution" to phylloxera epidemic; as well as importations of a wide range of other symptomless hosts could have provided numerous introductions (Hopkins & Purcell 2002; Janse & Obradovic 2010). Although froghoppers can be vectors, in the Americas, sharpshooters are the main vectors of *X. fastidiosa*-related diseases. Cicadellinae are a very diverse subfamily in the American continent but this group is not common in Europe (Redak et al. 2004) being restricted to a few genera that, in their majority, are not widespread. Knowing this, the possible lack of competent vectors as well as the unfamiliarity with disease symptoms (Janse & Obradovic 2010) were hypothesised as possible reasons for the previous "absence" of *X. fastidiosa* in Europe.

Due to the uncertainty of *X. fastidiosa* introductions, the phytopathogen has been considered as a quarantine organism in Europe. In 1989, European and Mediterranean Plant Protection Organization (EPPO) included *X. fastidiosa* in the A1 List of quarantine pests (a list of pests not present in European and Mediterranean territory that should be under surveillance). Recently, in September 2017, EPPO transferred the bacterium to the A2 List of quarantine pests (a list of pests present in the territory but not widely distributed and being officially controlled). The phytopathogen was also included in Annex I of Directive 2000/29/EC that contains protective measures against introduction and spread of organisms harmful to plants in European Union (EU) territory and that imposes eradication measures against quarantinable pathogens.

The variety of subspecies detected in several regions around Europe suggests that multiple introductions have been made throughout time but remained unnoticed until recently, as previously suggested. The reports of interceptions of several *X. fastidiosa*-infected *Coffea* plants in various countries like Netherlands, Switzerland, France or Italy provide sufficient example of how these introductions could have occurred through plant trade between countries (Loconsole et al. 2016; Denancé et al. 2017).

The emergence of *X. fastidiosa* diseases is determined by multiple interactions between four main elements: the bacterium, the host plant, the insect vector and the environment (Almeida et al. 2005a). Plant diseases can be managed by disrupting interactions between the elements involved in the pathosystem. If only one of these interactions could be fully interrupted, disease spread would completely stop, but there are no available methods to totally disrupt any of the main interactions in *X. fastidiosa* pathosystems. Knowing this, a combination of multiple strategies has been used to manage *X. fastidiosa*-related diseases in different pathosystems.

The efficacy of each technique is dependent on the local conditions and dynamics associated to an infected area. In the Americas, PD management still depends on eliminating alternative hosts, suppressing vector populations and using resistant grapevine varieties that lack other desirable characteristics (Appel et al. 2010). Systemic insecticides, especially neonicotinoids, and repellents, as kaolin, have been used in American vineyards (Almeida et al. 2005a). Different insecticides like neonicotinoids and pyrethroids have showed high mortality rates for *H. vitripennis* [mentioned as *H. coagulata*] (Akey et al. 2001) and are used effectively in California PD-affected vineyards, but chemical control has not been efficient to reduce vector populations in PPD-affected peach orchards in Florida (Overall & Rebek 2017). Some insecticides can also inhibit feeding and *X. fastidiosa* transmission by *H. vitripennis* [mentioned as *H. coagulata*] to oleander (Bethke et al. 2001). Application of kaolin to grapevine has been shown to reduce acquisition of *X. fastidiosa* from infected grapevines and inoculation of healthy grapevines by *H. vitripennis* [mentioned as *H. coagulata*] partly due to visual cues since kaolin-treated grapevines look white (Almeida et al. 2005a). In vineyards, "treatments with kaolin, either alone or in combination with neonicotinoids, had 50-57% less PD than untreated controls" (Almeida et al. 2005a).

Knowing from a previous study that "97% of immigrant leafhopper vectors enter a vineyard from adjacent citrus or native vegetation at heights <5 m", Blua et al. (2005) evaluated the effect of a 5-meter screen barrier positioned between a grapevine commercial nursery and two types of surrounding habitats (citrus groves and riparian vegetation) on the movement limitation of *H. vitripennis* [mentioned as *H. coagulata*] from surrounding vegetation to the commercial nurseries. From the 87 tested individuals (including males and females), the majority (70.5%) flew away from the barrier, showing the potential of the barrier as a mechanical control method (Blua et al. 2005). Removing infected hosts is useful to reduce secondary spread. Although removal of infected grapevines in California has not been successful to diminish PD incidence in vineyards since primary spread due to migration from neighbouring habitats as riparian vegetation is the main mechanism to chronic infections establishment (Almeida et al. 2005a);

removal of infected trees and alternative hosts in peach and plum orchards has been an effective measure to reduce incidence of PPD and PLS (Overall & Rebek 2017).

Other options for reducing vector populations include biological control that involves identifying the natural enemies of a target pest (Pilkington et al. 2005). Population regulation in an ecosystem is a biologic process involving natural enemies. These auxiliary species can be either predators, parasites, pathogens, parasitoids or competitors and reduce the population density of their prey, hosts or competitors, either directly or indirectly (Amaro 2003). In North America, several species of parasitoid wasps belonging to *Gonatocerus* (Hymenoptera: Mymaridae) have been shown to parasitize eggs of *H. vitripennis* (Triapitsyn et al. 1998). "The most commonly released natural enemies are the egg parasitoids, *Gonatocerus ashmeadi* Girault, 1915, *Gonatocerus trigutattus* Girault, 1916, a few other *Gonatocerus* spp., and *Anagrus epos* Girault, 1911" (Overall & Rebek 2017). While *G. ashmeadi* is the most abundant parasitoid in California, being the key natural enemy in the region, *G. trigutattus* (Triapitsyn et al. 1998) seems to be the key parasitoid in Texas (Pilkington et al. 2005). Egg parasitism rates vary from between different regions. In California, where two annual peaks of *H. vitripennis* populations occur, the average proportion of parasitized eggs was 12% for the spring peak and 19% for the summer peak; in Florida, the reported parasitism rates are higher and can reach 100%; and in Texas, parasitism rates varied between 38% and 100% (Pilkington et al. 2005). Variation of climatic conditions among regions is important for success of parasitoids as biological control of *H. vitripennis*. Son et al. (2012) analysed the survival rate of *Gonatocerus morgani* Triapitsyn, 2006 by measuring the adult emergence from *H. vitripennis* parasitized eggs at different temperatures and the results showed survival rates ranging between 59% at 30.4ºC and 0% at 33.8ºC. Development time also was influenced by temperature; lower temperatures required more time to adult emergence and the optimal temperature for development time was 28.7ºC (Son et al. 2012).

Introduction of certain strains of a pathogen can mitigate the properties of virulent strains of the same pathogen and be used as biological control in plant disease (Appel et al. 2010). Weakly virulent strains of *X. fastidiosa* multiply and move systematically but more slowly, inducing milder or minor disease symptoms in the plant host (Hopkins 2005). The virulence of several *X. fastidiosa* strains isolated from different hosts in *Vitis vinifera* L. 'Carignane' was tested by Hopkins (2005) who identified six avirulent or weakly virulent strains: PD-1 (isolated from *V. vinifera*); PD91-2 (isolated from muscadine grapevine); PD94-1 (isolated from wild grapevine); PD95-6 (isolated from hybrid bunch grapevine); Syc86-1 (isolated from sycamore); and EB92-1 (isolated from elderberry). The effectiveness of those "beneficial" strains against naturally virulent PD strains was evaluated according to the severity of observed PD symptoms. PD-1 and Syc86-1 strains were tested in *V. vinifera* 'Himrod' for two years. Plants treated with PD-1 strain did not differ from non-treated plants in terms of symptom development or severity, but Syc86-1 strain had a positive effect since only one of the three grapevines inoculated with Syc86-1strain developed symptoms and the symptoms started developing a year after the first PD symptoms appeared in non-treated and PD-1-inoculated grapevines (Hopkins 2005). On other two trials, the same author tested the effect of the six strains in *V. vinifera* 'Flame Seedless' for two years and in *V. vinifera* 'Cabernet Sauvignon' for four years. Contrary to the trial with *V. vinifera* 'Himrod', Syc86-1 strain did not show a beneficial effect in *V. vinifera* 'Cabernet Sauvignon' since Sy86-1-inoculated plants revealed similar symptom severity to non-treated plants. PD91-2 inoculated grapevines in the two trials started to develop symptoms later, but in the end of both study periods the symptoms in those plants were as severe or worse than in the non-treated plants. In both trials, EB92-1 strain was the only one to reduce the ability of the virulent native PD strain to cause disease. Grapevines inoculated with EB92-1 strain had the lowest mortality rate in the end of the trials, retarded symptom development and milder symptoms (Hopkins 2005).

The protective effect of weakly virulent strains seems to be dependent on the studied varieties. Appel et al. (2010) showed the preliminary results of the protective effect of EB92-1 strain in four grapevine varieties: *V. vinifera* 'Merlot', *V. vinifera* 'Viognier', *V. vinifera* 'Cabernet Sauvignon' and *V. vinifera* 'Blanc du Bois'. The greatest level of symptom development when inoculated only with the virulent strain of the pathogen was observed in *V. vinifera* 'Merlot' and *V. vinifera* 'Viognier', that are probably the most susceptible varieties in the study, but inoculation with both the virulent *X. fastidiosa* strain and EB92-1 strain lead to much milder symptoms in both varieties. In *V. vinifera* 'Blanc du Bois', considered to be tolerant, the effected of inoculation with EB92-1 strain was less evident, but not negative (Appel et al. 2010).

After the OQDS outbreak in Italy, the EU has created a set of measures to prevent further introductions into and to limit the spread of *X. fastidiosa* within EU territory which are specified by the Commission Implementing Decision (EU) 2015/789 of 18 May 2015 amended by Commission Implementing Decision (EU) 2015/2417 of 17 December 2015 and by Commission Implementing Decision (EU) 2016/764 of 12 May 2016 (EU 2015a, 2015b, 2016). Those measures include annual surveys for the presence of *X. fastidiosa*; conditioned movement of plants from infected areas; and visual inspections, sampling and testing of imported plants, depending on the origin country. In case of detection, it is predicted the establishment of demarcated areas consisting of an "infected zone" (where infected hosts exist) and a "buffer zone" of at least 10km surrounding the "infected zone". In the infected areas, EU countries are supposed to implement eradication measures which imply 1) destroying all infected plants and removing all alternative hosts despite of health status in a radius of 100m surrounding infected plants; 2) vector control; and 3) investigation on infection origin. When eradication is not possible, it is predicted the implementation of containment measures to limit further spread aimed to minimize inoculum in host plants and controlling vector populations. Due to the numerous interceptions of *X. fastidiosa*-positive *Coffea* plants from Costa Rica and Honduras in EU since October 2014 (EFSA 2015), importation of coffee plants originating from these countries is prohibited into EU countries (legislative control), although seeds can be imported.

The multiple foci encountered within Italy, Corsica, mainland France, Baleares Islands and mainland Spain reveal that the bacterium is already established in the territory. "The long-standing American experience reflects that when *X. fastidiosa* enters a territory endowed with favourable climatic conditions and a receptive flora, the bacterium becomes so firmly established that its eradication is no longer achievable" (Martelli 2016).

Eradication measures have not been successfully implemented in Italy, France or Spain. This is partially due to the understandable resistance of local farmers and environmental organizations to these measures since they basically imply destroying all vegetable life in the infected areas, and that no actions were initially predicted to compensate local farmers (Martelli 2016) at least for their economic losses, but the opposition and disbelief of local communities has contributed a lot to *X. fastidiosa* spread in Italy (Abbot 2015, 2016, 2017).

Once *X. fastidiosa* is firmly established, the focus should be on containment measures that minimize disease spread. Since there are no effective measures to combat directly *X. fastidiosa* (EFSA 2015), control is focused on plant hosts and insect vectors. The contention measures in Italy are directed to limiting OQDS northwards progression and include extensive monitoring of *X. fastidiosa* and its vectors; vector control (chemical control by spraying olive canopies with insecticides where adult vectors feed during spring and physical control by mechanical weeding directed at reduction of vector nymph populations that spend their time on weeds from autumn to early-spring); elimination of alternative hosts in highways and canals; and immediate unrooting of recently-infected olive trees as well as neighbouring olive trees (cultural control) (Martelli 2016; Martelli et al. 2016). Mechanical

weeding has showed a great impact at reduction of spittlebug nymphs (70% estimated reduction) and consequently on the adult population (Martelli 2016).

The potential use of the assassin bug *Zelus renardii* Kolenati, 1856 as a biological control agent to complement the chemical and mechanical control of *P. spumarius* is also being investigated in Italy (Granitto 2017; Salerno et al. 2017)

Other options are being studied to reduce the impact of *X. fastidiosa* in infected areas such like the use of resistant varieties (genetic control). In Puglia Region, olive groves are based on two traditional varieties: *O. europaea* 'Cellina di Nardò' and *O. europaea* 'Ogliarola salentina' that are susceptible to OQDS. However, there are some varieties locally cultured like *O. europaea* 'Leccino' that showed tolerance to OQDS, displaying green canopies with low levels of leaf scorch despite being in the neighbourhood of heavily OQDS-affected olive trees. A comparison between *O. europaea* 'Ogliarola salentina' and *O. europaea* 'Leccino' showed that the last has bacterial populations 10 to 100 times lower than the first variety (Martelli 2016; Martelli et al. 2016; EFSA 2017). Other varieties that have been studied (*O. europaea* 'Coratina', *O. europaea* 'Fratoio' and *O. europaea* 'Leccino') develop bacterial populations ten-times lower than *O. europaea* 'Cellina di Nardò' (EFSA 2017). More than 60 varieties are being investigated in Italy, including *O. europaea* 'Arbequina' (EFSA 2017) which is largely used in intensive and superintensive Portuguese olive groves. Several European countries are also doing trials with other *X. fastidiosa* strains and different varieties of plant hosts like oleander, grapevine, alfalfa, cherry tree or plum but results are not yet available (EFSA 2017).

The search for resistant varieties seems to be the most promising measure to reduce *X. fastidiosa* impact in infected areas, but, as mentioned by Martelli (2016) and Martelli et al. (2016), other options like the use of benign strains that reduce the pathogenicity of more virulent ones (Hopkins 2005; Appel et al. 2010); biocontrol with bacteriophages (Das et al. 2015); the regulation of growth and movement of *X. fastidiosa* (Lindow et al. 2014); the induction of symptom remission with N-acetylcysteine (Muranaka et al. 2013); and the introduction of nanoparticle-carrying toxic molecules to the bacterium may also be useful in infected areas.

1.9. Portugal as a risk area

To this date, there are no reports of *X. fastidiosa* in Portugal, but a national contingency plan based on introduction prevention and prospection of susceptible hosts and potential vectors has been elaborated (DGAV 2016b).

Favourable climate, diversity and abundance of host plants and competent vectors are the basic requirements for *X. fastidiosa* establishment and spread in a newly-invaded area (Almeida & Nunney 2015). As pointed by Pereira (2015), Portugal is a risk area to *X. fastidiosa* introduction due to its geographical position in European and global trade; the mild climate; and the presence of preferential hosts with economic importance such as grapevines, olive trees, citrus trees, cork oaks or almond trees.

The occurrence of *P. spumarius*, a widespread competent vector, in both mainland (Drosopoulos & Quartau 2002) and archipelagos (Quartau et al. 1992) as well as of other species belonging to Aphrophoridae, Cercopidae, Cicadellinae, Cicadidae and Tibicinidae, the potential vector groups in Europe identified by EFSA (2015) is, likewise, a risk factor (Pereira 2015). In Portugal, such species include, for instance, the aphrophorids *Neophilaenus angustipennis* (Horváth, 1909) and *Philaenus tesselatus* Melichar, 1899 (Quartau & André 1988; Drosopoulos & Quartau 2002); the cercopids *Cercopis sanguinolenta* (Scopoli, 1763) and *Haematoloma dorsata* (Ahrens, 1812) (Soulier-Perkins 2007-present); the sharpshooter *Cicadella viridis* (Linnaeus, 1758) (Pereira 2015); the cicadas *C. orni*, *Cicada barbara lusitanica* Boulard, 1982 and *Lyristes plebejus* (Scopoli, 1763) (Quartau 1988; Suer et al. 2004); and the European cicadas *Tibicina garricola* Boulard, 1983 *Tibicina quadrisignata* (Hagen,

1855), *Tibicina tomentosa* (Olivier, 1790), *Melampsalta varipes* (Waltl, 1837), *Tympanistalna gastrica* (Stål, 1854), *Euryphara contentei* Boulard, 1982; *Tettigetta argentata* (Olivier, 1970), *Tettigetta estrellae* Boulard, 1982; *Tettigetta josei* Boulard, 1982 and *Tettigetta mariae* Quartau & Boulard, 1995 (Quartau et al. 2001; Suer et al. 2004).

Given the growing detections of *X. fastidiosa* in Europe and knowing the favourable conditions that Portugal presents to the phytopathogen introduction and spread, it seems to be more a matter of time until *X. fastidiosa* is reported as present in the territory than otherwise.

1.10. Olive production in Portugal

Considering that almost all olive and olive oil production in Europe is restricted to the southern countries and that African production of these goods is practically restricted to the northern countries, the Mediterranean Region is responsible for practically all the olive and olive oil production worldwide (**Figure 1.4**).

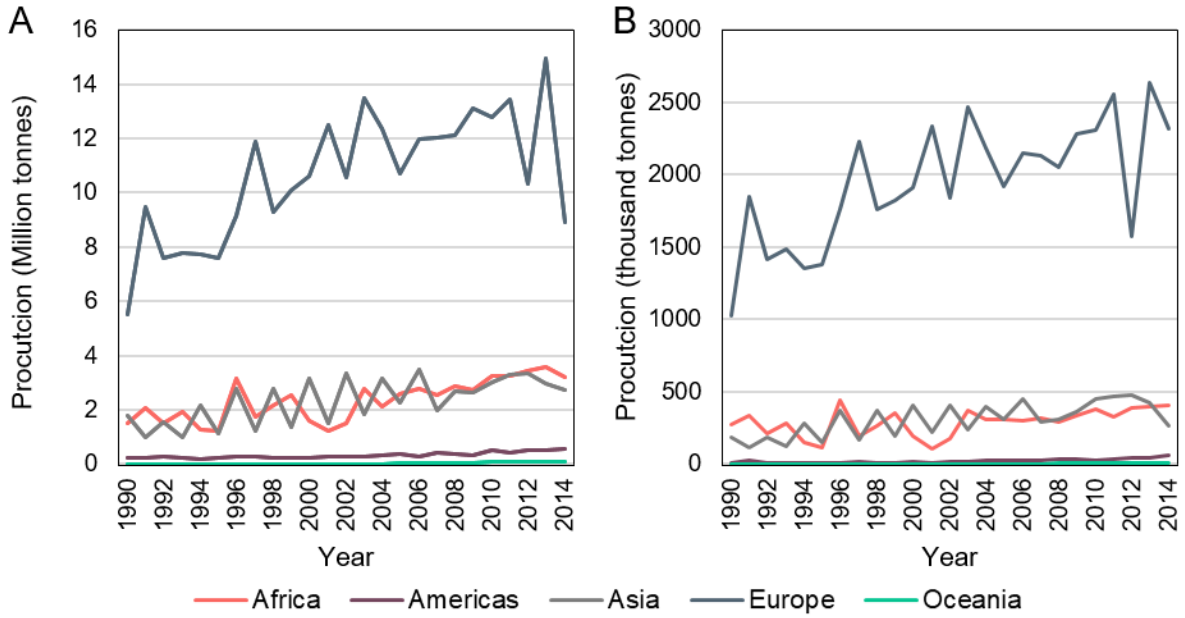

Figure 1.4. Worldwide oliviculture production by region between 1990 and 2014. **A** – Olive production (million tonnes). **B** – Virgin olive oil production (thousand tonnes). **Source data: FAOSTAT** (http://www.fao.org/faostat/en/#data/QC).

All top ten producers of olive are located in the Mediterranean Region (**Figure 1.5**) and in the top ten olive oil producers worldwide, only Argentina does not belong to the Mediterranean Region (**Figure 1.5B**). Spain, Italy and Greece are the largest producers, together being responsible for 74% of olive production and 45% of olive oil production globally (**Figure 1.5**). Portugal is part of the world top ten producers of both olive and olive oil and if one considers that Portugal is the smallest country in the group in terms of area, one can assume that oliviculture plays an important role nationally.

The olive culture is relevant in the country, not only because it is a significant source of economic income, but also because olive trees are a national icon, along with the cork oak, *Quercus suber* L., that have been part of the Mediterranean landscape for over 8000 years (Pereira 2015). According to the data resulting from the most recent farm structure survey, olive groves are the most relevant permanent culture in Portugal, occupying in total 340284 ha that, excluding stone pine, correspond to near half (48.0%) of the total area held by permanent cultures (INE 2014). Olive culture is dispersed through all mainland agricultural regions, but the most important is for sure Alentejo which has 48.5% of the total area of olive groves in Portugal, followed by Trás-os-Montes (22.6%) and Beira Interior (13.5%) (INE 2014). According to data resulting from the national annual survey of olive oil production, in 2016,

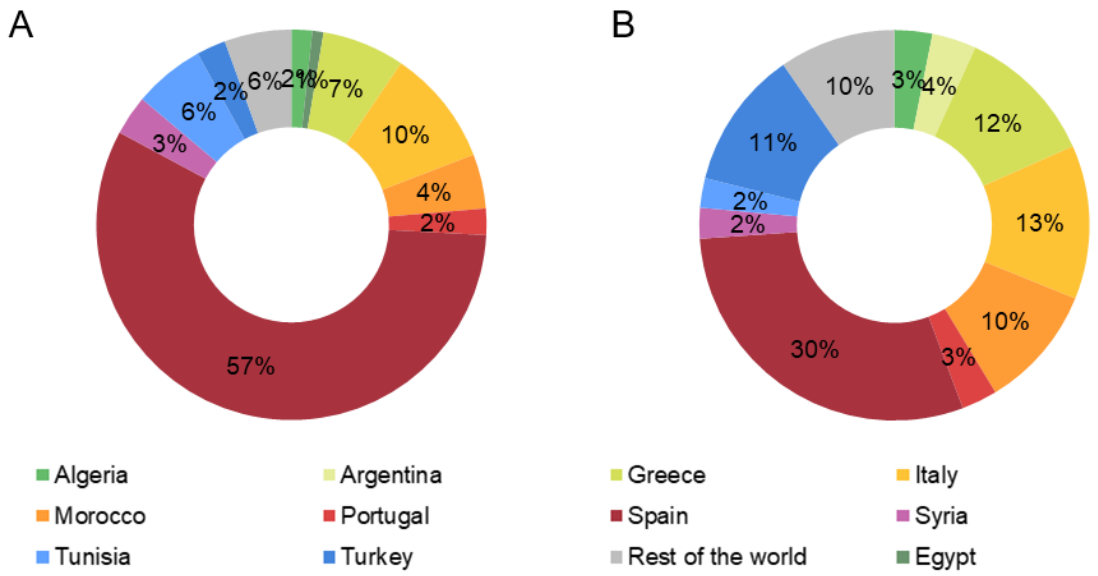

Figure 1.5. Word share of top olive and olive oil producers in 2014. **A** – Olive production; **B** – Virgin olive oil production. **Source data: FAOSTAT** (http://www.fao.org/faostat/en/#data/QC).

Portugal produced in total 476003 t of olives and 757373 hL of olive oil. More than three fourths of both goods were produced in Alentejo and Trás-os-Montes, that contributed with 71.4% and 16.1% to the total production of olives and 70.8% and 16.4% to the total production of olive oil, respectively (INE 2017).

1.11. Objectives

With the consciousness that *X. fastidiosa* is an emergent problem in Europe and that Portugal holds favourable conditions to introduction and spread of the pathogen; knowing that, to this date, olive groves have been the mainly impacted crop in Europe, that olive production is an important part of Portuguese culture and economy and that Alentejo is the main region of Portuguese olive production; and remembering that the presence of vectors is an essential part of *X. fastidiosa* establishment and spread, and that, so far, there are no previously published studies aimed directly at identification of potential vectors in Portugal, the focus of this study is 1) the identification of the potential vectors of *X. fastidiosa* in Alentejo olive orchards, 2) the assessment of auxiliary groups as predators and parasitoids that may be used to control potential vector populations, and 3) the evaluation of possible management and control measures.

2. Materials and methods

2.1. Study area

To determine the presence of xylem-feeding hemipteran insects that can potentially act as *Xylella fastidiosa* Wells et al. 1987 vectors in Portuguese olive groves, field surveys were conducted in Alentejo Region (south-central Portugal). This region is characterized by a semi-arid Mediterranean climate reflected in relatively low and concentrated rainfall during winter, high average temperatures and thermal amplitude, low humidity and cloudiness and high insolation during summer.

2.2. Field surveys

To have a significant cover of the study area, Alentejo was divided into a grid composed of 18 squares (30 × 30km). In each square, seven non-treated olive groves were selected as sampling sites through field prospection. The lack of insecticide treatments was the only criterion in which the local selection was based. Location of the 126 sampling points was registered with a GPS device in WGS-84 coordinate system and is shown in **Figure 2.1**.

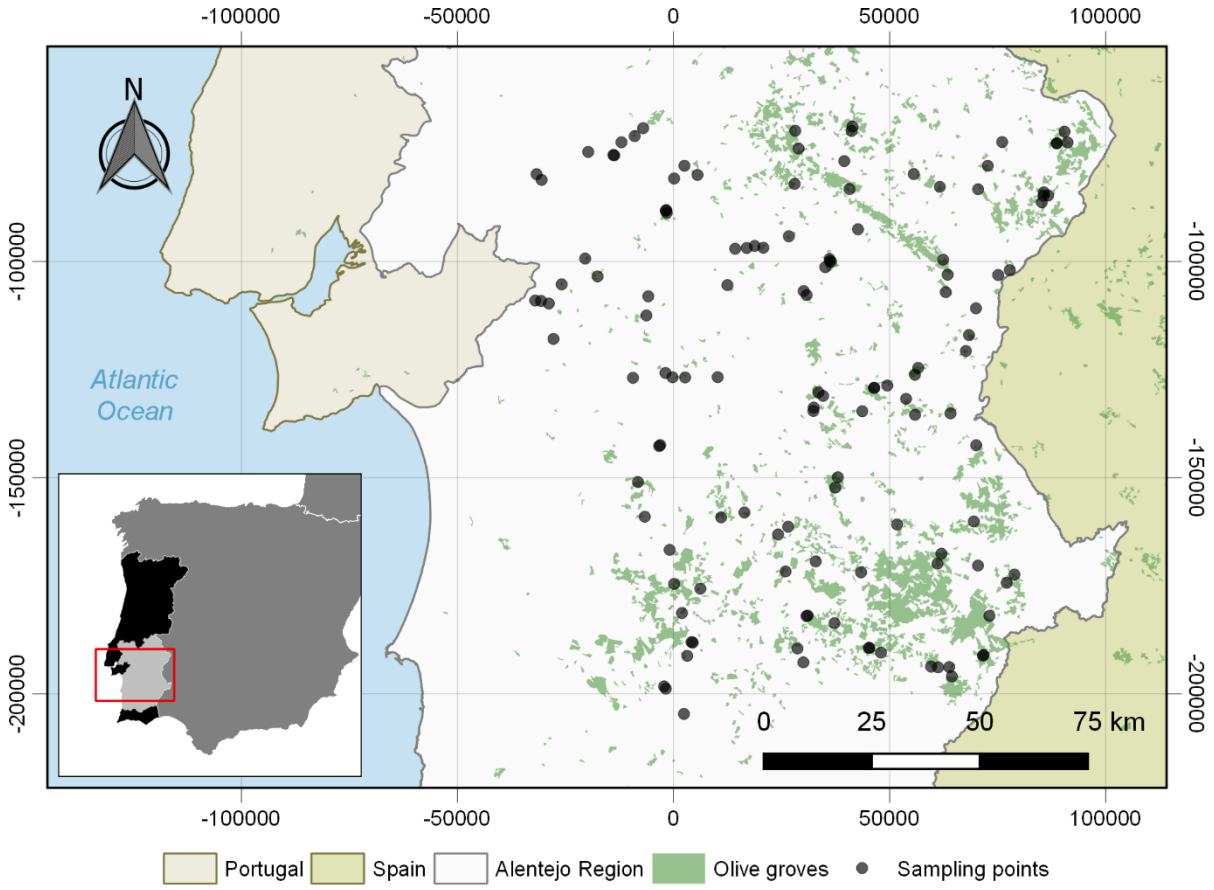

Figure 2.1. Spatial distribution of sampling points. This map is projected in ETRS89/PT-TM06. **Author's original.**

At each site, five randomly selected olive trees were vacuum-sampled at canopy level around the tree for ten seconds each, together composing a unique sample.

Key *X. fastidiosa* vectors to disease spread in several crop pathosystems are often associated with surrounding vegetation that serves as their breeding and overwintering habitat and has a particularly important role when epidemics emerge mainly through primary spread. Vectors with low importance in crop epidemics may have a crucial role in the maintenance of *X. fastidiosa* reservoirs outside the affected crops (Almeida et al. 2005a). Also, some potential vectors may feed in different plant hosts at different

stages of their life cycle (Tonkyn & Whitcomb 1987; Cornara et al. 2016b). Knowing the relevance of adjacent alternative plant hosts in *X. fastidiosa* related diseases, when present, nearby weeds were also vacuum sampled for fifty seconds. This way, each sampling point has one (olive canopy) or two samples (olive canopy and weeds), depending if weeds are locally absent or present.

Field surveys were performed during autumn of 2016 (from 25th October to 15th November) with the author participating in a sampling day. Vacuum sampling collection was performed using a gasoline-powered Agricultural Backpack 2-Cycle Aspirator Model 1612 with a 12.7cm diameter collection nozzle (126.68cm^2) where a collection cup or a sock was attached. The vacuum produced a 64km/h air intake. After collection, samples were preserved in a freezer until later sorting and identification.

2.3. Meteorological conditions during sampling period

Meteorological data relative to daily mean temperature (ºC), daily precipitation (mm) and daily relative humidity (%) from climatological stations of Meteorological Monitoring Network provided online by *Sistema Nacional de Informação de Recursos Hídricos* (http://snirh.apambiente.pt) were used to describe the meteorological conditions throughout the sampling period. Only active climatological stations inside the study area with complete series of data for the sampling period were considered (**Figure 2.2**).

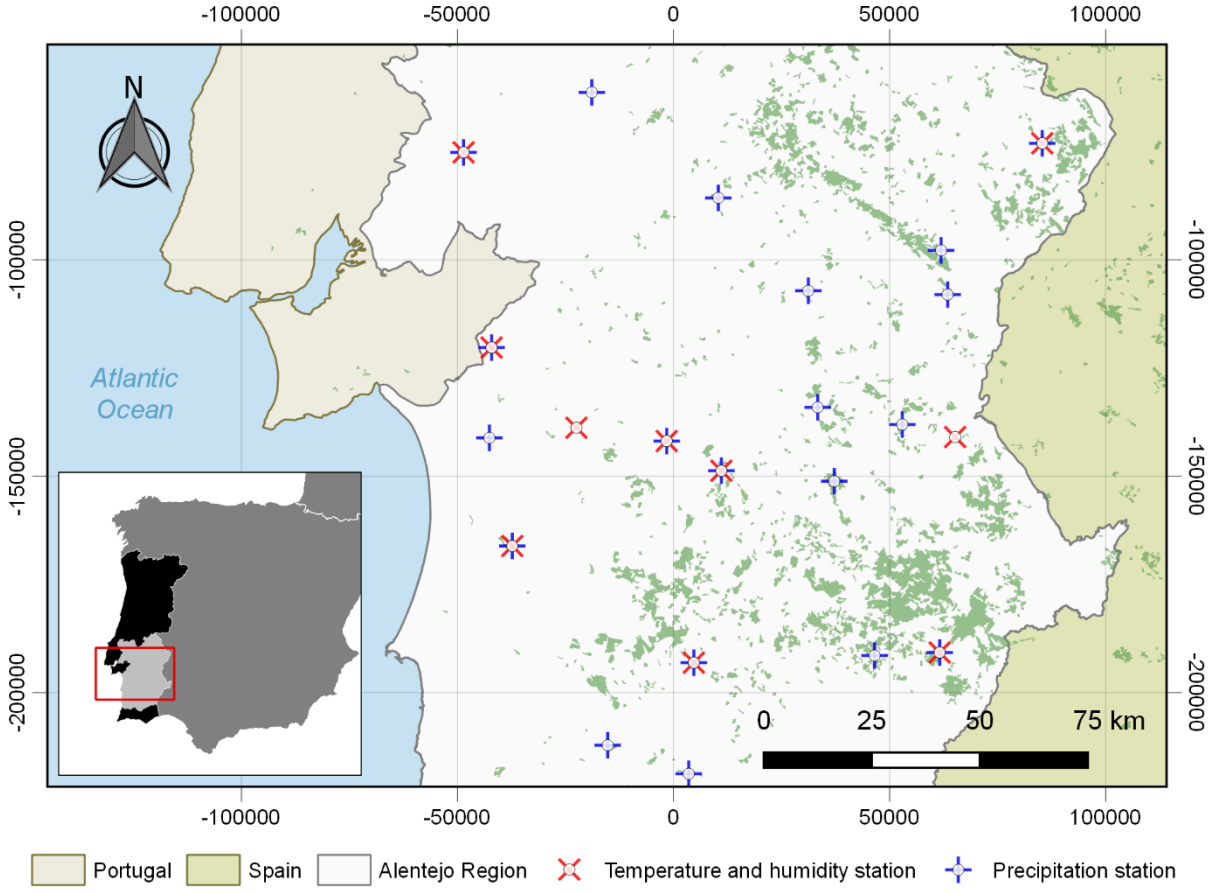

Figure 2.2. Spatial distribution of the meteorological stations used to characterize the meteorological conditions during the sampling period. Since the stations fulfilling the imposed conditions for the base data were not the same for precipitation, temperature and humidity, they are represented with different symbols to allow distinction. This map is projected in ETRS89/PT-TM06. **Author's original.**

In total, 24 meteorological stations fulfilled these requirements for precipitation data and 10 for mean temperature and relative humidity data. The base data used in this characterization and additional

information about the station characteristics are provided in **Appendix 1** – **Table A.1**, **Table A.2**, **Table A.3** and **Table A.4**.

2.4. Sorting and identification

Samples from 113 out of 126 sampling points from either olive trees and weeds were analysed in FCUL Entomology Laboratory. A total of 156 samples were sorted, 113 from olive trees and 43 from weeds. The location of the sorted samples in relation to all sampling points according to the host type and week of collection is represented in **Figure 2.3**.

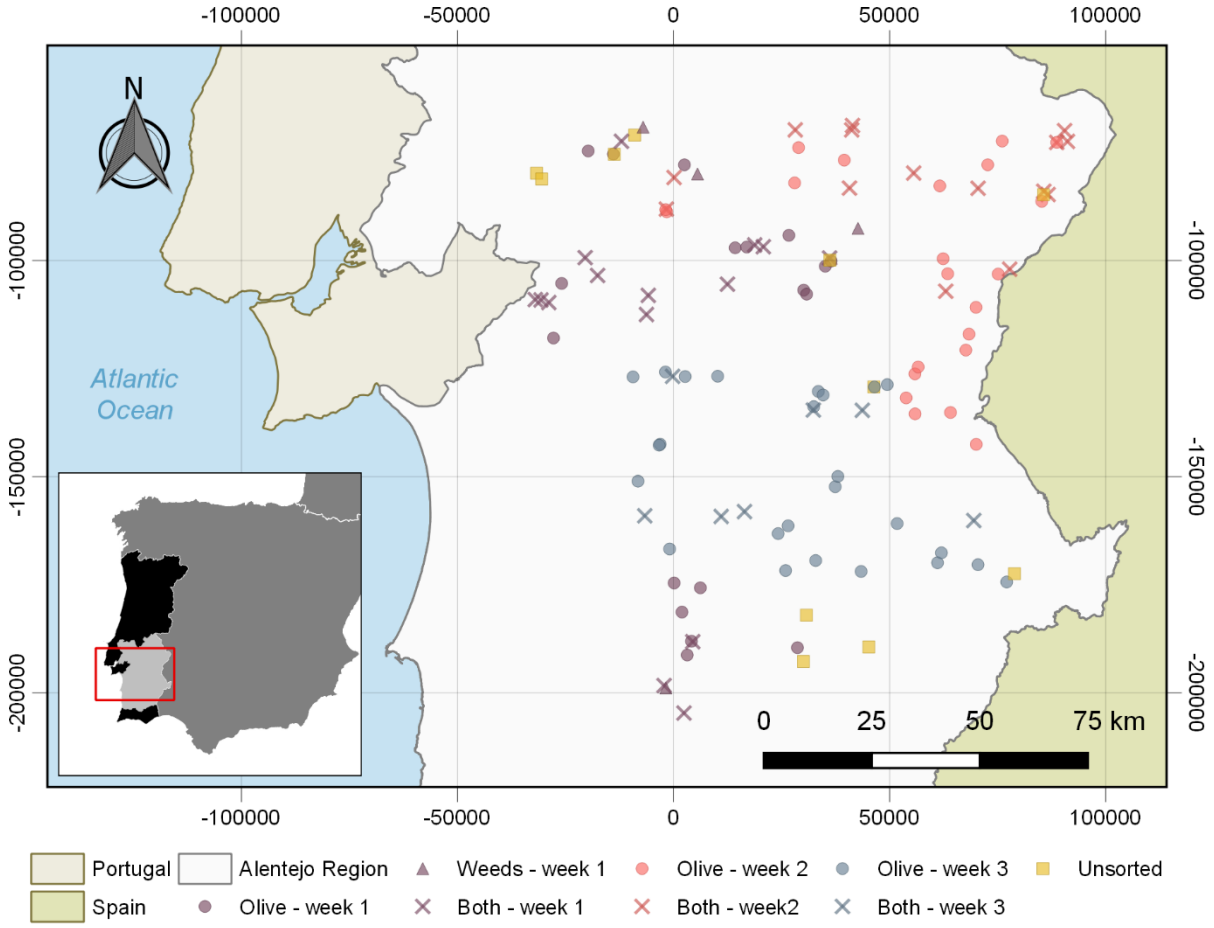

Figure 2.3. Distribution of sorted and unsorted samples in relation to the respective sampling sites. Sorted samples are represented according to host type and week of collection. Points referring only to weeds also have olive samples, but these were not sorted. Week 1 goes from 25/10/2016 to 01/11/2016, week 2 goes from 02/11/2016 to 08/11/2016 and week 3 goes from 09/11/2016 to 15/11/2016. This map is projected in ETRS89/PT-TM06. **Author's original.**

Collected insects were sorted to orders according to Chinery (1988) through observation with a binocular stereomicroscope Olympus SZX7. After sorting, all specimens were preserved in identified tubes containing ethanol (70%).

Some auxiliary groups of predators and parasitoids, were further sorted within the orders due to their potential as a pest-suppression force. Ladybugs (Coccinellidae) were sorted from Coleoptera using Chinery (1988) and Raimundo & Alves (1986). Parasitoid wasps (Chalcidoidea, Chrysidoidea, Cynipoidea, Ichneumonoidea and Platygastroidea) and ants (Formicidae) were sorted within Hymenoptera order according to Goulet & Huber (1993).

Aphrophoridae, Cercopidae, Cicadellidae (subfamily Cicadellinae), Cicadidae and Tibicinidae are pointed as the potential vectors for *X. fastidiosa* dissemination for Europe, since these groups correspond to the xylem sap-feeding hemipterans (EFSA 2015). Transmission by phloem sap- and mesophyll-

feeders has not been reported as successful but it has been shown that this type of feeders can acquire the bacterium (Elbeaino et al. 2014) which can be useful to *X. fastidiosa* detection. For this reason, all Auchenorrhyncha were analysed in this study. Auchenorrhyncha were sorted and identified to the lowest possible taxonomic level using several books, taxonomic keys and other references from the literature (Ribaut 1936; Le Quesne 1960, 1965, 1969; Ossiannilsson 1978; Le Quesne & Payne 1981; Quartau 1984; della Giustina 1989; Dmitriev 2003-present; Gnezlidov 2003; Holzinger et al. 2003; Reis & Aguin-Pombo 2003; Dietrich 2005; Zenner et al. 2005; Gonzon & Bartlett 2007; Zahniser 2007-present; Biedermann & Niedringhaus 2009; Gnezlidov et al. 2014; Wilson et al. 2015; Fletcher et al. 2017). Specimens that could not be identified to species were separated into morphospecies (i.e., taxa based on morphological similarity).

The counting data from the sorted orders, parasitoids and other auxiliary groups and Auchenorrhyncha were tabulated in Microsoft Excel for each sample. Some of these tables were exported into text files and represented graphically in several forms with R version 3.4.1 statistical computing platform (http://www.R-project.org) in R Studio version 1.0.153 (https://www.rstudio.com), an integrated development environment for R, recurring to "ggplot2" package for R software (https://cran.r-project.org/web/packages/ggplot2/index.html) . Some maps were also used to represent the information about species richness and relative abundance. All maps were rendered in QGIS version 2.18.12 (https://www.qgis.org). Metadata associated to the base layers used in all maps from this document is provided in **Appendix 2 – Table A.5**. All layers were projected in European Terrestrial Reference System 1989/ Portugal - Transverse Mercator 06 (ETRS89/PT-TM06), the recommended national projection of datum ETRS89.

2.5. Image acquisition from specimens

Whole body images from several perspectives and details of taxonomic importance such as legs, forewings, antennae, head and pronotum from planthoppers, leafhoppers and spittlebugs were obtained using a Zeiss SteREO Lumar.V12 stereomicroscope equipped with a The Imaging Source DFK 23U274 colour industrial camera before male genitalia dissection and preparation, needed to species identification. After mounted, genitalia were also photographed. ImageJ 1.51j8 was used to scale the obtained images after processing.

2.6. Preparation of genitalia

Traditionally, the female genitalia morphology of the leafhoppers (Cicadellidae) is considered as more conservative, having little use for species recognition. Some preliminary studies dedicated to compare female genitalia morphology in Cicadellidae, showed potential taxonomic interest in various features of the female genitalia (Carvalho & Mejdalani 2014) but most identification keys are based on male genitalia characters that tend to be more variable (Le Quesne 1983). Therefore, males are usually required to accomplish identification and male genitalia preparation is needed.

To prepare the genitalia, the pygofer was dissected from the insect by piercing its abdomen with a dissection needle, roughly between segments VII and VIII, and by softly pulling it from the rest of the specimen. Piercing a more anterior part of the abdomen is advisable when gaining practice to avoid potential damage of genital parts.

The separated part of the abdomen was placed in hot 10% potassium hydroxide (KOH) solution for about two minutes. Less time (30 to 60 seconds) was used for more fragile and light-coloured specimens, like most of the collected Typhlocybinae, to avoid "over clearing". This step was necessary since the caustic properties from the solution clear the sclerotized structures from the genitalia and facilitate the removal of soft parts.

After, the dissected part of the abdomen was placed in a drop of glycerine to gently remove the remaining abdomen segments and soft tissue, leaving only the genital parts. The genitalia was then mounted on glass slides in glycerine, sealed with nail polish and observed under a Nikon XSZ-107BN binocular optical microscope.

2.7. Principal component analysis

To describe the fauna associated to different samples and understand if the communities show any pattern regarding host type provenance, multivariate analysis was used, specifically a Principal Component Analysis (PCA).

The main aim of PCA is to project points from a high-dimensional space into a low-dimensional space (usually two- or three-dimensional), allowing the comprehension of the data and a more effective communication of results. In the new space, similar entities are projected near each other and dissimilar entities are projected far apart, revealing patterns of the data and relationships between variables and between variables and observations (Gauch 1982).

To do this, PCA transforms the original variables into uncorrelated linear combinations of them called principal components (PC). These principal components are the axes of the new coordinate system in which the observations are going to be projected. The first PC is in the direction that captures the largest portion of the total variance of the data, the second PC is the perpendicular axis to the first PC that explains the maximal remaining variance and so on (Gauch 1982). There are as many PCs as there are variables in the data. Dimension reduction is achieved by using the first PCs, but there is always some information loss.

PCA is a non-parametric method, but it assumes that the original dataset has a multinormal distribution (i.e. that all the variables are normally distributed) and that the variables have a linear relationship. If PCA results are going to be used *a posteriori* to statistical analysis, these assumptions should be closely met, but for descriptive purposes, as this, departures from the assumptions are tolerable (Gauch 1982). Knowing this, before computing PCA, an exploratory analysis of the dataset was performed by summarizing descriptive statistics (such as mean, standard deviation, minimum, maximum…) of orders abundance, by plotting frequency distribution histograms for the variables (**Appendix 3** – **Figure A.1** and **Figure A.2**) and by looking to dependence through Person correlation coefficient between variables and its significance (**Appendix 3 – Table A.6** and **Table A.7**). An exploratory PCA of centred orders abundance was performed but the results did not allow perception of any pattern since practically all samples were plotted close to one another.

The frequency distribution of orders abundances was mostly skewed to the right, which means that there are only a few samples with large counts, some samples with intermediate values of abundance, lots of samples with low counts, and a few or lots of samples with zero value (depending if the order is common or rare, respectively), which is typical among species frequency distributions (Legendre & Legendre 1998). A common transformation which is applied to this type of data is a logarithmic one since "logarithmic transformation of variables greatly aids in meeting the assumptions of linear models – including homogeneity (reduction in variability), normality (reduction in skewness), and additivity (conversion to a linear scale)" (Kenkel 2006). Other benefit of log-transformation is the reduction of the effect of large values that could be perceived as outliers (Kenkel 2006).

Knowing this, it was applied a logarithmic transformation to abundance data (natural logarithm of abundance plus one). Adding one to abundance before applying the natural logarithm allows to keep absence values as zero.

A PCA of the centred log-transformed orders abundance was performed with R version 3.4.1 in R Studio 1.0.153, using the function *prcomp()* from "stat" package, a standard package in R. Centring is done by the function by specifying argument "*center = TRUE*". Data were not scaled since abundance data are counts and, as such, are all in the same unit.

Another inbuilt function for performing PCA in R is *princomp()*. The differences between *prcomp()* and *princomp()* functions reside in the algorithm used for computing the principal components. The function *princomp()* computes the principal components via eigenvector decomposition of the covariance (for centred data) or correlation matrix (for standardized data, i.e. centred and scaled by unit variance) calculated from the original data matrix, while *prcomp()* computes the principal components via singular value decomposition of the original data matrix. For this reason, PCA is sometimes referred as SVD (Singular Value Decomposition). In practice, the returned results are the same, but while *princomp()* function is faster, *prcomp()* function offers more accurate values (Borcard et al. 2011). Another difference between both functions is that *princomp()* is not able to compute matrixes with more columns (variables) than rows (observations), which is not this case, so either function could be applied to the dataset. The larger the original data matrix, the more noticeable is the speed difference between both functions, but since the original data matrix used as base for PCA computation is not that large (156 × 21), *prcomp()* was the selected function to perform PCA due to better numerical accuracy. The PCA result charts were computed using "ggplot2" package for R software.

3. Results

3.1. Meteorological conditions during sampling period

The daily mean temperature (10 meteorological stations) and daily mean humidity (10 meteorological stations) variation throughout the sampling period can be observed in **Figure 3.1**. Mean temperatures ranged between 9 and 22ºC. The first sampling week was the hottest, with a mean temperature always higher than 16ºC. During the second week, the mean temperature dropped until near 10ºC and in the third week the mean temperature increased again until about 15ºC, therefore not reaching the values of the first week mean temperature. The relative humidity, in mean, was always higher than 60%, oscillating until about 95% throughout all sampling period, and it does not seem to have a specific tendency or differences between the three weeks.

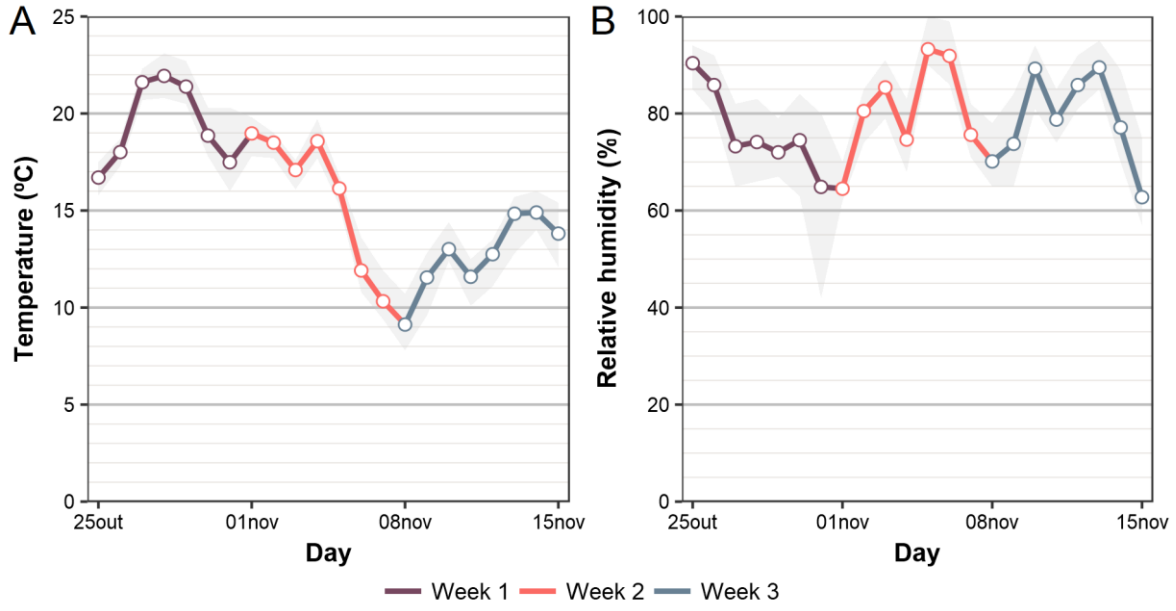

Figure 3.1. Meteorological characterization of the study area during sampling period (25/10/2016 – 15/11/2016). **A** – Mean temperature (ºC). Mean temperature refers to the mean of the daily mean temperatures registered in the meteorological stations. The upper/ lower grey envelope refers to the maximum/ minimum mean temperature among all meteorological stations observed in a specific day. **B** – Relative humidity (%). Relative humidity is the mean of the daily relative humidity observed in the meteorological stations. The upper/ lower grey envelope refers to the maximum/ minimum relative humidity among all stations observed in a specific day.

The mean daily precipitation (24 meteorological stations) was 1.4mm, and it was always lower than 2.0mm, (in most days, even lower than 1.0mm), except for the first sampling day (25th October), which was the day with highest mean precipitation (13.0mm), and 5th and 6th November (6.5mm and 4.0mm, respectively). Since the observed precipitation throughout the sampling period was residual, it is not represented graphically.

3.2. Samples composition

A total of 22149 individuals (both adults and immatures) from 156 samples of olive trees (113) and weeds (43) collected between 25th October and 15th November 2016 were sorted into 21 orders: Coleoptera, Collembola, Dermaptera, Diptera, Embioptera, Hemiptera, Hymenoptera, Lepidoptera, Mantodea, Neuroptera, Orthroptera, Psocoptera, Trichoptera, Thysanoptera, Thysanura, Acari, Aranea, Opiliones, Pseudoscorpiones, Polyxenida and Pulmonata. Twenty-eight specimens remained unsorted, either because they were in poor conditions or because they were larvae and proper distinction was not possible. In **Figure 3.2**, it is shown the relative proportion of all orders in all olive and weeds samples. The most abundant orders in olive samples were Hemiptera, Aranea, Diptera and Hymenoptera while

in weeds the most abundant orders were Acari, Hemiptera, Collembola, Diptera, Hymenoptera and Thysanoptera. Embioptera (one individual), Mantodea (one individual), Orthroptera (eight individuals) and Thysanura (seven individuals) were only found in weeds, while Opiliones (two individuals) were the only order absent from weeds.

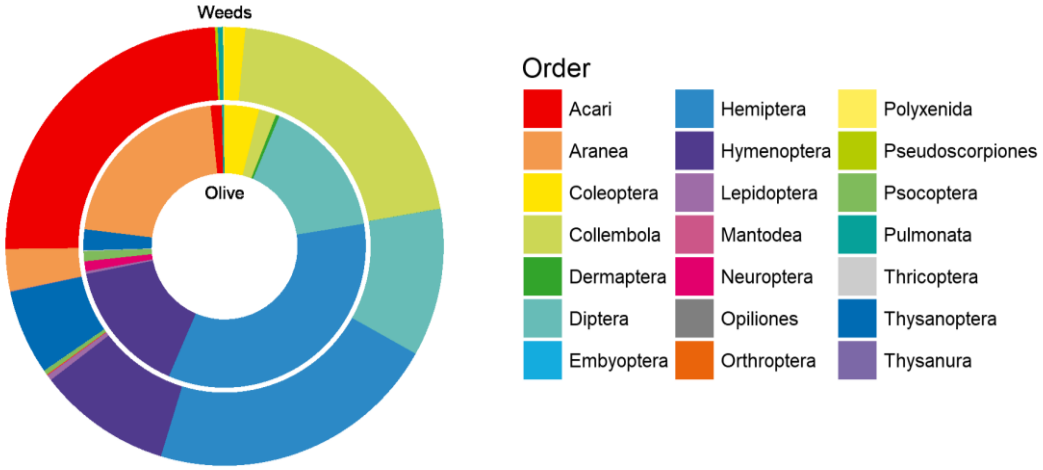

Figure 3.2. Relative abundance of the different orders found in all olive tree (inner ring) and weeds (outer ring) samples.

3.3. Principal component analysis

The orders abundances were log-transformed, centred and subjected to PCA. The first principal component accounts for 55.27% of the explained variance while the second principal component only explains 8.32% of the variance (**Figure 3.3**). Combined, the first two principal components only explain 63.59% of the data variability (**Figure 3.4**), but they give sufficient insight on some underlying ecological patterns. For instance, they allow enough distinction between olive tree and weeds samples, suggesting that there may be differences in the communities associated to both hosts (**Figure 3.3A**), and they show different dynamics between different orders (**Figure 3.3B**).

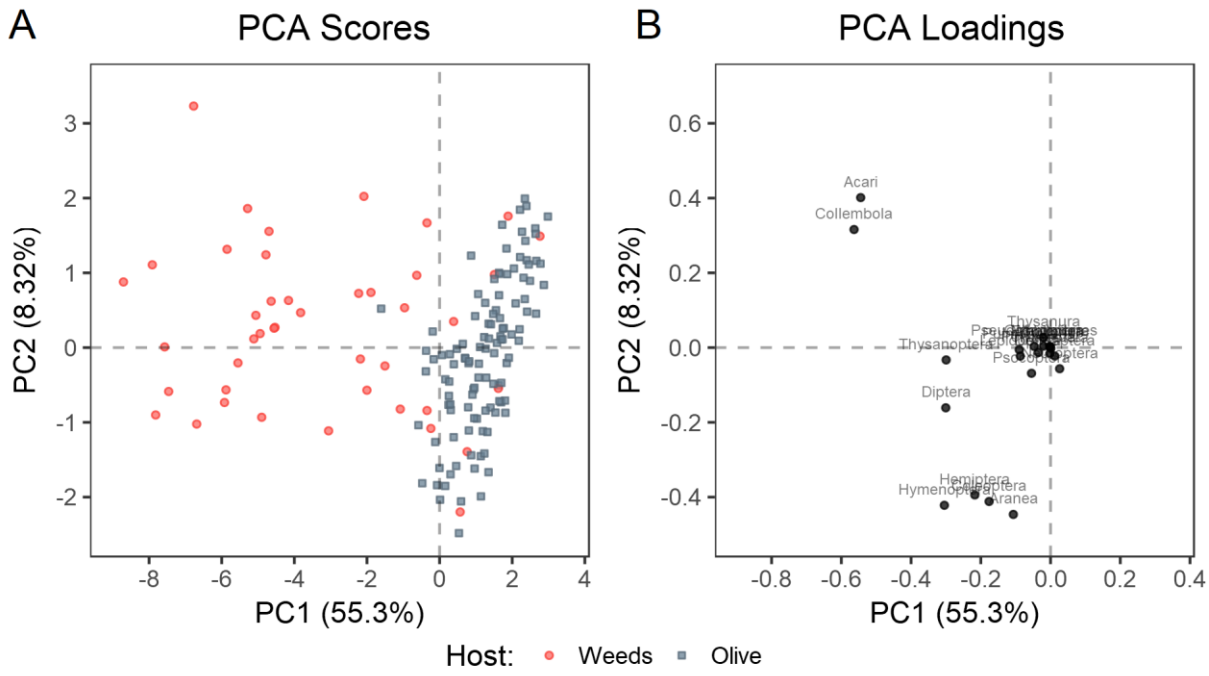

Figure 3.3. Principal component analysis of log-transformed orders abundances. **A** –PCA scores. **B** – PCA loadings.

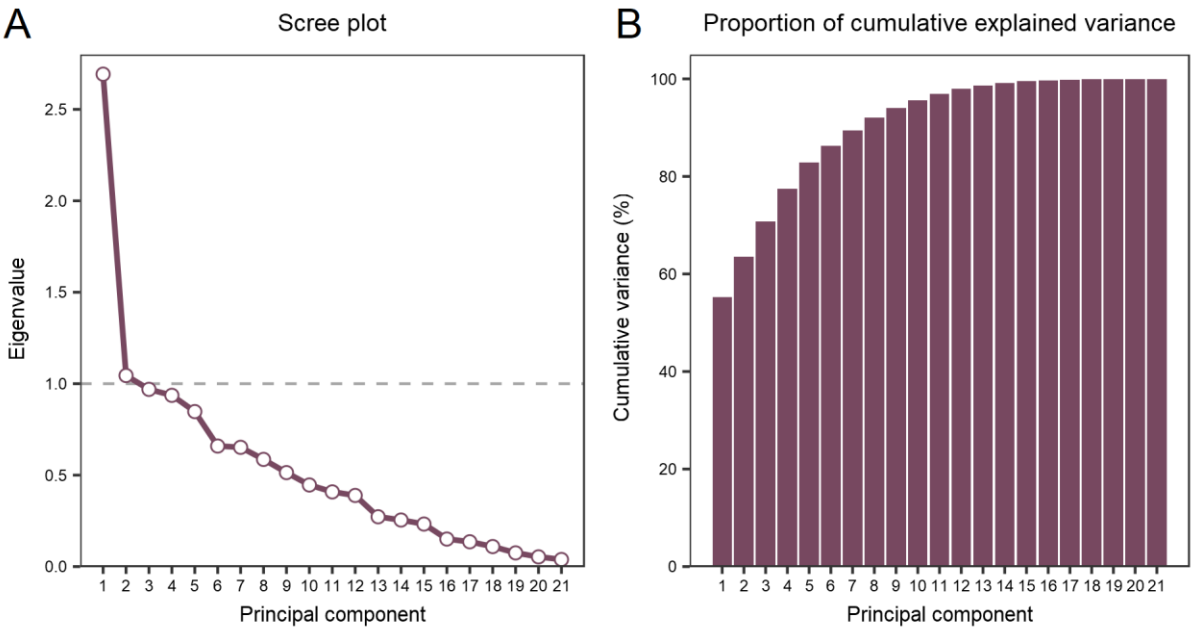

Figure 3.4. Principal component analysis of log-transformed orders abundances. **A** – Eigenvalues associated to the principal components. **B** – Cumulative explained variance (%) by the principal components.

Table 3.1 shows the Pearson correlation coefficients (r) between the untransformed orders abundances and the first three principal components scores. To test the significance of the correlation coefficients it was applied a t-test with the null hypothesis of the correlation coefficient equality to zero. These p-values refer to the probability of the correlation coefficient being equal to zero (no correlation), so the smaller the p-value, the more significant the relationship is. The chosen level of significance (α) was 0.05, so significant correlation coefficients have p-values lower than this value and are also marked in **Table 3.1**. Correlation coefficients and associated p-values for all variables against all principal components, are available in **Appendix 4** – **Table A.8** and **Table A.9**.

Table 3.1. Pearson correlation coefficients between order abundance and first three principal components scores. Strong correlations (absolute values above 0.5) are marked as red. Significant correlations are marked as bold with and asterisk.

Order	PC1	PC2	PC3	Order	PC1	PC2	PC3
Coleoptera	**-0.574***	**-0.395***	**-0.177***	Psocoptera	**-0.242***	-0.085	-0.135
Collembola	**-0.615***	**0.174***	-0.022	Thysanoptera	**-0.398***	-0.007	**0.197***
Dermaptera	0.087	-0.082	0.073	Thysanura	**-0.323***	**0.200***	**-0.237***
Diptera	**-0.630***	-0.108	-0.008	Trichoptera	-0.024	-0.125	-0.114
Embioptera	-0.125	0.049	0.079	Acari	**-0.504***	**0.286***	-0.109
Hemiptera	**-0.374***	**-0.184***	**0.405***	Aranea	**-0.215***	**-0.425***	**-0.507***
Hymenoptera	**-0.754***	**-0.200***	0.066	Opiliones	0.029	-0.024	0.104
Lepidoptera	**-0.394***	-0.058	**0.163***	Pseudoscorpiones	**-0.307***	-0.015	-0.024
Mantodea	-0.057	0.057	-0.105	Polyxenida	**-0.333***	-0.058	-0.056
Neuroptera	0.106	-0.150	0.024	Pulmonata	**-0.461***	-0.056	0.053
Orthroptera	**-0.314***	0.013	-0.038				

The first PC is strongly correlated (here considering r > |0.5| as strong correlations) with five of the variables: Hymenoptera (r = −0.754), Diptera (r = −0.630), Collembola (r = −0.615), Coleoptera (r = −0.574) and Acari (r = −0.504). All coefficient values for these variables were statistically significant

($p < 0.05$) as marked in **Table 3.1**. These five correlation coefficients are negative, which suggests two things: 1) that these orders abundances vary together, when one increases, the others increase, and 2) that the first PC increases with decreasing abundance of these orders. Low values of the first PC can be viewed as a measure of high abundance of Hymenoptera, Diptera, Collembola, Coleoptera and Acari. Since Hymenoptera has the highest correlation with the first PC, this component is primarily a measure of Hymenoptera abundance. The second PC has no strong correlation with any of the orders abundances. The highest correlation coefficient for the second PC is with Aranea abundance ($r = -0.425$), so the second PC reflects this variable the most, but it is not essentially a measure of it. Since the coefficient is negative, Aranea abundance increases when the second PC decreases.

The first PC *per se* allows the major distinction between communities from olive tree and weeds samples (**Figure 3.3A**) since blue squares (olive tree samples) are grouped to the right and pink dots (weeds samples) are grouped in the left part of the plot, even that they are more dispersed than the olive tree samples. This means that, typically, weeds are characterized by higher abundances of Hymenoptera, Diptera, Collembola, Coleoptera and Acari while olive trees tend to have lower abundances of these groups. Also, olive tree samples are more homogeneous, having similar communities between themselves, than weeds samples which have more variable associated communities, possibly due to different weeds species where sampling was performed.

3.4. Parasitoids

A total of 1388 parasitoid wasps from the superfamilies Chalcidoidea, Chrysidoidea, Cynipoidea, Ichneumonoidea and Platygastroidea were found in all the collected samples (506 specimens in olive trees 882 specimens in weeds). The distribution maps for these groups can be observed in **Appendix 5 – Figure A.3**, **Figure A.4** and **Figure A.5**. Other superfamilies of parasitoid wasps like Ceraphronoidea or Proctotrupoidea were found but since they were very rare and are not known to parasite Auchenorrhyncha, they were not accounted here.

Abundance of parasitoid wasps in all weeds and olive samples according to superfamily is showed in **Table 3.2**. Chalcidoidea and Ichneumonoidea were respectively the most and the second most abundant superfamilies in both olive and weeds samples, corresponding together to 90.85% of the total number of collected specimens from the considered parasitoid superfamilies. Chrysidoidea and Platygastroidea occurred occasionally, having a low representativeness in the overall number of captured parasitoids.

Table 3.2. Collected specimens by parasitoid wasp superfamily according to plant host. The total number of collected parasitoid wasps from the referred superfamilies in each plant host is between parenthesis. N – Absolute frequency. % - Percentage from all wasps collected in each host.

Superfamily	Olive canopy (506)		Weeds (882)		Both (1388)	
	N	%	N	%	N	%
Chalcidoidea	350	69.17	424	48.07	774	55.76
Chrysidoidea	3	0.59	8	0.91	11	0.79
Cynipoidea	14	2.77	74	8.39	88	6.34
Ichneumonoidea	127	25.10	360	40.82	487	35.09
Platygastroidea	12	2.37	16	1.81	28	2.02

In mean, all superfamilies were more abundant in weeds than in olive samples (**Figure 3.5**). The mean number of chalcids found in weeds samples was about three times higher than in the olive samples, while ichneumonoids were, in mean, about four times more abundant in weeds than olive trees. Despite

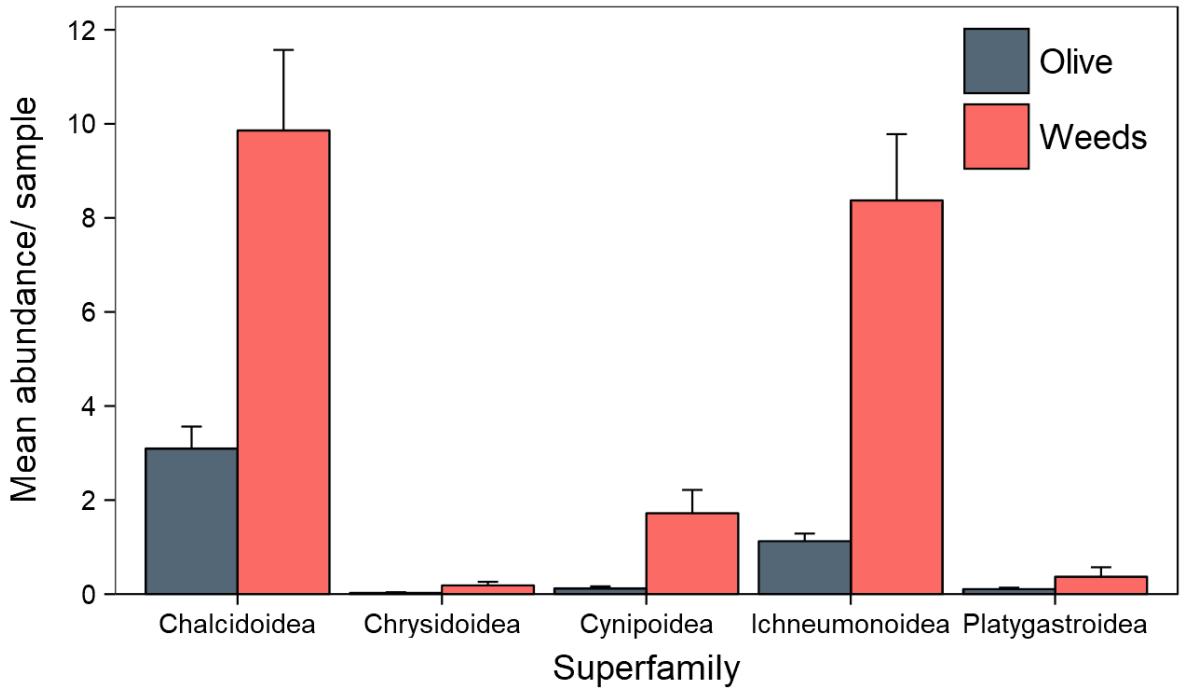

Figure 3.5. Mean abundance of parasitoid wasps per sample according to host type and superfamily. The error bars account for the standard error defined by the ratio between the standard deviation of the mean and the root square of the samples number (113 for olive trees, 43 for weeds).

the mean number of collected Cynipoidea being practically zero in olive tree samples, Cynipoidea had some representativeness in weeds occurring, in mean, two individuals per weeds sample.

Chalcidoidea was practically omnipresent in weeds samples and was the most widespread parasitoid superfamily being in 84.62% of the sampling sites with sorted samples (**Table 3.3**). In contrast Chrysidoidea was the least represented superfamily, being collected on only nine samples, all from different sampling sites.

Table 3.3. Number of samples and sampling sites with sorted samples in which each parasitoid wasp superfamily is present. The total number of collected samples on each plant and the total number of sampling sites with sorted samples are between parenthesis. N – Absolute frequency. % - Percentage.

Superfamily	Presence in sorted samples						Presence in sites with sorted samples	
	Olive canopy (113)		Weeds (43)		Both (156)		Total (117)	
	N	%	N	%	N	%	N	%
Chalcidoidea	87	76.99	42	97.67	129	82.69	99	84.62
Chrysidoidea	3	2.65	6	13.95	9	5.77	9	7.69
Cynipoidea	10	8.85	20	46.51	30	19.23	28	23.93
Ichneumonoidea	65	57.52	36	83.72	101	64.74	82	70.09
Platygastroidea	11	9.73	5	11.63	16	10.26	16	13.68

Four parasitoids were found directly over Auchenorrhyncha individuals. Three Auchenorrhyncha were parasitized by Dryinidae (Chrysidoidea) based on the characteristic dryinid “saculli”, usually black or dark brown, formed by the larvae that has a semi-external position in the host (Waloff & Jervis 1987; Biedermann & Niedringhaus 2009). The other hemipteran, a spittlebug, had a distended abdomen and

was certainly parasitized by a Hymenoptera, though the superfamily or family could not be determined. Several aphids were also found parasitized by parasitoid wasps.

3.5. Predators

Three thousand and twenty-three specimens belonging to essentially predatory groups Coccinellidae (Coleoptera), Formicidae (Hymenoptera), Neuroptera, Mantodea, Aranea, Opiliones and Pseudoscorpiones were found in all samples. The distribution maps of these groups are available in **Appendix 6 – Figure A.6**, **Figure A.7**, **Figure A.8** and **Figure A.9**. Aranea and Formicidae were the most common groups in both olive trees and weeds, corresponding together to 94.44% of all collected specimens from predatory groups (**Table 3.4**).

Table 3.4. Collected specimens by predatory group according to plant host. N – Absolute frequency. % - Percentage.

Group	Olive canopy (1878)		Weeds (1145)		Both (3023)	
	N	%	N	%	N	%
Coccinellidae	47	2.50	10	0.87	57	1.89
Formicidae	412	21.94	604	52.75	1016	33.61
Mantodea	0	0.00	1	0,09	1	0,03
Neuroptera	71	3.78	4	0.35	75	2.48
Aranea	1346	71.67	493	43.06	1839	60.83
Opiliones	2	0.11	0	0,00	2	0.07
Pseudoscorpiones	0	0.00	33	2.88	33	1.09

However, while the mean number of Aranea specimens found per sample was practically the same for both host plants, Formicidae specimens were, in mean, about three times more abundant on weeds than on olive trees (**Figure 3.6**). Spiders were practically omnipresent, missing only two of the sampling sites with sorted samples (**Table 3.5**). Formicidae, Neuroptera and Coccinellidae were all present in more than one-fourth of the sampling sites with sorted samples.

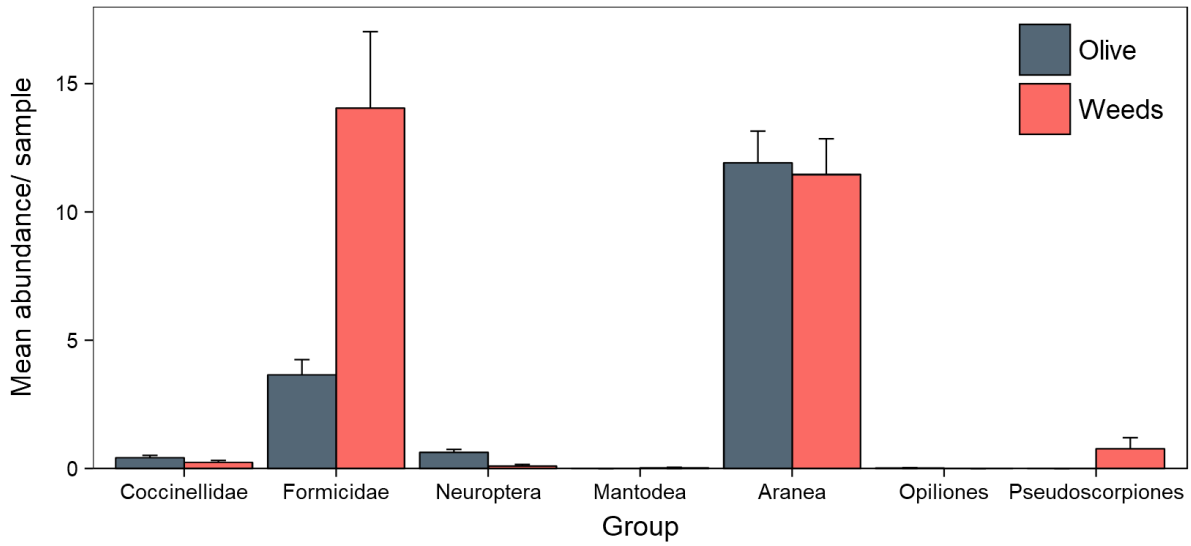

Figure 3.6. Mean abundance of predators per sample according to host type and predatory taxon. The error bars account for the standard error defined by the ratio between the standard deviation of the mean and the root square of the samples number (113 for olive trees, 43 for weeds).

The only Mantodea specimen was found on weeds and the only two Opiliones individuals were collected from olive trees. Twenty-three Pseudoscorpiones were found on weeds, but none on olive trees. All specimens from Neuroptera belong to the species *Chrysoperla carnea* (Stephens, 1836). In olive tree samples, most of them were adults but the only four individuals collected from weeds were all larvae.

Within Coccinellidae, seven species were identified: *Coccinella septempunctata* Linnaeus, 1758; *Hippodamia variegata* Goeze, 1777; *Rhyzobius litura* (Fabricius, 1787); *Scymnus interruptus* (Goeze, 1777); *Scymnus mediterraneus* Iablokoff-Khnzorian, 1972; *Stethorus punctillum* (Weise, 1891); and *Subcoccinella vigintiquatuorpunctata* Linnaeus, 1758. Among the identified coccinellid species, only *H. variegata* and *S. mediterraneus* were encountered on weeds.

Table 3.5. Number of samples and sampling sites with sorted samples in which each predatory group is present. The total number of collected samples on each plant and the total number of sampling sites with sorted samples are between parenthesis. N – Absolute frequency. % - Percentage.

Group	Presence in sorted samples						Presence in sampling sites with sorted samples	
	Olive canopy (113)		Weeds (43)		Both (156)		Total (117)	
	N	%	N	%	N	%	N	%
Coccinellidae	26	23.01	8	18.60	34	21.79	33	28.21
Formicidae	77	68.14	35	81.40	112	71.79	91	77.78
Mantodea	0	0.00	1	2.33	1	0.64	1	0.85
Neuroptera	42	37.17	2	4.65	44	28.21	43	36.75
Aranea	109	96.46	42	97.67	151	96.79	115	98.29
Opiliones	2	1.77	0	0.00	2	1.28	2	1.71
Pseudoscorpiones	0	0.00	8	18.60	8	5.13	8	6.84

3.6. Auchenorrhyncha

Six hundred and forty-nine leafhoppers, spittlebugs and planthoppers (254 adults and 395 nymphs) belonging to 68 samples (30 from olive trees, 38 from weeds) were sorted from other Hemiptera, corresponding to 11.7% of the collected hemipterans. The majority of other Hemiptera found in olive trees belonged to *Euphyllura olivina* Costa, 1839, a psyllid known as a secondary pest in olive groves (**Santos et al. 2007**). It may be relevant to report that a single olive tree sample contained a large amount (more than 400 individuals) of *Oxycarenus lavatera* (Fabricius, 1787), which is also considered a pest in some countries (**Nedvěd et al. 2014**). In weeds, the large abundance of Hemiptera was mainly due to aphids.

The abundance of Auchenorrhyncha nymphs and adults among found families in olive tree and weeds samples is shown in **Figure 3.7**. Aphrophoridae, Cixiidae and Tettigometridae nymphs were not found in any of the sorted samples. The only Cixiidae present in all samples was found in an olive tree. Cicadellidae was the most abundant group, followed by Delphacidae and Aphrophoridae. Leafhoppers and delphacids were both more frequently collected in weeds samples, however, if leafhopper adults and nymphs were collected in about the same amount, delphacid nymphs were about four times more abundant than adults.

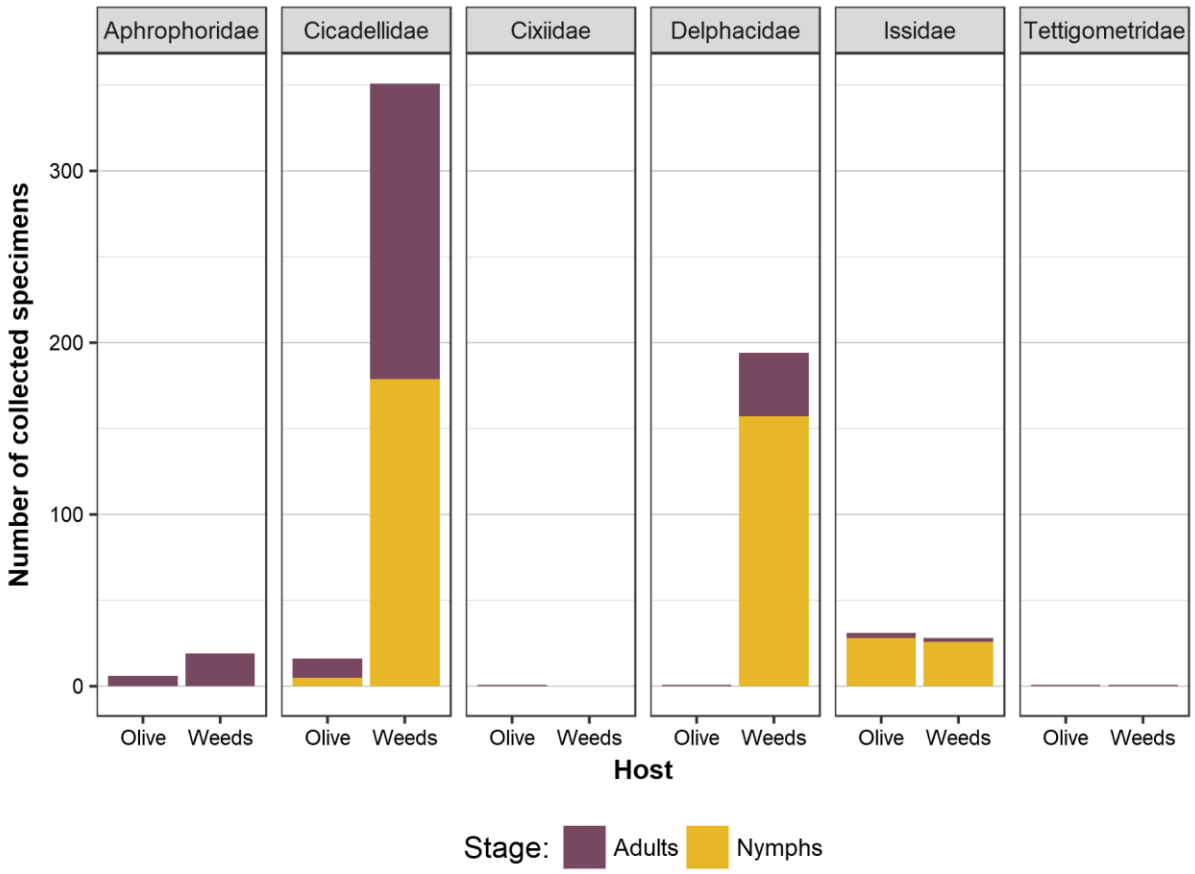

Figure 3.7. Number of collected Auchenorrhyncha adults and nymphs per family in all olive trees and weeds samples.

Auchenorrhyncha adults were identified into 24 species and 20 morphospecies belonging to six families: Aphrophoridae, Cicadellidae, Cixiidae, Delphacidae, Issidae and Tettigometridae. All the found species and morphospecies are listed in **Table 3.6**. A more complete table with reference to number and gender of adults according to the collection site, host and sampling date is provided in (**Appendix 7 – Table A.10**). Due to space limitations, the plates with photographs from somatic and genital characters of most of the identified Auchenorrhyncha species are presented in **Appendix 8**. References to the the specific plates for each species are included in **Table 3.6**. Some draws of somatic and genital characters are also available in **Appendix 9 – Figure A.39** and **Figure A.40**.

Since *Philaenus spumarius* Linnaeus, 1758 is a known vector in Italy (Cornara et al. 2016b; Cornara et al. 2017), it is important to note the presence of *Philaenus* sp. in the samples. Although identification to the species based on morphology was not possible since only females were found, it is likely that the specimens belong to *Philaenus tesselatus* Melichar, 1899 or *P. spumarius* (Drosopoulos & Quartau 2002). *Neophilaenus campestris* (Fallén, 1805) and *Euscelis lineolatus* Brullé, 1832 from which several individuals have been positive-tested for *Xylella fastidiosa* Wells et al. 1987 in Italy (Elbeaino et al. 2014; Saponari et al. 2014b; Cornara et al. 2016b) were also found in this study. *P. spumarius* and *N. campestris* are the only xylem-feeders among the identified species and the distribution of the collected specimens within the study area can be observed in **Appendix 10 – Figure A.41**.

Table 3.6. Number of Auchenorrhyncha adults by gender. Males – ♂♂; Females – ♀♀. *Adult specimens where sex could not be determined due to poor conditions of the specimen, usually the lack of the posterior part of the abdomen.

Auchenorrhyncha species	♂♂	♀♀	Adults*	Total	Plate
Aphrophoridae					
Philaenus sp.	0	5	0	5	Figure A.10
Neophilaenus campestris (Fallén, 1805)	9	11	0	20	Figure A.11 Figure A.12
Cicadellidae: Agallinae					
Agallia consobrina Curtis 1833	0	1	0	1	Figure A.15
Anaceratagallia laevis (Ribaut 1935)	3	6	0	9	Figure A.13 Figure A.14
Austroagallia sinuata (Mulsant & Rey, 1855)	0	3	0	3	Figure A.16
Agallinae 1	0	1	0	1	
Cicadellidae: Deltocephalinae					
Euscelidius variegatus (Kirschbaum, 1868)	2	0	0	2	Figure A.17
Euscelis lineolatus Brullé, 1832	0	2	0	2	Figure A.18
Exitianus capicola (Stål, 1855)	5	6	0	11	Figure A.19 Figure A.20
Goniagnathus brevis (Herrich-Schäffer, 1835)	0	1	0	1	Figure A.21
Orosius albicinctus Distant, 1918	1	0	0	1	Figure A.22 Figure A.23A-B
Psammotettix sp.	2	1	0	3	Figure A.24 Figure A.25
Deltocephalinae 1	0	1	0	1	
Deltocephalinae 2	0	1	0	1	
Deltocephalinae 3	0	1	0	1	
Deltocephalinae 4	1	0	0	1	
Deltocephalinae 5	0	1	0	1	
Deltocephalinae 6	0	2	0	2	
Deltocephalinae 7	2	0	0	2	
Deltocephalinae 8	0	1	0	1	
Deltocephalinae 9	0	1	0	1	
Cicadellidae: Idiocerinae					
Idiocerinae 1	0	1	0	1	
Cicadellidae: Typhlocybinae					
Arboridia parvula (Boheman, 1845)	1	1	0	2	Figure A.26
Empoasca sp.	1	0	0	1	
Frutioidia bisignata (Mulsant & Rey, 1855)	0	1	0	1	Figure A.27

Auchenorrhyncha species	♂♂	♀♀	Adults*	Total	Plate
Cicadellidae: Typhlocybinae (continuation)					
Zygina nivea (Mulsant & Rey, 1855)	2	1	0	3	Figure A.28A-B Figure A.23C-D
Zygina ordinaria (Ribaut, 1936)	1	4	1	6	Figure A.28C-D Figure A.23E-F
Zyginidia scutellaris (Herrich-Schäffer, 1838)	48	55	4	107	Figure A.29 Figure A.30
Typhlocybinae 1	0	3	0	3	
Typhlocybinae 2	0	6	0	6	
Typhlocybinae 3	0	1	0	1	
Typhlocybinae 4	0	3	0	3	
Typhlocybinae 5	0	1	0	1	
Typhlocybinae 6	0	1	0	1	
Typhlocybinae 7	0	1	0	1	
Typhlocybinae 8	0	1	0	1	
Cixiidae					
Cixius nervosus (Linnaeus, 1758)	1	0	0	1	Figure A.34E-F
Delphacidae					
Laodelphax striatella (Fallén, 1826)	2	0	0	2	
Metadelphax propinqua (Fieber, 1866)	20	15	1	36	Figure A.37 Figure A.38
Issidae					
Fieberium impressum (Fieber, 1877)	2	1	0	3	Figure A.31 Figure A.33
Tingissus guadarramense (Melichar, 1906)	0	1	0	1	Figure A.34A-D
Issidae 1	0	1	0	1	
Tettigometridae					
Tettigometra impressopunctata (Dufour, 1846)	1	0	0	1	Figure A.35
Tettigometra virescens (Panzer, 1799)	1	0	0	1	Figure A.36

Despite leafhoppers being the most represented group among adult Auchenorrhyncha, no sharpshooter (Cicadellinae) was found. Amid leafhoppers, Typhlocybinae and Deltocephalinae had the largest (137) and second largest number of individuals (31), but Deltocephalinae were more diverse (15 species and morphospecies) than Typhlocybinae (14 species and morphospecies). The large abundance of Typhlocybinae is mainly due to *Zyginidia scutellaris* (Herrich-Schäffer, 1838) which was found in several locations, but was present in an exceptionally large amount (81 individuals) in a single weeds sample.

Four Auchenorrhyncha individuals were parasitized: one *N. campestris* male (**Figure 3.8**), one delphacid nymph (**Figure 3.9**), one *Laodelphax striatella* (Fallén, 1826) male (**Figure 3.10**) and one *Metadelphax propinqua* (Fieber, 1877) male (**Figure 3.11**). The three delphacids were parasitized by

dryinid larvae whose identification was based on the typical "saculli" formed by larvae from this family in their hosts. The parasitoid attached to *N. campestris* was almost fully developed and certainly belongs to Hymenoptera, but a lower taxonomic level could not be assigned.

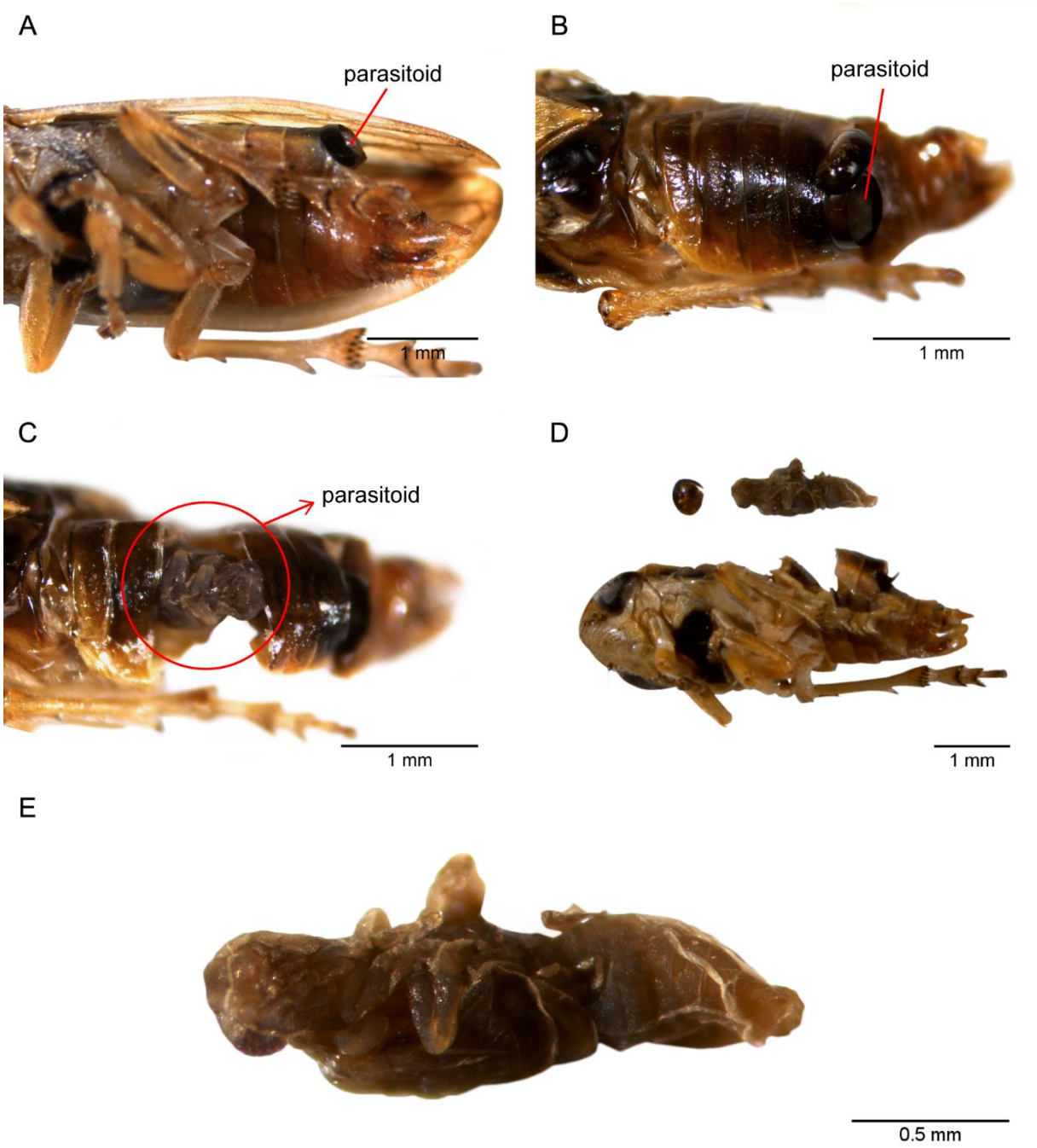

Figure 3.8. Parasitized *Neophilaenus campestris* (Fallén, 1805) male. **A** – Distended abdomen and parasitoid insertion (ventral view). **B** – Distended abdomen and parasitoid insertion (dorsolateral view). **C** – Detail of parasitoid inside the host. **D** – Side by side comparison between host and parasitoid larva. **E** – Parasitoid. **Author's original.**

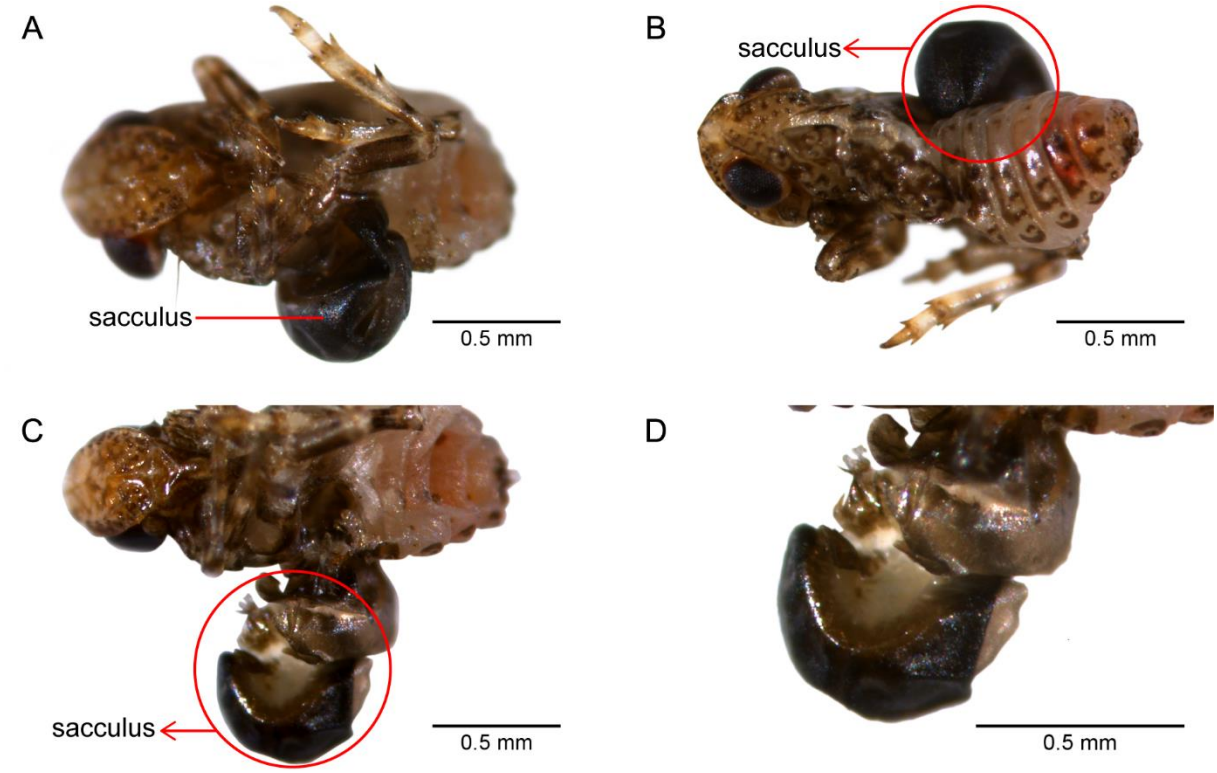

Figure 3.9. Parasitized delphacid nymph. **A** – General morphology (ventral view). **B** – General morphology (dorsolateral view). **C** – Side by side comparison between host and dryinid larva. **D** – Detail of "dryinid sacullus" with larva. **Author's original.**

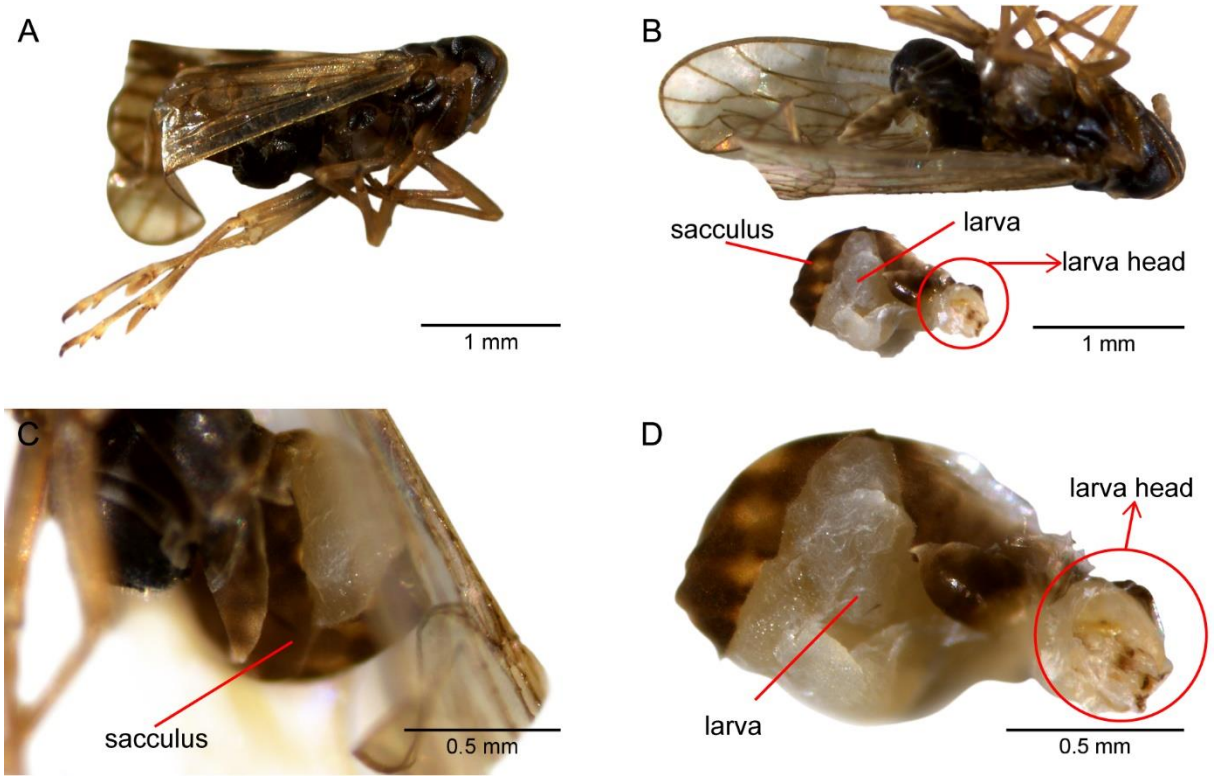

Figure 3.10. Parasitized *Laodelphax striatella* (Fallén, 1826) male. **A** – General morphology. **B** – Side by side comparison between host and dryinid larva. **C** – Details of "dryinid sacculus". **D** – Dryinid larva. **Author's original.**

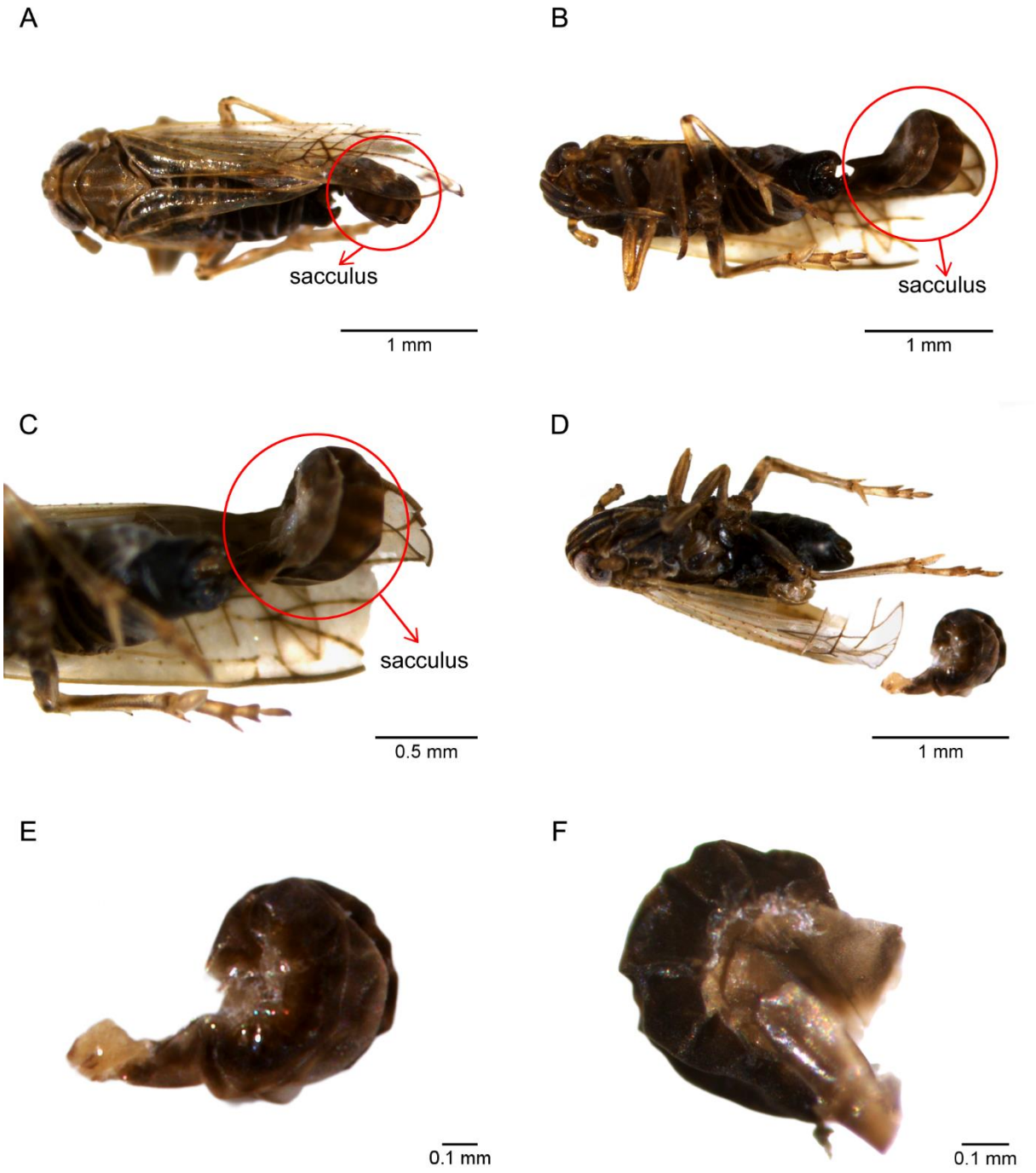

Figure 3.11. Parasitized *Metadelphax propinqua* (Fieber, 1877) male. **A** – General morphology (dorsal view). **B** – General morphology (ventral view). C –Detail of "dryinid sacculus". **D** – Side by side comparison between host and dryinid larva. **E-F** – Details of dryinid larva. **Author's original.**

Species richness of Auchenorrhyncha adults and Auchenorrhyncha abundance in olive tree and weeds samples regarding the sampling location can be observed in **Figure 3.12** and **Figure 3.13**, respectively. Although adult Auchenorrhyncha were found in only 53 out of 156 samples, the sampling sites from which they were collected are spread throughout all the sampling region and not concentrated in a particular zone. While olive trees had one or two species of leafhoppers, spittlebugs and froghoppers, species richness on weeds ranges from one to six different species. Besides being more diverse, weeds samples also showed more abundance of Auchenorrhyncha adults and nymphs

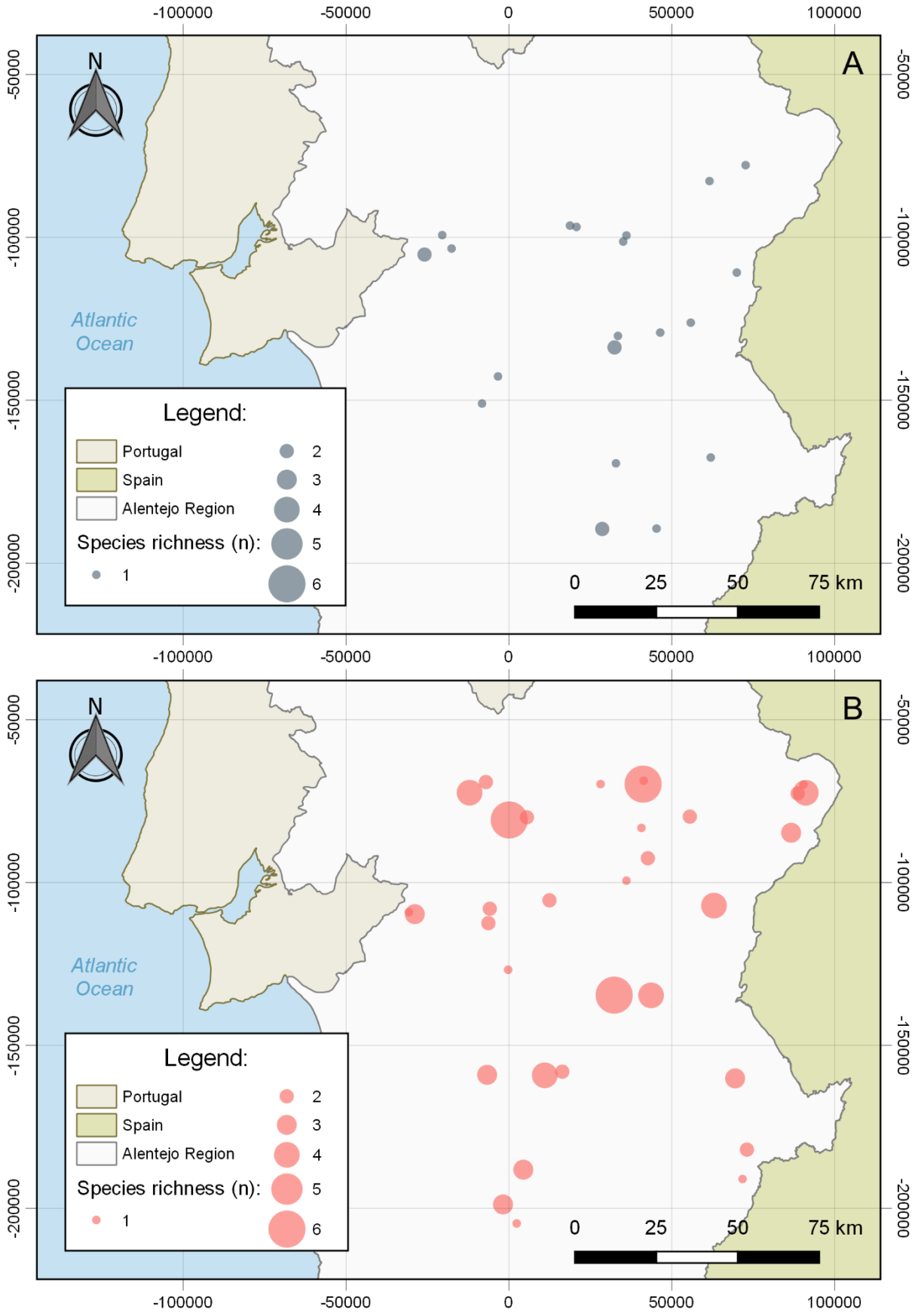

Figure 3.12. Distribution of Auchenorrhyncha adults species richness in the sampling sites with sorted samples. **A** – Olive tree samples. **B** – Weed samples. This map is projected in ETRS89/PT-TM06. **Author's original.**

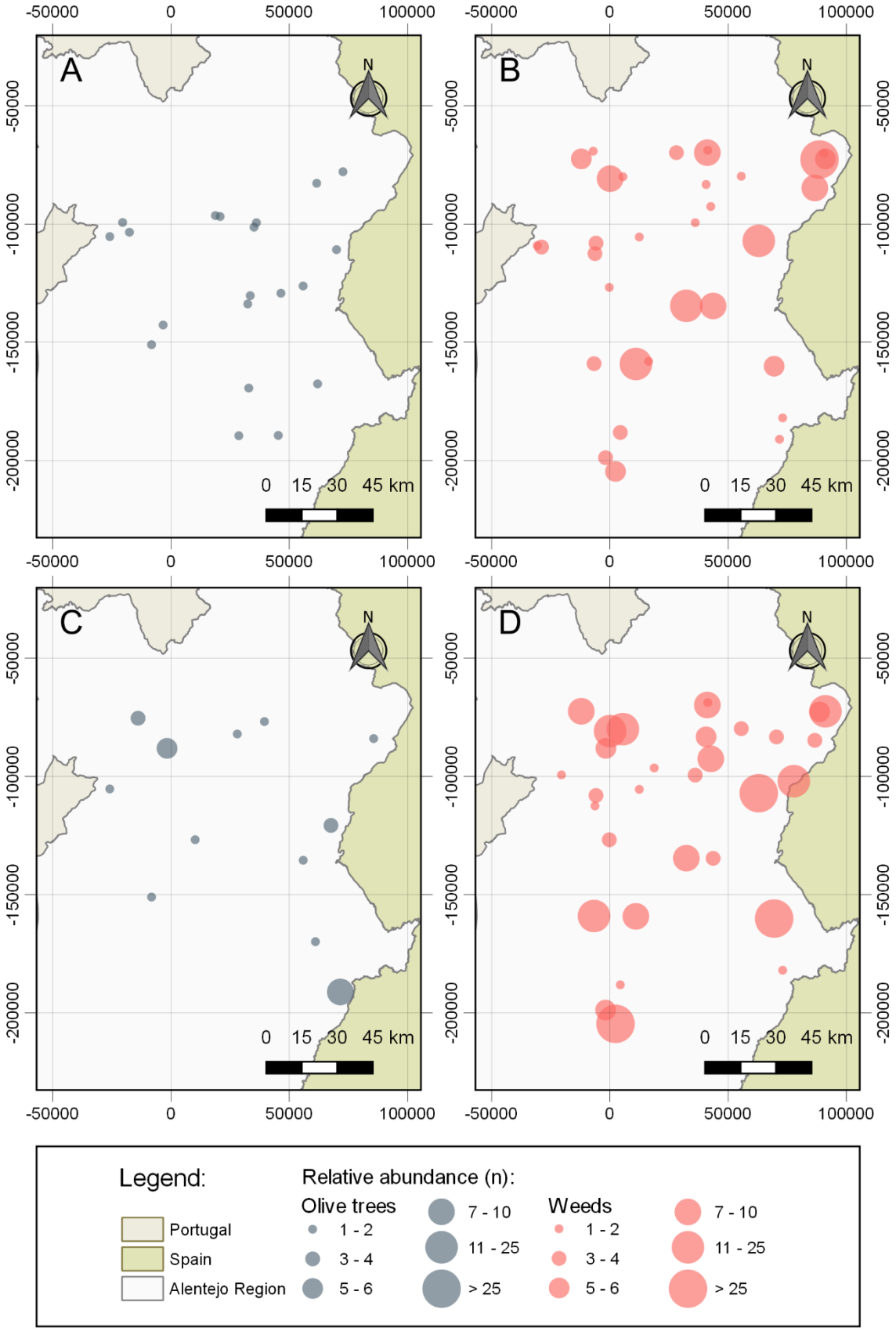

Figure 3.13. Distribution of Auchenorrhyncha abundance in the sampling sites with sorted samples. **A** – Adults in olive tree samples. **B** – Adults in weed samples. **C** – Nymphs in olive tree samples. **D** – Nymphs in weeds samples. This map is projected in ETRS89/PT-TM06. **Author's original.**

4. Discussion

The recent introduction of *Xylella fastidiosa* Wells et al. 1987 in Italy and its emergence in several other European countries has highlighted the importance of extend monitoring to other countries, especially on the susceptible host plants and potential vectors. The presence of capable vectors is essential to *X. fastidiosa* establishment and spread when an introduction occurs. In Europe, *X. fastidiosa* is responsible for Olive Quick Decline Syndrome (OQDS) and so far, the most negatively impacted plants in Europe have been olive trees. For this reason, and since no previous studies dedicated specifically to the identification of the potential vectors of *X. fastidiosa* have been made in Portugal, the aims of this study were to identify the potential vectors of *X. fastidiosa*, to evaluate the presence of eventual natural enemies in Alentejo olive groves, and to make some considerations about management measures.

4.1. Auchenorrhyncha and potential vectors

Among the identified species, only two xylem-feeders, *Philaenus* sp. and *Neophilaenus campestris* (Fállen, 1805) were collected from 16 out of 117 sampling sites with sorted samples. Although a limited sampling period during autumn was considered in this study and seasonal variation of species richness and abundance can occur throughout the year and between years (Morris et al. 1999), the occurrence of these potential vectors shows that there is a real risk of *X. fastidiosa* dissemination on Alentejo´s olive orchards, in case of pathogen introduction. According to Redak et al. (2004), xylem-feeding seems to be the only condition necessary for *X. fastidiosa* transmission so, if the bacterium is introduced in Portugal, these species will likely act as vectors in olive groves. Having little importance in *X. fastidiosa* epidemics in the Americas, *Philaenus spumarius* Linnaeus, 1758 has been shown to transmit the phytopathogen for a long time, and is the main vector of OQDS in Italy (Severin 1950; Cornara et al. 2016b; Cornara et al. 2017). Also, albeit successful *X. fastidiosa* transmission by *N. campestris* to olive trees has not yet been demonstrated (Cornara et al. 2016b), acquisition ability has already been verified (Elbeaino et al. 2014; Saponari et al. 2014b; Cornara et al. 2016b).

Species distinction within *Philaenus*, as in most Auchenorrhyncha, is based on morphological aspects of the male genitalia. In this genus, the appendages of the male aedeagus are particularly important for certainty in identification. Since in this study, only *Philaenus* females were collected, species distinction was not possible. It is likely that the collected specimens belong to either *P. spumarius* or *Philaenus tesselatus* Melichar, 1899. *P. spumarius* occurs in all Portuguese mainland, but it is more abundant north of Lisbon, becoming rare in the south, while *P. tesselatus* only occurs south of Lisbon and tends to be larger than *P. spumarius* (Drosopoulos & Quartau 2002). In Germany, only *P. spumarius* occurs and female size varies between 5.4 and 6.9 mm (Biedermann & Niedringhaus 2009). The five collected females size is about 7 mm, but size by itself is not sufficient to determine that they do not belong to *P. spumarius*. Since *P. spumarius* is probably the most studied and widespread species within its genus and is already a known *X. fastidiosa* vector, discussion will be focused on this particular species. Furthermore, for some authors, *P. tesselatus* is still considered as a subspecies of *P. spumarius* and molecular studies are not conclusive about *P. tesselatus* speciation.

P. spumarius is a highly polyphagous species, as *P. tesselatus* (Drosopoulos 2003), with hundreds of recorded host plants around the world (Yurtsever 2000). Nymphs and adults feed on numerous herbs, shrubs and trees, but tend to use dicotyledonous plants more often that monocotyledonous plants (Yurtsever 2000; Nickel 2003; Mazzoni 2005). This spittlebug is univoltine, meaning that it produces one generation per year, and overwinters in the egg form (Nickel 2003; Mazzoni 2005). The occurrence of different life phases varies regionally, depending mostly on climatic conditions (Yurtsever 2000). In temperate regions of Europe like Germany, nymphs emerge from April to May, the first adults appear about a month after egg hatching and reproduce over summer (Nickel 2003). In warmer regions, nymphs and adults tend to develop earlier: in southern Italy, spittlebug masses have been observed since March (Cornara et al. 2016b) and adults emerge in late April (Cornara et al. 2017); and in Portugal, there is a report of first instar nymphs in the beginning of February and of adults in the end of April (Rodrigues

2010). Adults can be found until November/December (Nickel 2003; Mazzoni 2005; Elbeaino et al. 2014) but the proportion of males in relation to the females declines over time (Yutsever 2000). Given the sampling period of this survey overlapped with the end of adult season, when males are rarer, it is not surprising that only *Philaenus* sp. females were collected.

N. campestris is an oligophagous grass-feeding species common in ruderal and grazed sites (Nickel 2003). Although this species is mostly found on Poaceae, during particularly hot days, *N. campestris* may migrate to woody plants to exploit them as shelter, having occasionally been collected on scots pine in Germany (Nickel 2003) and on cypress in Italy (Mazzoni 2005) during summer. *N. campestris* is also a univoltine species that overwinters in the egg form and has life cycle similar to *P. spumarius* (Elbeaino et al. 2014). Adults may be observed since the end of May to the beginning of October in Germany (Nickel 2003) and from May to November in Italy (Mazzoni 2005).

Despite the relative low abundance and diversity of potential vectors collected during this study, *N. campestris* and *Philaenus* sp. were both present on olive trees and, more frequently, on weeds (**Figure 3.7**). No nymphs were collected which is not surprising since spittlebugs overwinter in the egg form. Although collection on a certain plant does not necessarily mean that insects feed of it, the major vector presence on weeds suggests that they may act as alternative hosts for these spittlebugs, in olive orchards. Knowing that in Apulian olive orchards it has been observed a *P. spumarius* population shift from weeds to olive trees, in May, with a subsequent reverse migration in the end of July (Cornara et al. 2016b) and that identified potential vectors in this study occur in both weeds and olive trees, the population dynamics of the potential vectors should be investigated in the future for both plant hosts. The results also suggest that, even if *N. campestris* does not demonstrate transmission ability or efficient transmission to olive trees, it is likely that *N. campestris* will have an important role in *X. fastidiosa* inoculum maintenance on alternative hosts, like weeds in Alentejo olive orchards, as well as *Philaenus* sp., if bacterium introduction occurs.

P. spumarius is known for its "exuberant" balanced dorsal colour polymorphism. In fact, there are reports of more than sixteen dorsal colour patterns in *P. spumarius* (Yurtsever 2001), however their frequency varies locally. Dorsal colour polymorphism also occurs in other species belonging to *Philaenus* genus, but there is variation in the number of phenotypes displayed by other species (Drosopoulos et al. 2010). Morphs are usually classified as non-melanic, when having essentially a pale background with limited markings; or melanic, when they are mostly dark with several patterns of pale markings. Thirteen phenotypes are known to occur in Portugal mainland (Quartau & Borges 1997). In this study two non-melanic phenotypes were found in *Philaenus* sp. individuals, *populi* and *typicus* (**Appendix 8** – **Figure A.10**), which are usually the most frequent.

The relative proportion of different phenotypes in *P. spumarius* varies locally between populations and may be influenced by several factors like gender, habitat composition, climatic conditions or air pollution. In Finnish populations, melanic forms seem to be limited to females (Yurtsever 2000), as well as in Turkish populations (Yurtsever 2001), and in Portuguese populations melanic females tend to be more frequent than males (Quartau & Borges 1997), but in British populations no differences have been noticed in melanic morphs incidence between females and males. In North America, only eight phenotypes are reported (Yurtsever 2000) and in Azores only three phenotypes occur (Quartau et al. 1992), probably due to a founder effect in the latter case. Habitat composition and climatic conditions can affect the colour patterns displayed by some *P. spumarius* populations (Quartau & Borges 1997), but not others (Yurtsever 2001). British populations have shown that industrial melanism occurs in *P. spumarius*, since higher frequencies of melanic individuals were observed in populations near urban centres where atmospheric pollution is more intense than in populations from areas with lower air pollution (Yurtsever 2000).

To the author's knowledge, only Severin (1950) has studied *X. fastidiosa* transmission by *P. spumarius* considering its different dorsal colour phenotypes. In that study, Pierce's Disease (PD) transmission rate

to grapevines varied between different morphs. At the time, PD causal agent was thought to be a virus and there were no available methods to determine acquisition rates, so the transmission rate variation might not be due to different morph transmission efficiency, but related to lack of acquisition in the first place. Nonetheless, it might be relevant to understand if different colour phenotypes have different transmission efficiencies or feeding preferences within plants since it is known that *X. fastidiosa* is irregularly distributed within plants (Hopkins 1981; Daugherty et al. 2010b) and that its differential distribution may influence acquisition by vectors (Marucci et al. 2004; Daugherty et al. 2010b). If *Philaenus* sp. morphs display background matching behaviour, like it was observed in PD vectors *Draeculacephala minerva* (Ball, 1927) *Homalodisca vitripennis* (Germar, 1821) and *Graphocephala atropunctata* (Signoret, 1854) by Rashed et al. (2011), that may influence probing site selection and consequently transmission.

N. campestris was not yet shown to transmit *X. fastidiosa*, so its transmission efficiency cannot be compared to the transmission efficiency of *P. spumarius*. However, in this study, *N. campestris* was more abundant than *Philaenus* sp., in opposition to what was observed in Italian olive groves, where *P. spumarius* corresponds to more than 90% of the collected spittlebugs (Cornara et al. 2016b). Although only a few individuals were found on a short sampling period not coincident with the seasonal population peaks, if the tendency for more numerous *N. campestris* in Alentejo olive groves prevails throughout the year, this species will likely have a more significant role in *X. fastidiosa* spread than *Philaenus* sp., independently of its transmission efficiency, as the American experience often shows that less efficient vectors with denser populations are the key vectors instead of more efficient vectors with smaller populations (Turner & Pollard 1959; Redak et al. 2004; Janse & Obradovic 2010). Nonetheless, *P. spumarius* distribution is very limited by humidity and temperature. "Egg hatching and nymph development stages are temperature dependent, moreover, adults may die above and below certain temperature limits" (Yurtsever 2000). The fact that the summer of 2016 was characterized by extreme climatic conditions (IPMA 2016a, 2016b, 2016c) certainly has influenced *Philaenus* sp. populations, being a possible reason for its low representativeness in the collected samples.

Weeds occurred in only 43 out of the 126 sampled olive groves. Auchenorrhyncha species richness and abundance was globally higher in weeds (36 species/ morphospecies) than in olive trees (17 species/ morphospecies). The presence of weeds seems to be associated with higher abundance of spittlebug adults and leafhopper nymphs so olive groves where weeds occur should be considered more susceptible to *X. fastidiosa* establishment, if introduction occurs. The summer of 2016 was extremely hot and dry. July had the highest maximum temperature since 1931 and the second highest mean temperature since there are records. Two heat waves occurred during this month, one of them, between 23th and 30th of July, affected Alentejo Region (IPMA 2016a). A heat wave also occurred from 5th to 13th of August and the precipitation during August was about 30% of the normal precipitation for the period (IPMA 2016b). In the beginning of September, the records for the maximum registered temperature were broken in about three-fourths of the meteorological stations throughout all mainland territory and a heat wave impacted several regions, especially the interior of Alentejo (IPMA 2016c). These persistent exceptional climatic conditions had certainly affected the vegetation in the study region. Most weeds probably dried, making the arthropods concentrate in the limited weeds available or search for food and refugee on trees. So, despite no extreme climatic conditions occurred during the sampling period (**Figure 3.1**), the severe climate observed previously during summer has certainly influenced the distribution of the arthropodofauna and may be one of the reasons for the differences in the numbers of collected specimens on weeds and on olive trees.

Among other captured Auchenorrhyncha, some species of economic interest have been identified in the surveyed olive groves, as the subsequently described. *Euscelis lineolatus* Brullé, 1832 specimens were collected from weeds, as all Deltocephalinae adults. This species is a known vector of clover phyllody and of witches' broom virus to several plants in England (Nielson 1968) and has also been collected in Italy, where several specimens tested positive for *X. fastidiosa* (Elbeaino et al. 2014). Despite *X.*

fastidiosa acquisition revealing that this species may accidentally or occasionally probe on xylem sap, as a phloem-feeder, *E. lineolatus* role as a potential vector is highly unlikely. *Euscelidius variegatus* (Kirschbaum, 1858) is a vector of several phytopathogens, transmitting diseases like the aster yellows in North America, the clover phyllody disease in France or the *Chrysanthemum* yellows and it has shown ability to infect grapevine with *Flavescence Dorée* in laboratory tests (Nielson 1968; Reis & Aguin-Pombo 2003).

To the author's knowledge, *Orosius* spp. have never been previously reported in Portugal, and this study might be providing the first record of *Orosius albicinctus* Distant, 1918 in Portugal mainland. Fletcher et al. (2017) refers to *Orosius* as "one of the more economically significant leafhopper genera" as it contains important vectors of serious phytoplasma diseases in several Old World regions and Oceania. Due to confusion with the nomenclature of several species within the genus (Fletcher et al. 2017), it is difficult to determine which diseases are transmitted by which species, but, for instance, *O. albicinctus* is reported as a vector of plant diseases like sesame phyllody, lucerne witches' broom or garden beet witches' broom, among others, in Iran (Omidi et al. 2010).

Several species of Typhlocybinae are common during autumn in central Europe (Nickel 2003; Mazzoni 2005). Such species include *Frutioidia bisignata* (Mulsant & Rey, 1855), *Zyginidia scutelaris* (Herrich-Schäffer, 1838), *Zygina nivea* (Mulsant & Rey, 1855) and *Zygina ordinaria* (Ribaut, 1936) that were collected in this study samples. Although *F. bisignata*, *Z. nivea* and *Z. ordinaria* were not common, *Z. scutelaris* was the most regularly collected Auchenorrhyncha species, being present in 14 samples, the same number as *Metadelphax propinqua* (Fieber, 1866), and corresponding to more than 40% of the collected Auchenorrhyncha adults. *M. propinqua* was the second most numerous Auchenorrhyncha species and was mainly collected from weeds making about 15% of the collected Auchenorrhyncha on weeds. While *M. propinqua* is not considered a particular agricultural threat, it is a vector of maize rough dwarf disease in Israel and of *Cynodon* chlorotic streak virus (Gonzon & Bartlett 2007).

4.2. Natural enemies

In this study two distinct guilds of natural enemies were considered: parasitoid insects and arthropod predators. The regulation of host populations by parasitoids is related to direct mortality and interference with host reproduction since suppression or reduction of the internal reproductive organs known as "parasitic castration" is a noticeable common effect of parasitism in Auchenorrhyncha (Waloff & Jervis 1987). Among parasitoids, five wasp superfamilies were considered, although others occurred in a vestigial way. Chalcidoidea and Ichneumonoidea were the dominant superfamily of parasitoid wasps, composing about 55% and 35% of the total amount of collected parasitoids, respectively. All considered superfamilies were more important on weeds than on olive trees (**Figure 3.5**). Out of the sorted parasitoid wasps' superfamilies, Chalcidoidea and Chrysidoidea include families known to parasitize Auchenorrhyncha (Waloff & Jervis 1987). Chalcids are mostly egg parasitoids in Auchenorrhyncha, but Encyrtidae parasitize nymphs and adults, including spittlebugs. In the Americas, *Gonatocerus* spp. (Chalcidoidea: Mymaridae) is used as biological control of *H. vitripennis* populations in several crops (Overall & Rebek 2017). Since weeds are typical overwintering plant hosts of spittlebug eggs and nymphs and that chalcids were very common in weeds samples, they may be important in the regulation of immature stages of *P. spumarius* and *N. campestris* in olive groves.

Most of the collected Chrysidoidea belonged to Bethylidae, but Dryinidae also occurred. Dryinid larvae have a semi-external position within their hosts, laying in a prominent sac (Le Quesne 1983, Waloff & Jervis 1987; Biedermann & Niedringhaus 2009) and were found parasitizing two delphacid adults (*Laodelphax striatella* (Fallén, 1826) shown in **Figure 3.10** and *M. propinqua* shown in **Figure 3.11**) and one delphacid nymph (**Figure 3.9**), however, dryinids are not known to parasitize spittlebugs (Waloff & Jervis 1987). The finding of a parasitized *N. campestris* (**Figure 3.8**) suggests the existence of local spittlebug-parasitoid relationships that could regulate spittlebug populations, which may be exploited as biological control without the need of introductions. Identification of the parasitoids

associated with spittlebug populations in Alentejo olive groves and the study of their ecological requirements is relevant to establish management measures that conserve and enhance parasitoid populations.

Other Auchenorrhyncha parasitoids include Pipunculidae (Diptera) and Strepsiptera (Le Quesne 1983, Waloff & Jervis 1987). Pipunculidae are exclusively parasitic in Auchenorrhyncha, attacking both nymphs and/ or adults. In Europe, the genus *Verrallia* attacks exclusively froghopper adults, actively avoiding the spittle surrounding the nymphs. Whittaker (1969) analysed the attack by pipunculids on British spittlebug adults: *P. spumarius*, *N. campestris* and *Neophilaenus lineatus* (Linnaeus, 1758). He sampled pipunculids from the beginning of June to mid-September, but reduced numbers were captured and *Verralia* spp. were only collected until mid-August. The results led to concluding that *Verralia aucta* Fallén, 1817 attacked *P. spumarius* and *N. lineatus* in proportion to their relative abundance (Whittaker 1969). Given that the sampling period of the survey in Alentejo olive groves was from late-October to mid-November, it is possible that pipunculids occur earlier and an elongated sampling period should allow the investigation on the presence of this group of parasitoids, that might be relevant for biocontrol of potential vectors, since *Verralia* spp. exclusively parasitizes spittlebugs.

Spiders are generalist arthropod predators in natural and managed agroecosystems. Contrary to specialist predators, spiders may have a broad range of prey types and affect non-dominant species despite their smaller populations (Sunderland & Samu 2000). Generally, Araneae was the most abundant group, corresponding to about 60% of considered predatory groups. Spiders were practically omnipresent in olive tree and weeds sorted samples (**Table 3.5**). Although species diversity was not evaluated, the mean number of collected specimens on olive trees and weeds was similar (**Figure 3.6**), suggesting a similar importance of spiders in terms of abundance in both plant hosts.

Not all ant species are predatory, but immediately after spiders, Formicidae was the second most common taxon from the considered groups of predators. Although not as "widespread" as spiders, ants occurred in 91 of the sampling sites with sorted samples (**Table 3.5**). Comparing the mean number of collected ants per sample between weeds and olive tree samples, ants were near four times more numerous on weeds. The inverse tendency in terms of relative abundance between these two groups was observed in Spanish olive groves where spiders are the predators with the bigger species diversity and are generally the second most populous group corresponding to about 20% of collected predators after ants that are much less diverse in terms of species richness, but much more abundant (Morris et al. 1999). Santos et al. (2007) observed the same trend as Morris et al. (1999) in olive canopies from northern Portuguese olive groves. However, one should consider once again the restricted sampling period of this study and that there are methodological differences like the sampling method: Santos et al. (2007) used a beating tray and Morris et al. (1999) used a hybrid beating tray for sampling while in this study the arthropodofauna was vaccum-sampled.

Even that spiders were more frequent than ants on olive trees and that the opposite predisposition was observed on weeds (**Figure 3.6**), spiders are likely to be the most relevant predators on both hosts, since only some species of ants are predatory.

In North America, the prairie mound ant, *Formica montana* Wheeler, 1910 [mentioned as *Formica montana* Emery, 1893], is known to prey *P. spumarius* and to use the spittle produced by nymphs in the construction of tents to protect aphids from which they harvest honeydew, having a significant impact in the reduction of cercopid populations. "Ant-less" plots showed reduced numbers of spittlebugs when compared with plots where ant nests are present (Henderson et al. 1990). In the same way, other species of ants may evidence usefulness in spittlebug control in olive orchards.

The green lacewing *Chrysoperla carnea* (Stephens, 1836) (Chrysopidae) was the only Neuroptera species found in this survey. This chrysopid is a generalist predator known to prey on aphids, psyllids, mites, leafhoppers and scale insects in several cultures, depending on prey availability (Pantaleoni et al.

2001; Porcel et al. 2013). In Italian olive groves *C. carnea* is the dominant chrysopid species (Pantaleoni et al. 2001), as well as in Spanish olive groves under different management systems (Corrales & Campos 2004; Porcel et al. 2013), consuming mostly olive tree pests like the black scale *Saissetia oleae* (Olivier, 1791), *Euphyllura olivina* Costa, 1839 (both found in several olive tree samples, especially *E. olivina* that was present in practically all olive tree samples, sometimes largely outnumbering other hemipterans or being the only hemipteran even in small numbers) and immature stages of *Prays oleae* (Bernard, 1788). Collected specimens were mainly adults from olive canopy, but larvae were also collected, being the only stage occurring on weeds which is in accordance with studies reporting that chrysopids lay their eggs on non-olive plant hosts in olive groves (McEwen & Ruiz 1994 in Corrales & Campos 2004). In the Mediterranean, *C. carnea* adults occur between May and October, reaching considerable numbers between August and September (Pantaleoni et al. 2001; Corrales & Campos 2004), but different management systems, especially regarding insecticide application and weed cover, influence the magnitude of the population peak (Corrales & Campos 2004; Porcel et al. 2013). Green lacewing larvae occur from April until December, reaching their abundance peak a little earlier than adults around June/ July (Pantaleoni et al. 2001). The occurrence of the green lacewing largely coincides with adult spittlebugs seasonal abundance (April/ May – October/ November) in Italy (Cornara et al. 2016b). Given the generalist predatory habit of both *C. carnea* adults and larvae, the green lacewing may help regulate potential *X. fastidiosa* vectors if they reach high densities in Alentejo olive groves. The reduced number of collected chrysopids in comparison to ants or spiders should not undervalue the biocontrol potential of these group since low population numbers of chrysopids are typical of the sampling period.

Coccinellids were not numerically significant on both olive trees and weeds, but were present in more than one-fourth of the sampling sites with sorted samples. Not all coccinellids were identified, but from all the identified species only *Subcoccinella vigintiquartuorpunctata* Linnaeus, 1758 is phytophagous, feeding on alfalfa, clover, soy and other Fabaceae, and is known to cause economic damage in some crops in Romania. *Coccinella septempunctata* Linnaeus, 1758 and *Hippodamia. variegata* Goeze, 1777 are mainly aphidophagous species (Raimundo & Alves 1986); in Turkish olive groves, *C. setempunctata* was observed feeding on scale insects, psyllids and immature stages of Lepidoptera and *H. variegata* was also found feeding on eggs and larvae of Lepidoptera (Kacar 2015). *Rhyzobius litura* (Fabricius, 1787) is known to prey aphids and scale insects (Raimundo & Alves 1986); *Scymnus interruptus* (Goeze, 1777) and *Scymnus mediterraneus* Iablokoff-Khnzorian, 1972 are predators of scale insects like eggs and first instar nymphs of the economically important pest *S. oleae* (Santos et al. 2010); and *Stethorus punctillum* (Weise, 1891), the smallest and most frequently observed coccinellid ini this study, is known to prey mites, aphids, trips and scale insects (Raimundo & Alves 1986).

Most coccinellid species were found only on olive trees except for *H. variegata* and *S. mediterraneus* that were present on weeds. Given that aphids were not commonly found on olive trees, it is likely that coccinellids that usually prey on aphids, exploit other prey like psyllids or scale insects when hunting on olive trees. Prey consumption by coccinellids largely depends on the relation between predator and prey sizes, the nutritional quality of the prey and the tegument characteristics of the prey (Santos et al. 2010). Coccinellids are generally predators of aphids and scale insects and the observed coccinellid species are mostly smaller than the observed spittlebug adults, making them improbable prey, but coccinellids might consume immature forms. If coccinellid species on olive groves tend to hunt on olive trees and not on alternative hosts and if spittlebug eggs and nymphs are usually observed on weeds, Coccinellidae role as biocontrol agents of potential *X. fastidiosa* potential vectors is highly unlikely.

Only two Opiliones specimens were captured on olive trees. Opiliones prey on small arthropods, including insects, but some are omnivorous (Gonçalves et al. 2013). It is known from the literature that the opilion *Mitopus morio* (Fabricius, 1779) consumes *Philaenus* sp. as part of its diet (Philipson 1960). Some Pseudoscorpiones specimens and one mantis were collected from weeds, both generalist predators. The likelihood of these three groups having an important role as control agents is low due the

reduced presence in Alentejo olive groves, but once again, only a small sampling period was accounted in this study.

Although not considered in this study, Anthocoridae and Miridae are two heteropteran families, typically predatory that may be of importance as control agents and have been previously collected on Portuguese olive groves (Santos et al. 2007). Some mites (Acari: Anystida) known to prey on some phytophagous mites, trips, leafhoppers and aphids in vineyards (Gonçalves et al. 2013) were also found on olive trees and weeds in this study and may help regulate leafhopper populations, especially on weeds from where practically all cicadellids were collected (**Figure 3.7**).

4.3. Management measures

To this time, *X. fastidiosa* has not been reported in Portugal but, being a high-risk country for the pathogen introduction and spread (Pereira 2015), preventive measures should be taken. Without a detection in Portugal, introduction prevention and monitoring of plant hosts with regular tests to *X. fastidiosa* presence should be one of the focuses. Maximizing the care in the plant material trade, particularly from areas where *X. fastidiosa* has been detected is the main way to prevent introduction since it seems to be the principal path to new introductions (EFSA 2015). This has been supported by the several interceptions in countries around Europe of *X. fastidiosa*-positive *Coffea* plants imported from countries where *X. fastidiosa* occurs (Bergsma-Vlami et al. 2015; EFSA 2015; Denancé et al. 2017; Loconsole et al. 2016).

Usually disease symptoms tend to take some time to develop and not all hosts are susceptible to *X. fastidiosa*, so plants should be tested regardless of their apparent health status. Further identification of potential vectors and investigation of their distribution and population dynamics, as well as of susceptible plants, is also necessary. The knowledge about potential vectors diversity, abundance and ecological requirements and susceptible vegetation density and diversity is essential to the identification and mapping of more susceptible areas that, ultimately, will prove useful in the elaboration of adapted monitoring programmes that can be more exhaustive in more susceptible areas.

Immediate communication of a suspected presence of *X. fastidiosa* to the Plant Health Authorities is essential. In this matter, the education of general population, farmers and other stakeholders about the *X. fastidiosa* problematic, as well as the divulgation of updated information about the disease evolution, is essential. However, as the Italian experience has shown (Abbot 2015, 2016, 2017), building trust among farmers and producers is equally or even more important because the lack of it can jeopardize a precocious detection, in case of introduction, and may dictate the possibility of disease eradication or containment. If the initial area of infection is small and restricted, like a plant nursery or a garden centre, aggressive actions like vegetation destruction and heavy pesticide use to kill the vectors may be able to eliminate the infection, but in that case compensation measures should be provided to producers.

From the previous experience, a new *X. fastidiosa* introduction is usually detected only when there are plants displaying symptoms which may take months to years to develop so, if detection occurs in open field, it is likely that the bacterium is already widespread and only containment measures should be applied. Besides, plants with symptomless infections can be more relevant as inoculum sources since several vectors are known to discriminate plants, based on their infection status, preferring healthy looking plants to symptomatic plants (Marucci et al. 2005; Daugherty et al. 2011).

Xylem sap is nutritionally very poor, containing nitrogen concentrations between 0.01%-015%(w/v) and in some cases, as low as 0.0002% (w/v) (Tonkyn & Whitcomb 1987). For this reason, nitrogen access is a limiting factor to most species feeding on xylem sap. Yurtsever (2000) pointed that nitrogen-fixing plants like *Medicago sativa, Trifollium* spp. or *Vicia* spp. are favoured by *P. spumarius*. Hartley & Gardner (1995) investigated if *P. spumarius* plant host selection was influenced by its nutritional status, comparing spittlebug abundance on *Calluna vulgaris* (L.) between non-treated, fertilized, shading and fertilized-and-shading plots. Their results showed that *P. spumarius* adults were significantly more

abundant on the fertilized plots where *C. vulgaris* displayed higher levels of nitrogen and lower levels of fibre and lignin than on non-treated or shaded plots where no change in *C. vulgaris* nutritional status was observed. Knowing this, olive groves' fertilization should be considered in relation to potential vector populations.

Cao et al. (2012) investigated the potential of high-grafting almond branches to peach rootstock, which is resistant to the *X. fastidiosa* strain causing Almond Leaf Scorch (ALS), into limiting *X. fastidiosa* spread to other branches in the same plant. For this, they grafted several almond branches in peach rootstock and mechanically inoculated *X. fastidiosa* into some of the grafted almond branches. Later, almond branches that had not been inoculated with the bacterium tested positive for *X. fastidiosa* presence showing that it moved from inoculated almond branches to the peach rootstock and to other almond branches. These results imply that grafting infected plant material may be a way to transmit *X. fastidiosa* as well as vegetative multiplication with infected olive tree cuts. For this reason, the use of vegetative material from infected areas should be specifically avoided or forbidden, since this is considered the main form of long-distance disease dissemination. Also, tests for *X. fastidiosa* presence on graft plants prior to grafting should be done with a certain regularity to assure that there is no contamination and subsequent disease spread.

Dimethoate application is a common chemical control procedure for the two key pests in olive groves: *B. oleae* and *P. oleae*. As dimethoate is a systemic insecticide it can contribute to reduce potential vector populations and it should be effective if its application overlaps with the seasonal population peak of spittlebugs (Purcell & Franzier 1985). However, it is also likely that natural enemies are negatively impacted by dimethoate application (Santos et al. 2007, Santos et al. 2010) and there is always the possibility of resistance development to insecticide treatments by culture enemies (Amaro 2003), which already occurs for *B. oleae* (Pereira-Castro et al. 2015).

The economic threshold of an insect as a pathogen vector is very different of the one when an insect is a pest directly damaging the crop. For these reason, given the current situation of *X. fastidiosa* in Portugal, there is no need to take direct measures against vectors (like chemical control), but indirect measures like cultural practices that help maintaining or boost natural enemies' populations are important, regardless of the presence or absence of *X. fastidiosa*.

Several studies have shown the importance of plant cover in several crops including olive orchards in the conservation of natural enemies (Sunderland & Samu 2000; Porcel et al. 2013). In this study, weeds seem to have a positive impact in both natural enemies and potential *X. fastidiosa* vectors. If on one side, weeds presence is desirable since they provide important habitat for natural enemies of pests, their presence is also beneficial to potential *X. fastidiosa* vectors which is not so desirable. Weed removal might help decreasing spittlebug populations (Martelli 2016), but it also might promote migration to new areas, potentially contributing to bacterium spread. At least it will be very useful to understand if vectors show preferences for some weeds species, for a better and safer future weed management, since weeds presence in olive orchards can provide several advantages, such as soil conservation and enrichment on organic matter.

In case of *X. fastidiosa* introduction, the importance of weed management on the disease spread will depend if the infection cycle is primary or secondary. If weeds are the main inoculum of the bacterium and transmission is mainly through primary spread (from weeds to olive trees), weed removal might be essential to slow disease spread. However, if secondary spread (from olive tree to olive tree) is the main transmission mechanism, like it seems to be in Italy (Cornara et al. 2016b), weed removal is not as important to reduce *X. fastidiosa* inoculum. Removing diseased trees and pruning branches with early symptoms in adult trees has been effective in slowing Citrus Variegated Chlorosis (CVC) in Brazil, where the main dispersal mechanism is secondary spread (Redak et al. 2004; Janse & Obradovic 2010).

In Italy, at least three olive trees varieties have been shown to develop lower bacterial populations and milder symptoms (Martelli et al. 2016; EFSA 2017). So far, there is no cure to *X. fastidiosa*, but in case of introduction and establishment, the most promising measure to diminish its negative impact on olive culture, as well as on others, is the search for resistant cultivars (that do not develop disease symptoms) or tolerant cultivars (that develop milder symptoms).

5. Conclusions

The results of this survey showed a very low diversity and relative abundance of potential *Xylella fastidiosa* Wells et al. 1987 vector species in olive groves. *Neophilaenus campestris* (Fallén, 1817) and *Philaenus* sp. were the only xylem-feeding species collected in Alentejo olive groves and are highly likely to spread *X. fastidiosa* if introduction occurs, however, some aspects should be considered. This study covered a very wide area, throughout the main olive production region in Portugal, but only a limited temporal period, in autumn. For this reason, the identification of potential vectors should happen at a larger time scale in the future. This might allow the identification of other xylem-feeding species in olive groves, not detected in this study, and the confirmation of *Philaenus* sp. identity. Further studies on potential vectors of *X. fastidiosa* should focus, not only on their diversity, but also on temporal and spatial population dynamics and on testing potential vectors for *X. fastidiosa* presence. As vector preferences have a big role on *X. fastidiosa* transmission, studies focused on potential vectors behaviour should also be conducted. Being a less studied species and having been collected in a higher number than *Philaenus* sp., it is especially important to better understand aspects of the biology and ecology of *N. campestris*.

Since potential vectors were more abundant on weeds and that these plants seem to have an important role for overwintering Auchenorrhyncha nymphs, weeds are expected to play an important role on the maintenance of potential vector populations, and species identification of these alternative hosts should be considered in further studies. Besides helping the identification of alternative susceptible hosts, it provides basic ecological information on occurring Auchenorrhyncha in Portugal mainland, where little studies were made. This also allows the determination of possible associations between plant species and potential *X. fastidiosa* vectors and the identification and mapping of more vulnerable areas based on the present vegetation and potential vectors.

During this study, one specimen of *Orosius albicinctus* Distant, 1918 (Deltocephalinae) was identified on weeds. This species is not a potential vector of *X. fastidiosa*, but it is an important vector of phytoplasma-related diseases in other countries and it might be the first record of this species in mainland Portugal.

This study showed the presence of several groups of natural enemies, including predators and parasitoids, in Alentejo olive groves which may aid in limiting potential vector populations. Weeds seem to have a positive impact on the abundance of both predators and parasitoids and its maintenance may be important for this objective.

Although still undetected, given the existence of favourable climatic conditions and of susceptible hosts of economic importance, as olive trees, Portugal is prone to *X. fastidiosa* introduction and spread. With the occurrence of potential vectors, as verified by this study, the application of preventive management measures, as functional biodiversity conservation and enhancement could be essential and useful to reduce *X. fastidiosa* spread on olive orchards.

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Appendix 1 – Meteorological data

Table A.1. Base data from the daily mean air temperature (ºC) provided by SNIRH (http://snirh.apambiente.pt). *Values of the automatic network are marked with the letter A and values from the conventional network are marked with the letter C.

DATE / STATION	23G/01F^{C}	22M/05F^{C}	19O/02F^{C}	26I/02F^{A}	23I/01C^{A}	20E/01C^{C}	24F/01C^{A}	26M/01C^{C}	22F/03C^{A}	24I/01C^{A}
25/10/2016	17.8	17.1	16	17.4	16.3	17.5	17	16.4	17.2	15.8
26/10/2016	19.1	19	18.7	18.6	17.5	18.7	17.3	17.7	18	17.6
27/10/2016	22.8	21.5	21.5	21.5	22	22.1	20.7	21.8	21	22.3
28/10/2016	22.6	21.7	22	21.8	22.7	21.9	20.8	21.7	21.5	23.1
29/10/2016	22.3	21.3	21.9	21.2	21.6	22.7	20.5	20.6	20.9	21.7
30/10/2016	19.5	19.7	18.9	18.9	19.8	18.7	17.8	18.1	18.4	20.3
31/10/2016	17.5	18.2	17.5	17.8	18.3	16.7	16.1	17.2	16	20.3
01/11/2016	20.2	19	17.8	19.6	18.9	19.3	18.9	18.7	19.8	18.8
02/11/2016	19.5	19.4	17.7	18.9	18.2	19	18.5	18.6	18.8	18.3
03/11/2016	17.2	18.9	17.4	17.6	16.8	17.3	16.1	17.1	16.9	17.5
04/11/2016	19.3	19.6	18.3	19.7	18.7	17.7	18	19.3	18.1	18.8
05/11/2016	17	16.8	16.3	16.7	15.5	16.6	16.8	15.9	16	15.3
06/11/2016	12.6	13.2	11.4	13.6	11.4	11.6	12.9	12.3	10.8	11.3
07/11/2016	11.4	11.5	9.7	11.2	10.3	10.4	11.9	9.4	9.7	11
08/11/2016	9.9	10.3	9.2	10	9.1	9.3	10.7	7.8	8	8.9
09/11/2016	11.4	12.4	12.5	11.8	11.4	12.8	12.2	9.6	11	11.1
10/11/2016	13.8	13.6	12.7	14.4	12.7	13.1	14	12.4	12.4	12.4
11/11/2016	12.5	13.1	11.9	12.5	12.1	11.5	11.9	10.1	10.6	12.1
12/11/2016	13.5	12.8	12	13.5	13.3	12.9	13.4	11.1	12.7	13.1
13/11/2016	15.7	14.7	14.2	15.4	15.1	15.5	15.7	12.8	15.4	14.6
14/11/2016	15.6	16.3	15.2	16	15.3	14.3	14	14.9	14.3	15.2
15/11/2016	16	14.7	13.2	15.4	14	14.4	12.1	14.2	13.5	13.7

Table A.2. Base data from the daily mean relative humidity (%) provided by SNIRH (http://snirh.apambiente.pt). All values are from the automatic network.

DATE/ STATION	23G/01F	22M/05F	19O/02F	26I/02F	23I/01C	20E/01C	24F/01C	26M/01C	22F/03C	24I/01C
25-10-2016	85	98	88	89	94	85	91	91	94	91
26-10-2016	81	92	80	84	91	83	92	83	91	83
27-10-2016	68	87	71	77	68	72	79	72	82	65
28-10-2016	73	89	70	76	71	76	79	72	83	66
29-10-2016	71	88	68	71	73	72	76	70	79	67
30-10-2016	76	85	71	77	70	79	81	71	84	63
31-10-2016	69	76	64	68	54	80	75	57	79	42
01-11-2016	63	69	63	66	64	65	70	62	64	62
02-11-2016	79	83	74	80	82	82	84	78	85	79
03-11-2016	88	84	79	85	91	84	89	82	89	84
04-11-2016	74	74	71	69	74	82	82	68	83	68
05-11-2016	88	99	90	91	96	93	92	90	100	94
06-11-2016	93	96	91	86	97	90	90	90	99	92
07-11-2016	77	73	72	75	75	79	74	77	82	71
08-11-2016	73	73	69	70	67	72	67	73	78	65
09-11-2016	79	76	65	73	73	74	75	75	84	71
10-11-2016	87	97	81	88	94	86	90	92	94	89
11-11-2016	79	77	76	77	77	81	81	79	85	74
12-11-2016	86	89	81	84	87	91	86	84	92	82
13-11-2016	90	92	85	88	92	90	91	86	95	89
14-11-2016	78	79	72	72	74	85	83	72	89	70
15-11-2016	56	67	62	63	58	59	75	57	70	58

Table A.3. Base data from the daily precipitation (mm) provided by SNIRH (http://snirh.apambiente.pt). All values are from the automatic network.

DATE/ STATION	21M/02UG	19O/02F	26I/02F	23I/01C	21K/01UG	20E/01C	17M/01G	27I/01G	17G/02G	18G/01G	24F/01C	26M/01C
25-10-2016	27	0	0	12	7.4	6.2	9.7	21	0	0.9	0.6	36.8
26-10-2016	0.9	0	0	0.9	1.7	0	0	0.9	0.1	0	0	0.2
27-10-2016	0	0	0	0	0.5	0	0	0	0	0	0.3	0
28-10-2016	0	0	0	0	0.2	0	0	0.1	0	0	0.2	0
29-10-2016	0	0	0	0	0	0	0	0	0	0	5.8	0
30-10-2016	0	0	0	0	0	0	0	0.2	0	0	0.1	0.1
31-10-2016	0	0	0	0	0	0	0	0	0	0.1	0	0
01-11-2016	0	0	0	0	0.1	0.2	0	0.1	0	0	0	0
02-11-2016	0	0	0	0	0	0	0	0.1	0	0.7	0	0.1
03-11-2016	0.1	0	0	0.4	0.1	0.2	0	0.3	0	0.1	0	0.2
04-11-2016	0	0	0	0.3	0.1	2.9	2.3	0.5	0	0.6	0	0.5
05-11-2016	6	0	0	5.9	2.6	5	3	24.9	0	3.4	6.5	8.7
06-11-2016	6.8	0	0	0.3	1.8	5.8	12.9	4.3	0	3.2	1	0.9
07-11-2016	0	0	0	0.1	1.2	0	0	0	0	0.1	0.3	0.4
08-11-2016	0	0	0	0.1	0.7	0	0	0	0	0	0.2	0.9
09-11-2016	0	0	0	0	0.7	0	0.6	0	0	0.6	0	0.1
10-11-2016	0.4	0	0	0	0.7	2.5	0	1.6	0	0.8	0.1	1.5
11-11-2016	0	0	0	0	0.3	0.2	0	0	0	0.2	0.1	0.3
12-11-2016	0	0	0	0	0.4	0.2	0	0.2	0	0	2.2	0.2
13-11-2016	0.2	0	0	0.1	0.8	18.5	2.8	0.1	0	11.6	1.2	0
14-11-2016	0.1	0	0	0.1	0.9	0	0	0	0	0.2	0.5	0.1
15-11-2016	0	0	0	0	0.6	0	0	0	0	0	2.9	0

Table A.3 (continuation). Base data from the daily precipitation (mm) provided by SNIRH (http://snirh.apambiente.pt). All values are from the automatic network.

DATE/ STATION	19G/01UG	22F/03C	23F/01UG	27H/01CG	20I/01G	24K/01UG	23L/01G	26L/01UG	23K/01UG	17L/02UG	24I/01C	21M/01UG
25-10-2016	1.6	3.6	2.9	23.7	3.2	50.6	35	17.3	13.7	6.1	12.1	21.1
26-10-2016	0	0	0.1	0.7	0	2.1	0.9	0.9	1.4	0.2	2.2	0.7
27-10-2016	0	0	0	0	0	0	0	0	0	0	0	0
28-10-2016	0	0	0	0	0	0	0	0	0	0	0	0
29-10-2016	0	0.1	0.1	0	0	0.1	0	0	0	0	0	0
30-10-2016	0	0.1	0.1	0	0	0.1	0	0	0	0.1	0	0
31-10-2016	0	0.1	0.2	0	0	0.1	0	0	0.1	0.1	0	0
01-11-2016	0.1	0	0	0	0.2	0	0.2	0	0.1	0	0	0
02-11-2016	0	0.1	0.1	0.1	0	0	0	0	0	0	0	0
03-11-2016	0.2	0.3	0.6	0.2	0.1	0.2	0.1	0.2	0.1	0	0.2	0.1
04-11-2016	7.2	11.6	0.1	0	0.1	0	0	3	0.1	2.2	0.1	0.4
05-11-2016	5.1	7.9	3.2	15.7	0.2	12.2	6.8	14.4	6.3	5.7	10.3	3.9
06-11-2016	3	6.7	4	7.9	6.2	2.2	6.5	2.6	4	2	4.5	9.4
07-11-2016	0	0.1	0.1	0	0.1	0	0	0	0	0.1	0	0
08-11-2016	0.1	0.1	0	0	0	0	0	0.1	0	0.2	0	0
09-11-2016	0.7	0.1	0.3	0	0	0	0	0	0	0.3	0	0
10-11-2016	1	2.2	1.3	2.2	0.8	1.2	1	3	1.2	0.2	0.1	0.9
11-11-2016	0.1	0.2	0.2	0.1	0.2	0.1	0	0.2	0.1	0.1	0.1	0.1
12-11-2016	0	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.2	0.1	0.1	0
13-11-2016	3.9	1.5	3.8	0.4	1.3	0.1	0	0.1	0.1	0.2	0.3	0.3
14-11-2016	0	0.1	0.1	0	0	0	0	0	0	0	0	0
15-11-2016	0	0	0	0	0	0	0	0	0	0	0	0

Table A.4. Information about used meteorological stations provided by SNIRH (http://snirh.apambiente.pt). [a,b] Clim. – Climatological station; Udog. – Udographic station. Udom. – Udometric station. Drift. Clim. – Drifting climatological station. [c] Quality index of the data series: values between 5 and 8 are bad; values between 9 and 12 have reasonable quality; values larger than 12 have high reliability; NA – Not available.

Code	Name	Altitude	Latitude	Longitude	Type of station (automatic)[a]	Type of station (conventional)[b]	Quality index[c]
21M/02UG	Alandroal	302	38° 41' 35"	-8° 35' 46"	Udog.	Udom.	15
23G/01F	Albufeira de Pego do Altar	48	38° 25' 4"	-9° 36' 34"	Drift. Clim.	-	NA
22M/05F	Albufeira do Alqueva (Mourão)	103	38° 23' 47"	-8° 36' 46"	Drift. Clim.	-	NA
19O/02F	Albufeira do Caia	221	39° 0' 22"	-8° 51' 7"	Drift. Clim.	-	NA
26I/02F	Albufeira do Roxo	135	37° 55' 44"	-9° 55' 13"	Drift. Clim.	-	NA
23I/01C	Alcáçovas	218	38° 23' 25"	-9° 50' 55"	Clim.	Clim.	12
21K/01UG	Azaruja	270	38° 42' 10"	-8° 13' 31"	Udographic	Udom.	12
20E/01C	Barragem de Magos	43	38° 59' 24"	-9° 18' 22"	Clim.	Clim.	15
17M/01G	Castelo de Vide	552	39° 24' 42"	-8° 32' 51"	Udog.	Udog.	14
27I/01G	Castro Verde	217	37° 41' 51"	-9° 54' 24"	Udog.	Udog.	14
17G/02G	Chamusca	18	39° 21' 40"	-9° 30' 47"	Udog.	Udog.	15
18G/01G	Chouto	126	39° 16' 26"	-9° 38' 56"	Udog.	Udog.	15
24F/01C	Grândola	95	38° 10' 16"	-9° 26' 27"	Clim.	Clim.	14
26M/01C	Herdade da Valada	223	37° 56' 53"	-8° 34' 3"	Clim.	Clim.	NA
19G/01UG	Machoqueira do Grou	133	39° 6' 58"	-9° 38' 53"	Udog.	Udom.	15
22F/03C	Moinhola	41	38° 35' 3"	-9° 23' 1"	Clim.	Clim.	15
23F/01UG	Montevil	24	38° 23' 46"	-9° 22' 44"	Udog.	Udometric	14
27H/01CG	Panóias	164	37° 45' 25"	-9° 41' 38"	Udog.	Clim.	15
20I/01G	Pavia	189	38° 53' 47"	-9° 59' 11"	Udog.	Udographic	15
24K/01UG	Portel	302	38° 18' 22"	-8° 17' 31"	Udog.	Udom.	14
23L/01G	Reguengos	218	38° 25' 24"	-8° 28' 23"	Udog.	Udom.	13
26L/01UG	Serpa	209	37° 56' 33"	-8° 23' 46"	Udog.	Udom.	13
23K/01UG	São Mancos	190	38° 27' 36"	-8° 14' 56"	Udog.	Udom.	15
17L/02UG	Vale do Peso	285	39° 20' 45"	-8° 21' 8"	Udog.	Udom.	15
24I/01C	Viana do Alentejo	314	38° 19' 42"	-9° 59' 36"	Clim.	Clim.	15
21M/01UG	Vila Viçosa	417	38° 47' 7"	-8° 34' 46"	Udog.	Udom.	14

Appendix 2 – Metadata of map layers

Table A.5. Metadata of the base layers used in all maps. This table intends to give a summary of the metadata, but more metadata are provided in the sources of the data.

Data	Source	Metadata	Site
Portugal administrative limits (Carta Administrativa Oficial de Portugal)	Direção Geral do Território (DGT)	**Format**: Shapefile (.shp) | **Coordinate reference system**: ETRS89/PT-TM06 | **Projection**: Mercator Transverse | **Datum**: ETRS89 | **Ellipsoid**: GRS80 | **Reference period**: 2016	http://www.dgterritorio.pt/cartografia_e_geodesia/cartografia/carta_administrativa_oficial_de_portugal_caop_/caop__download_/
Spain administrative limits (Límites jurisdiccionales de España)	Centro Nacional de Información Geográfica	**Format**: Shapefile (.shp) | **Coordinate reference system**: ETRS89 (Peninsula and Baleares) and WGS84 (Canary Islands) | **Reference period**: 2015	http://centrodedescargas.cnig.es/CentroDescargas/equipamiento.do?method=mostrarEquipamiento
Land cover (Corine Land Cover 2012 - version 18.5)	Copernicus (European Land Monitoring Service)	**Format**: ESRI geodatabase (.gdb) | **Coordinate reference system**: EUR_ETRS89/LAEA1052 | **Projection**: Lambert Azimuthal | **Datum**: ETRS89 | **Ellipsoid**: GRS 80 | **Reference period**: 2012 | **Other formats available**: Raster (.GeoTiff)	http://land.copernicus.eu/pan-european/corine-land-cover/clc-2012/view
Portugal administrative limits (used in overview map)	Global Administrative areas (GADM)	**Format**: Shapefile (.shp) | **Coordinate reference system**: WGS 84 | **Other available formats**: Google Earth (.kmz). R Spatial Polygons Data Frame (.rds). ESRI file geodatabse	http://www.gadm.org/country
Spain administrative limits (used in overview map)	Global Administrative areas (GADM)	**Format**: Shapefile (.shp) | **Coordinate reference system**: WGS 84 | **Other available formats**: Google Earth (.kmz). R Spatial Polygons Data Frame (.rds). ESRI file geodatabse	http://www.gadm.org/country
France administrative limits (used in overview map)	Global Administrative areas (GADM)	**Format**: Shapefile (.shp) | **Coordinate reference system**: WGS 84 | **Other available formats**: Google Earth (.kmz). R Spatial Polygons Data Frame (.rds). ESRI file geodatabse	http://www.gadm.org/country
Meteorological stations	Sistema Nacional de Informação de Recursos Hídricos (SNIRH)	**Format**: Excel (.csv) | **Coordinate reference system**: WGS 84 and Hayford-Gauss Datum 73	http://snirh.apambiente.pt/index.php?idMain=2&idItem=1
Daily mean temperature	Sistema Nacional de Informação de Recursos Hídricos (SNIRH)	**Format**: Excel (.csv) | **Reference period**: 25/10/2016 - 15/10/2016.	http://snirh.apambiente.pt/index.php?idMain=2&idItem=1
Daily mean relative humidity	Sistema Nacional de Informação de Recursos Hídricos (SNIRH)	**Format**: Excel (.csv) | **Reference period**: 25/10/2016 - 15/10/2016.	http://snirh.apambiente.pt/index.php?idMain=2&idItem=1
Daily precipitation	Sistema Nacional de Informação de Recursos Hídricos (SNIRH)	**Format**: Excel (.csv) | **Reference period**: 25/10/2016 - 15/10/2016.	http://snirh.apambiente.pt/index.php?idMain=2&idItem=1

Appendix 3 – Exploratory analysis

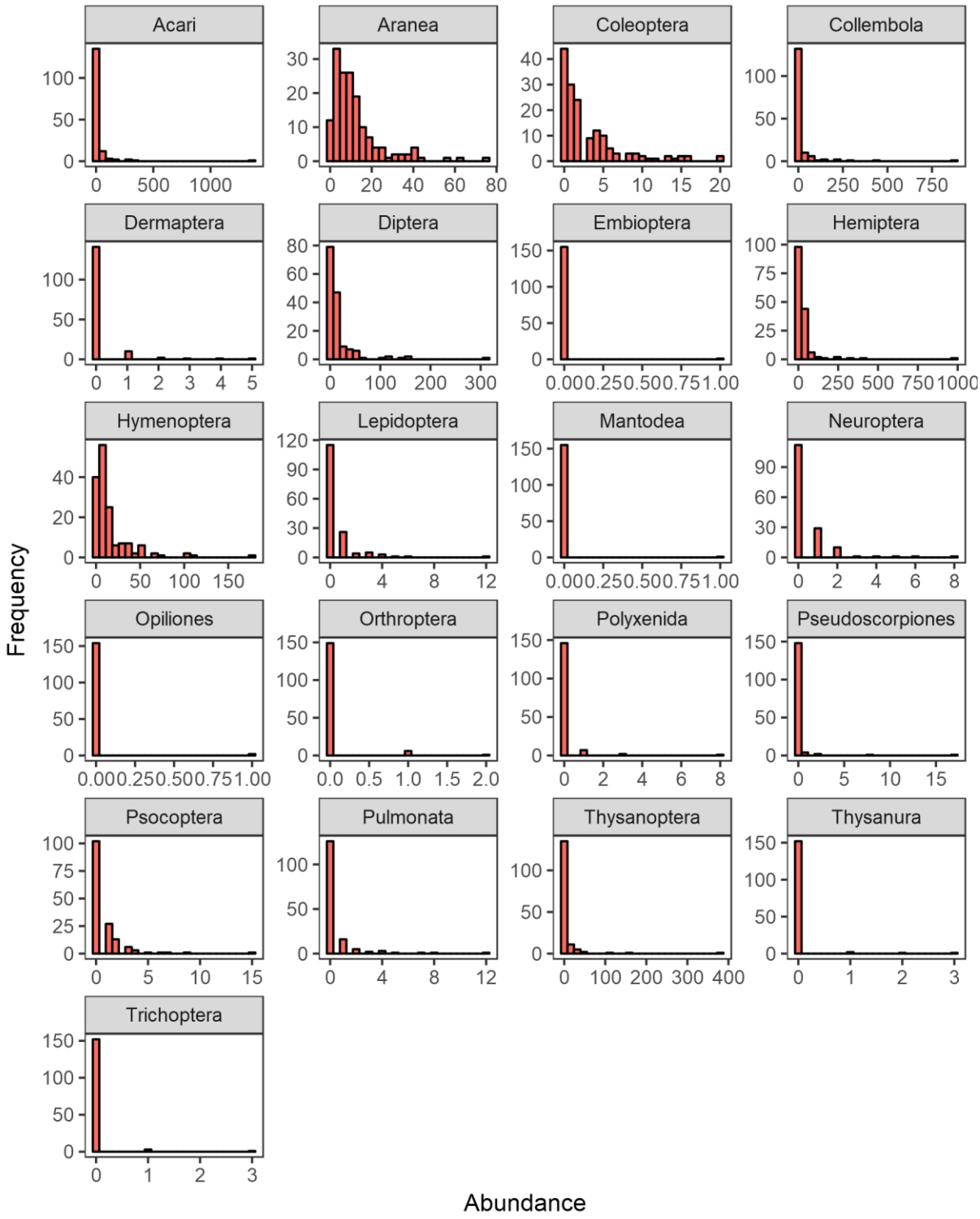

Figure A.1. Histograms of the frequency distribution of orders abundances.

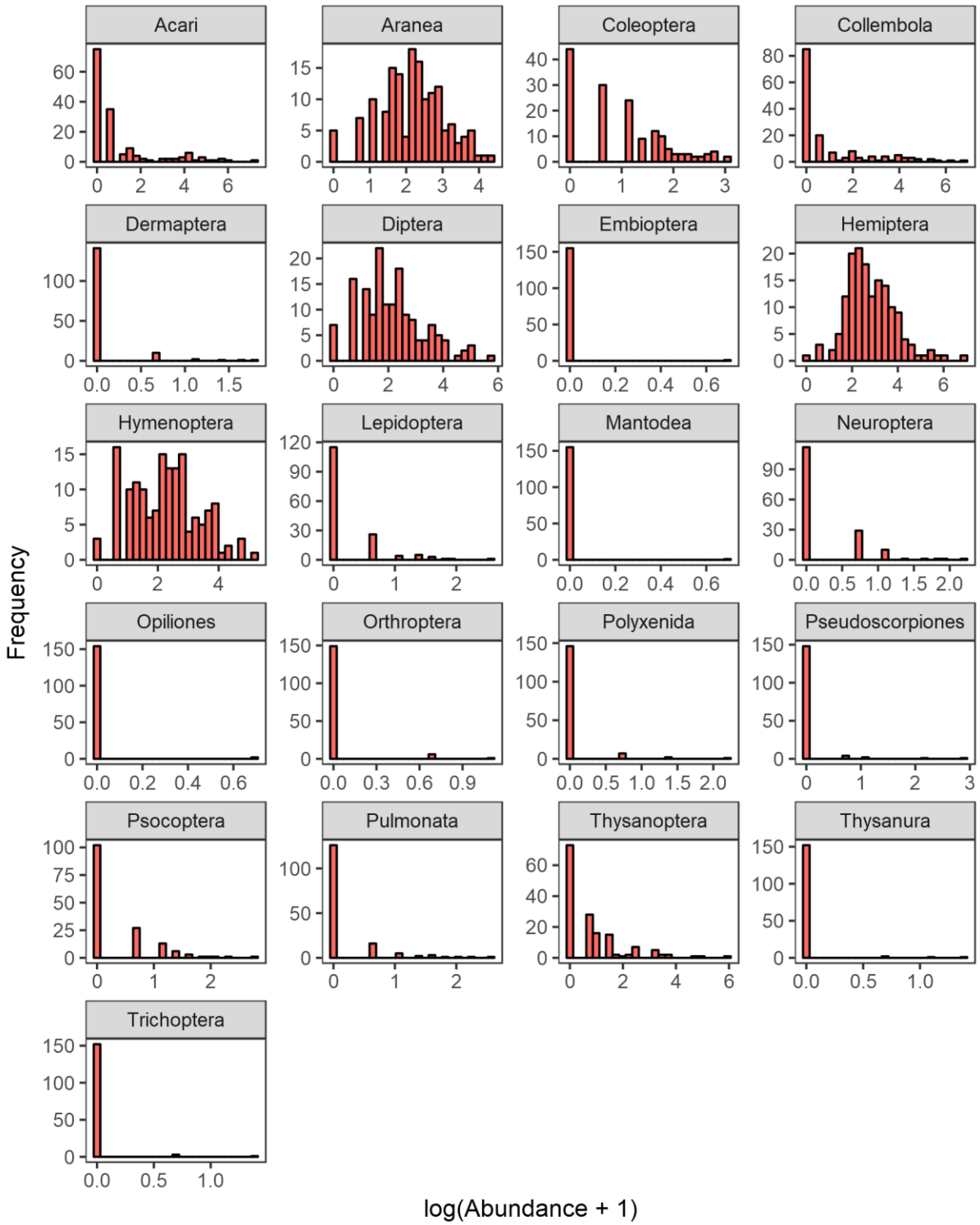

Figure A.2. Histograms of the frequency distribution of log-transformed orders abundances.

Table A.6. Correlation matrix of the orders abundances. Strong correlations (Pearson correlation coefficient larger than the absolute value 0.5) are marked in red. Significant correlations at the 0.05 level (two-tailed) are marked as bold with as asterisk. HEM – Hemiptera; ARA – Aranea; NEU – Neuroptera; HYM – Hymenoptera; COL1 – Coleoptera; THY1 – Thysanoptera; PSO – Psocoptera; MAN – Mantodea; DIP – Diptera; THY2 – Thysanura; TRI – Trichoptera; LEP – Lepidoptera; DER – Dermaptera; ORT – Orthroptera; EMB – Embioptera; COL2 – Collembola; PSEU – Pseudoscorpiones; OPI – Opiliones; ACA – Acari; PUL -Pulmonata; POL – Polyxenida.

Order	HEM	ARA	NEU	HYM	COL1	THY1	PSO	MAN	DIP	THY2	TRI	LEP	DER	ORT	EMB	COL2	PSEU	OPI	ACA	PUL	POL
HEM	1.00																				
ARA	0.01	1.00																			
NEU	-0.03	0.04	1.00																		
HYM	**0.45***	0.11	-0.02	1.00																	
COL1	0.08	**0.30***	-0.08	**0.48***	1.00																
THY1	0.07	0.02	-0.07	**0.21***	**0.36***	1.00															
PSO	0.12	**0.20***	0.07	0.15	0.07	-0.02	1.00														
MAN	-0.02	0.02	-0.04	-0.01	0.04	-0.02	0.01	1.00													
DIP	**0.18***	0.08	-0.10	**0.74***	**0.41***	**0.19***	-0.01	-0.03	1.00												
THY2	-0.02	**0.17***	0.01	0.10	0.13	-0.01	0.08	-0.01	**0.16***	1.00											
TRI	-0.03	**0.28***	**0.37***	0.10	-0.01	-0.02	**0.28***	-0.01	-0.02	0.13	1.00										
LEP	**0.16***	0.04	-0.08	**0.31***	**0.31***	0.07	-0.06	-0.03	**0.27***	0.10	-0.06	1.00									
DER	0.01	-0.03	**0.34***	0.06	-0.11	-0.04	-0.08	-0.02	-0.10	-0.04	-0.04	-0.03	1.00								
ORT	0.05	-0.00	-0.04	**0.16***	**0.19***	0.03	0.02	-0.02	**0.22***	0.05	-0.03	0.09	-0.05	1.00							
EMB	**0.17***	0.00	0.11	-0.02	0.02	-0.02	-0.04	-0.01	-0.02	-0.01	-0.01	**0.20***	-0.02	**0.31***	1.00						
COL2	0.12	0.13	-0.09	**0.32***	**0.42***	**0.43***	**0.18***	-0.01	**0.28***	**0.17***	-0.02	0.09	-0.06	0.15	0.00	1.00					
PSEU	0.12	**0.19***	0.00	0.14	**0.34***	0.05	-0.03	0.04	**0.27***	**0.23***	-0.02	**0.37***	-0.04	0.13	**0.41***	0.08	1.00				
OPI	-0.02	-0.05	0.05	-0.05	-0.05	0.04	-0.05	-0.01	-0.04	-0.02	-0.02	-0.05	0.06	-0.02	-0.01	-0.03	-0.02	1.00			
ACA	0.09	0.07	-0.07	**0.24***	**0.16***	0.10	0.14	0.00	**0.25***	**0.78***	-0.00	0.15	-0.05	0.11	0.07	**0.49***	0.15	-0.02	1.00		
PUL	0.06	0.12	-0.09	**0.42***	**0.40***	**0.27***	-0.06	-0.03	**0.63***	**0.23***	-0.01	**0.31***	-0.09	**0.31***	0.03	0.22*	**0.56***	0.00	**0.21***	1.00	
POL	0.09	0.08	-0.00	**0.54***	**0.21***	0.00	**0.35***	0.10	**0.55***	-0.03	0.07	0.07	-0.05	0.03	0.10	0.14	0.06	-0.02	0.10	**0.33***	1.00

Table A.7. P-value associated to the two-tailed t test of the Pearson correlation coefficients of the orders abundances. P-values lower than 0.05 are marked as red. NA – Not applicable. HEM – Hemiptera; ARA – Aranea; NEU – Neuroptera; HYM – Hymenoptera; COL1 – Coleoptera; THY1 – Thysanoptera; PSO – Psocoptera; MAN – Mantodea; DIP – Diptera; THY2 – Thysanura; TRI – Trichoptera; LEP – Lepidoptera; DER – Dermaptera; ORT – Orthroptera; EMB – Embioptera; COL2 – Collembola; PSEU – Pseudoscorpiones; OPI – Opiliones; ACA – Acari; PUL -Pulmonata; POL – Polyxenida.

Order	HEM	ARA	NEU	HYM	COL1	THY1	PSO	MAN	DIP	THY2	TRI	LEP	DER	ORT	EMB	COL2	PSEU	OPI	ACA	PUL	POL
HEM	NA																				
ARA	0.929	NA																			
NEU	0.724	0.624	NA																		
HYM	0.000	0.155	0.825	NA																	
COL1	0.326	0.000	0.295	0.000	NA																
THY1	0.398	0.779	0.357	0.008	0.000	NA															
PSO	0.122	0.011	0.367	0.065	0.408	0.803	NA														
MAN	0.782	0.856	0.659	0.891	0.652	0.855	0.912	NA													
DIP	0.029	0.320	0.195	0.000	0.000	0.016	0.909	0.727	NA												
THY2	0.791	0.034	0.879	0.200	0.100	0.914	0.353	0.884	0.048	NA											
TRI	0.679	0.000	0.000	0.207	0.953	0.820	0.000	0.889	0.845	0.101	NA										
LEP	0.042	0.604	0.353	0.000	0.000	0.405	0.431	0.697	0.001	0.240	0.496	NA									
DER	0.864	0.708	0.000	0.492	0.166	0.606	0.334	0.795	0.210	0.636	0.651	0.651	NA								
ORT	0.534	0.972	0.585	0.041	0.019	0.699	0.775	0.837	0.005	0.503	0.720	0.273	0.503	NA							
EMB	0.037	0.986	0.161	0.791	0.835	0.855	0.655	0.936	0.769	0.884	0.889	0.011	0.795	0.000	NA						
COL2	0.149	0.095	0.253	0.000	0.000	0.000	0.027	0.864	0.000	0.035	0.773	0.251	0.431	0.070	0.989	NA					
PSEU	0.114	0.016	0.995	0.089	0.000	0.564	0.693	0.604	0.001	0.004	0.809	0.000	0.652	0.121	0.000	0.301	NA				
OPI	0.811	0.577	0.498	0.577	0.573	0.602	0.526	0.910	0.592	0.836	0.843	0.580	0.461	0.770	0.910	0.721	0.844	NA			
ACA	0.264	0.390	0.407	0.003	0.048	0.239	0.084	0.989	0.002	0.000	0.965	0.058	0.523	0.188	0.420	0.000	0.070	0.777	NA		
PUL	0.490	0.145	0.289	0.000	0.000	0.001	0.475	0.745	0.000	0.004	0.858	0.000	0.290	0.000	0.732	0.007	0.000	0.990	0.010	NA	
POL	0.258	0.338	0.992	0.000	0.007	0.965	0.000	0.246	0.000	0.743	0.393	0.363	0.558	0.691	0.246	0.091	0.456	0.798	0.213	0.000	NA

Appendix 4 – Correlation matrix of PCA

Table A.8. Correlation matrix of orders abundances against principal components scores. Strong correlations (Pearson correlation coefficient larger than the absolute value 0.5) are marked in red. Significant correlations at the 0.05 level (two-tailed) are marked as bold. HEM – Hemiptera; ARA – Aranea; NEU – Neuroptera; HYM – Hymenoptera; COL1 – Coleoptera; THY1 – Thysanoptera; PSO – Psocoptera; MAN – Mantodea; DIP – Diptera; THY2 – Thysanura; TRI – Trichoptera; LEP – Lepidoptera; DER – Dermaptera; ORT – Orthroptera; EMB – Embioptera; COL2 – Collembola; PSEU – Pseudoscorpiones; OPI – Opiliones; ACA – Acari; PUL -Pulmonata; POL – Polyxenida.

Order	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13	PC14	PC15	PC16	PC17	PC18	PC19	PC20	PC21
HEM	**-0.37**	**-0.18**	**0.41**	**-0.22**	0.14	0.15	0.16	-0.02	0.00	-0.04	0.12	-0.02	-0.13	-0.01	0.07	0.01	-0.05	0.09	0.02	-0.03	0.01
ARA	**-0.21**	**-0.43**	**-0.51**	-0.12	**-0.32**	0.12	**0.35**	-0.13	-0.13	0.01	-0.03	0.02	-0.03	-0.02	0.09	-0.09	0.08	0.06	-0.01	-0.01	0.03
NEU	0.11	-0.15	0.02	**-0.18**	0.04	-0.14	0.06	**0.21**	-0.05	**0.85**	-0.09	**0.18**	0.07	-0.03	-0.02	-0.01	0.04	0.07	0.05	0.02	0.02
HYM	**-0.75**	**-0.20**	0.07	-0.03	**0.19**	0.00	-0.11	**0.20**	-0.12	-0.05	-0.09	-0.03	0.02	0.00	**-0.23**	-0.08	0.01	0.09	0.04	-0.05	0.10
COL1	**-0.57**	**-0.40**	**-0.18**	-0.01	**-0.21**	**-0.22**	**-0.38**	**-0.22**	0.16	0.01	0.04	-0.10	-0.05	0.07	0.04	-0.06	-0.01	-0.02	0.03	-0.03	0.09
THY1	**-0.40**	**-0.01**	**0.20**	**0.32**	**-0.38**	-0.14	-0.01	0.11	0.02	0.03	-0.02	**-0.16**	0.03	0.09	0.10	-0.03	-0.08	0.02	0.03	-0.01	-0.14
PSO	**-0.24**	-0.09	-0.14	**-0.37**	0.03	-0.15	**0.40**	**0.25**	**0.53**	-0.13	**-0.22**	0.13	0.00	0.05	-0.09	0.00	0.04	0.00	-0.01	-0.06	0.05
MAN	-0.06	0.06	-0.10	-0.10	-0.03	0.05	-0.07	0.00	0.08	-0.01	0.02	-0.03	**-0.18**	0.07	-0.14	-0.02	-0.04	0.05	0.00	**0.93**	**-0.16**
DIP	**-0.63**	-0.11	-0.01	**0.35**	**0.24**	0.04	-0.03	-0.09	-0.02	0.04	**-0.20**	-0.05	0.02	0.06	**-0.24**	-0.06	-0.03	-0.02	0.04	-0.04	0.10
THY2	**-0.32**	**0.20**	**-0.24**	-0.11	0.08	**0.21**	-0.01	-0.11	0.03	0.13	-0.12	0.01	0.15	-0.11	**0.28**	**-0.46**	**0.24**	**-0.54**	0.01	0.00	-0.07
TRI	-0.02	-0.12	-0.11	-0.04	-0.10	-0.06	**0.20**	**0.21**	0.09	**0.22**	-0.16	0.09	-0.02	-0.14	-0.02	**-0.33**	**0.68**	**0.40**	-0.02	0.00	0.02
LEP	**-0.39**	-0.06	**0.16**	-0.02	0.00	-0.04	**-0.21**	**-0.36**	**-0.22**	-0.11	-0.13	**0.63**	0.06	-0.05	-0.01	-0.03	-0.02	0.06	0.00	0.00	0.02
DER	0.09	-0.08	0.07	**-0.25**	0.06	0.09	-0.07	**0.24**	**-0.33**	**0.21**	0.05	0.05	**0.32**	**0.72**	0.03	-0.02	0.02	0.01	0.04	0.00	0.00
ORT	**-0.31**	0.01	-0.04	0.03	0.11	0.03	-0.10	-0.15	0.01	0.05	**-0.17**	-0.13	0.04	0.01	0.05	**0.74**	**0.46**	**-0.18**	0.00	0.03	0.04
EMB	-0.12	0.05	0.08	**-0.21**	0.04	0.14	-0.06	**-0.27**	-0.02	**0.22**	-0.06	0.14	**-0.38**	0.07	-0.04	**0.30**	0.14	0.07	-0.01	**-0.22**	**-0.67**
COL2	**-0.62**	**0.17**	-0.02	0.00	**-0.19**	-0.10	0.09	-0.01	0.12	0.02	0.09	-0.12	0.07	0.06	0.07	0.02	-0.09	0.06	0.02	-0.06	0.04
PSE	**-0.31**	-0.01	-0.02	-0.04	-0.04	**0.20**	-0.15	**-0.35**	-0.05	**0.16**	**-0.30**	0.16	**-0.57**	**0.23**	**0.29**	-0.08	-0.03	0.00	0.00	-0.06	0.07
OPI	0.03	-0.02	0.10	0.04	-0.10	0.13	-0.07	0.09	0.02	0.13	-0.03	-0.02	0.07	0.03	-0.02	0.01	-0.06	-0.03	**-0.96**	0.00	0.00
ACA	**-0.50**	**0.29**	-0.11	-0.12	0.03	**0.17**	0.02	-0.06	0.08	0.03	-0.04	0.02	**0.18**	-0.08	0.11	**-0.25**	0.06	**-0.38**	0.02	-0.03	-0.12
PUL	**-0.46**	-0.06	0.05	**0.22**	-0.02	0.03	-0.13	**-0.30**	-0.12	0.06	**-0.68**	**-0.20**	-0.02	0.08	0.04	-0.05	-0.03	-0.02	0.02	0.00	0.14
POL	**-0.33**	-0.06	-0.06	-0.09	0.08	-0.04	0.09	0.02	0.16	-0.01	**-0.28**	-0.03	-0.15	0.15	**-0.78**	-0.10	-0.03	-0.08	0.01	-0.07	0.04

Table A.9. P-value associated to the two-tailed t test of the Pearson correlation coefficients of the orders abundances against PC scores. P-values lower than 0.05 are marked as red. HEM – Hemiptera; ARA – Aranea; NEU – Neuroptera; HYM – Hymenoptera; COL1 – Coleoptera; THY1 – Thysanoptera; PSO – Psocoptera; MAN – Mantodea; DIP – Diptera; THY2 – Thysanura; TRI – Trichoptera; LEP – Lepidoptera; DER – Dermaptera; ORT – Orthroptera; EMB – Embioptera; COL2 – Collembola; PSEU – Pseudoscorpiones; OPI – Opiliones; ACA – Acari; PUL -Pulmonata; POL – Polyxenida.

Order	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13	PC14	PC15	PC16	PC17	PC18	PC19	PC20	PC21
HEM	0.000	0.021	0.000	0.006	0.072	0.065	0.051	0.814	0.986	0.614	0.135	0.844	0.093	0.930	0.357	0.940	0.551	0.270	0.805	0.725	0.879
ARA	0.007	0.000	0.000	0.133	0.000	0.145	0.000	0.110	0.105	0.868	0.723	0.826	0.690	0.790	0.275	0.291	0.337	0.439	0.892	0.898	0.722
NEU	0.188	0.062	0.768	0.026	0.629	0.076	0.424	0.007	0.542	0.000	0.266	0.024	0.383	0.743	0.830	0.870	0.590	0.413	0.566	0.849	0.767
HYM	0.000	0.012	0.415	0.745	0.017	0.983	0.166	0.013	0.138	0.576	0.267	0.740	0.838	0.994	0.003	0.295	0.896	0.258	0.633	0.517	0.221
COL1	0.000	0.000	0.027	0.904	0.009	0.006	0.000	0.006	0.052	0.862	0.623	0.208	0.516	0.407	0.622	0.466	0.928	0.829	0.742	0.731	0.282
THY1	0.000	0.929	0.014	0.000	0.000	0.086	0.877	0.177	0.787	0.750	0.829	0.047	0.701	0.277	0.223	0.673	0.303	0.790	0.750	0.881	0.083
PSO	0.002	0.290	0.093	0.000	0.695	0.055	0.000	0.002	0.000	0.112	0.006	0.102	0.959	0.512	0.257	0.963	0.655	0.968	0.899	0.455	0.541
MAN	0.483	0.480	0.192	0.210	0.668	0.499	0.403	0.988	0.320	0.856	0.799	0.687	0.029	0.395	0.090	0.783	0.598	0.536	0.964	0.000	0.048
DIP	0.000	0.178	0.923	0.000	0.002	0.586	0.702	0.253	0.810	0.654	0.011	0.554	0.757	0.452	0.002	0.465	0.753	0.798	0.649	0.663	0.228
THY2	0.000	0.012	0.003	0.169	0.339	0.009	0.920	0.190	0.672	0.105	0.137	0.929	0.066	0.166	0.000	0.000	0.002	0.000	0.947	0.973	0.360
TRI	0.763	0.121	0.155	0.619	0.219	0.457	0.012	0.010	0.283	0.006	0.052	0.249	0.828	0.082	0.764	0.000	0.000	0.000	0.811	0.970	0.764
LEP	0.000	0.473	0.042	0.847	0.955	0.623	0.007	0.000	0.005	0.164	0.100	0.000	0.453	0.520	0.855	0.722	0.805	0.483	0.991	0.969	0.849
DER	0.282	0.309	0.362	0.002	0.482	0.291	0.389	0.003	0.000	0.008	0.528	0.551	0.000	0.000	0.679	0.776	0.784	0.907	0.654	0.969	0.961
ORT	0.000	0.874	0.634	0.676	0.179	0.734	0.220	0.067	0.887	0.519	0.034	0.111	0.645	0.884	0.516	0.000	0.000	0.028	0.959	0.756	0.613
EMB	0.121	0.546	0.329	0.008	0.657	0.074	0.469	0.001	0.792	0.007	0.431	0.074	0.000	0.418	0.656	0.000	0.091	0.405	0.924	0.007	0.000
COL2	0.000	0.030	0.788	0.989	0.017	0.231	0.257	0.947	0.147	0.848	0.289	0.133	0.408	0.463	0.397	0.766	0.278	0.467	0.825	0.469	0.662
PSE	0.000	0.855	0.768	0.580	0.634	0.011	0.060	0.000	0.513	0.046	0.000	0.051	0.000	0.004	0.000	0.316	0.730	0.973	0.981	0.467	0.392
OPI	0.717	0.762	0.198	0.660	0.202	0.108	0.361	0.251	0.786	0.118	0.711	0.792	0.413	0.689	0.807	0.940	0.449	0.680	0.000	0.977	0.992
ACA	0.000	0.000	0.174	0.136	0.667	0.030	0.812	0.478	0.349	0.682	0.598	0.817	0.028	0.316	0.178	0.001	0.455	0.000	0.810	0.686	0.132
PUL	0.000	0.489	0.512	0.007	0.789	0.693	0.103	0.000	0.127	0.464	0.000	0.013	0.796	0.295	0.623	0.522	0.683	0.836	0.790	0.974	0.072
POL	0.000	0.470	0.487	0.281	0.316	0.590	0.289	0.848	0.052	0.940	0.000	0.726	0.055	0.069	0.000	0.200	0.696	0.313	0.895	0.357	0.608

Appendix 5 – Distribution maps of parasitoid wasps

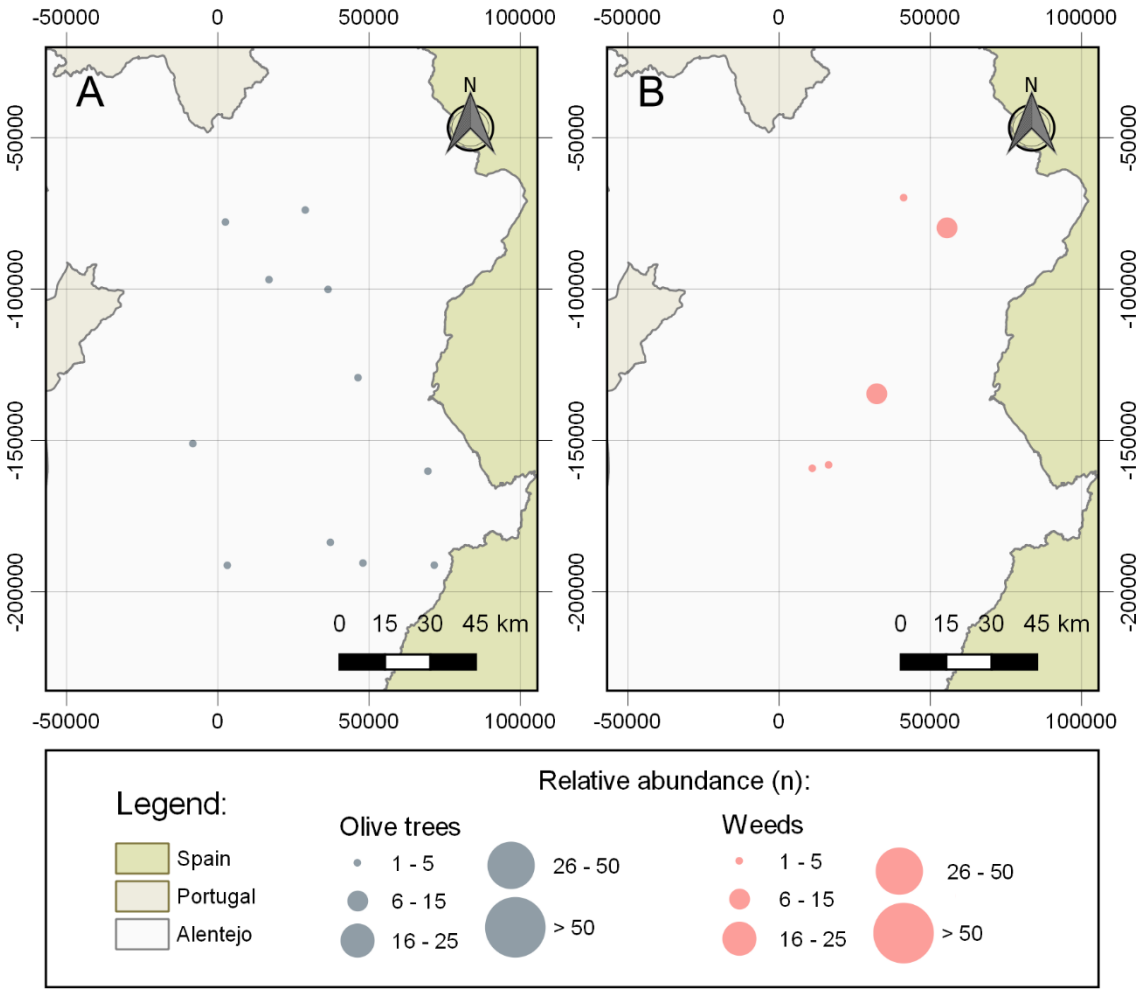

Figure A.3. Distribution of parasitoid wasps' abundance in the sampling sites with sorted samples. **A** – Platygastroidea in olive tree samples. **B** – Platygastroidea in weeds samples. This map is projected in ETRS89/PT-TM06. **Author's original.**

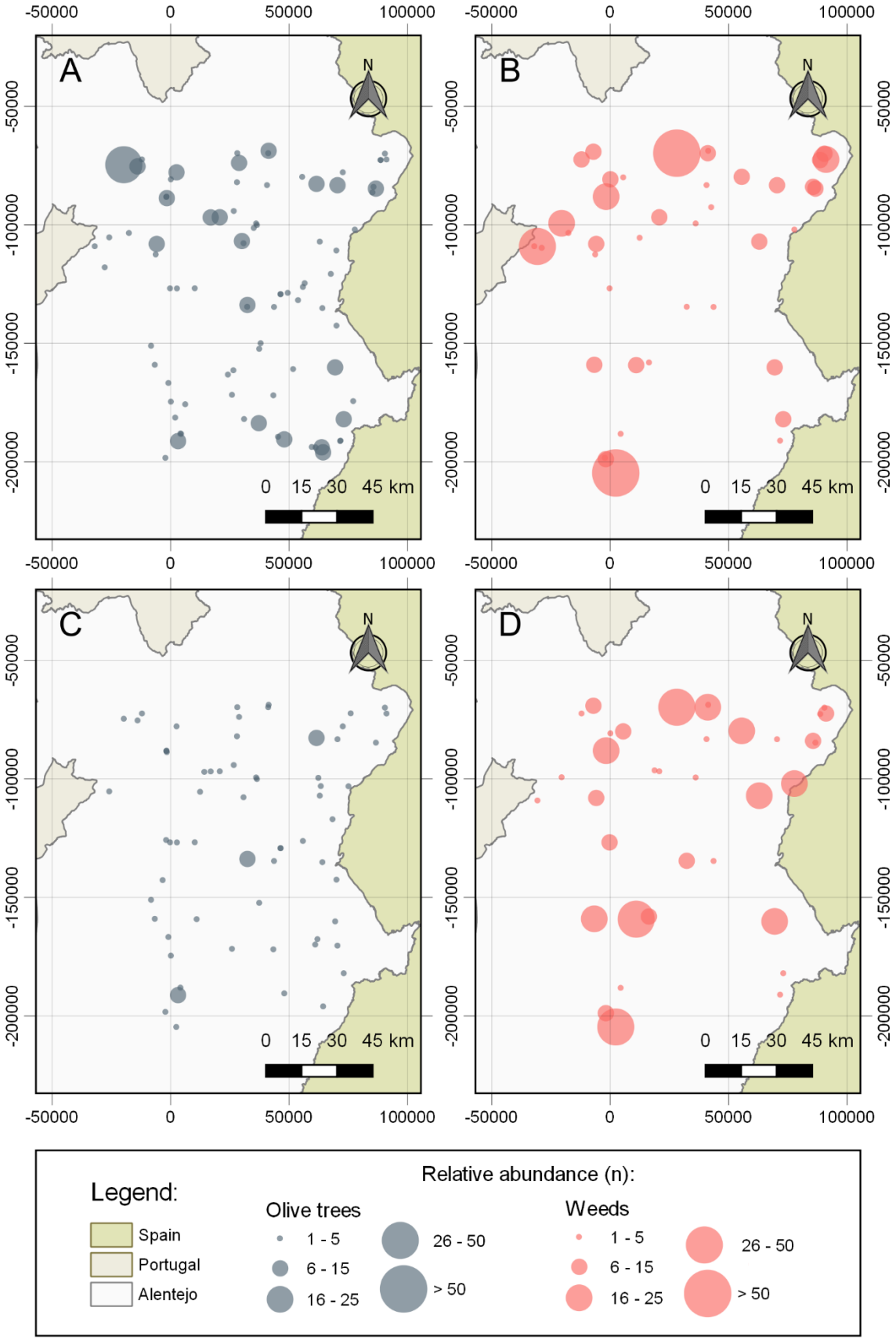

Figure A.4. Distribution of parasitoid wasps' abundance in the sampling sites with sorted samples. **A** – Chalcidoidea in olive tree samples. **B** – Chalcidoidea in weeds samples. **C** – Ichneumonoidea in olive tree samples. **D** – Ichneumonoidea in weeds samples. This map is projected in ETRS89/PT-TM06. **Author's original.**

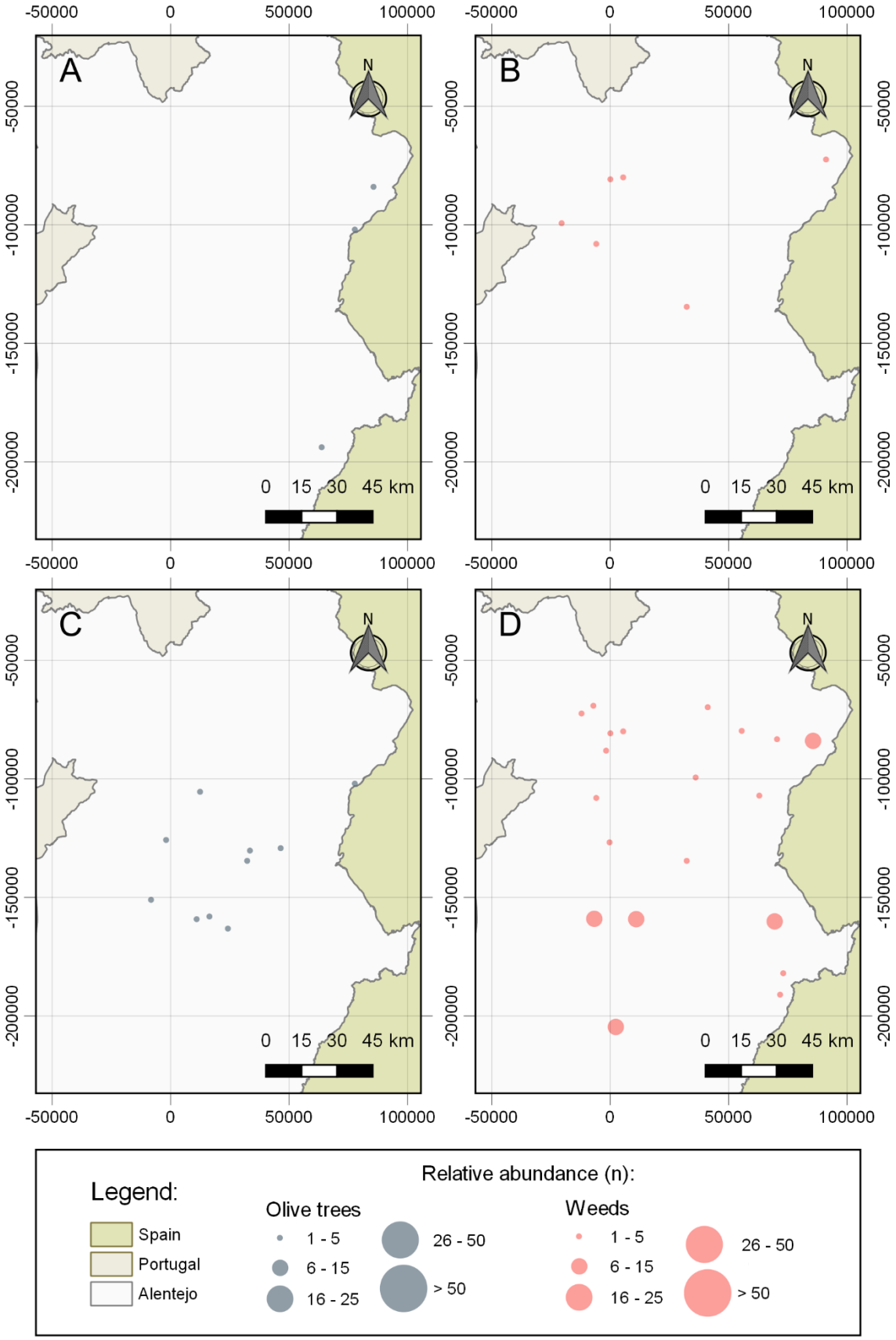

Figure A.5. Distribution of parasitoid wasps' abundance in the sampling sites with sorted samples. **A** – Chrysidoidea in olive tree samples. **B** – Chrysidoidea in weeds samples. **C** – Cynipoidea in olive tree samples. **D** – Cynipoidea in weeds samples. This map is projected in ETRS89/PT-TM06. **Author's original.**

Appendix 6 – Distribution maps of predators

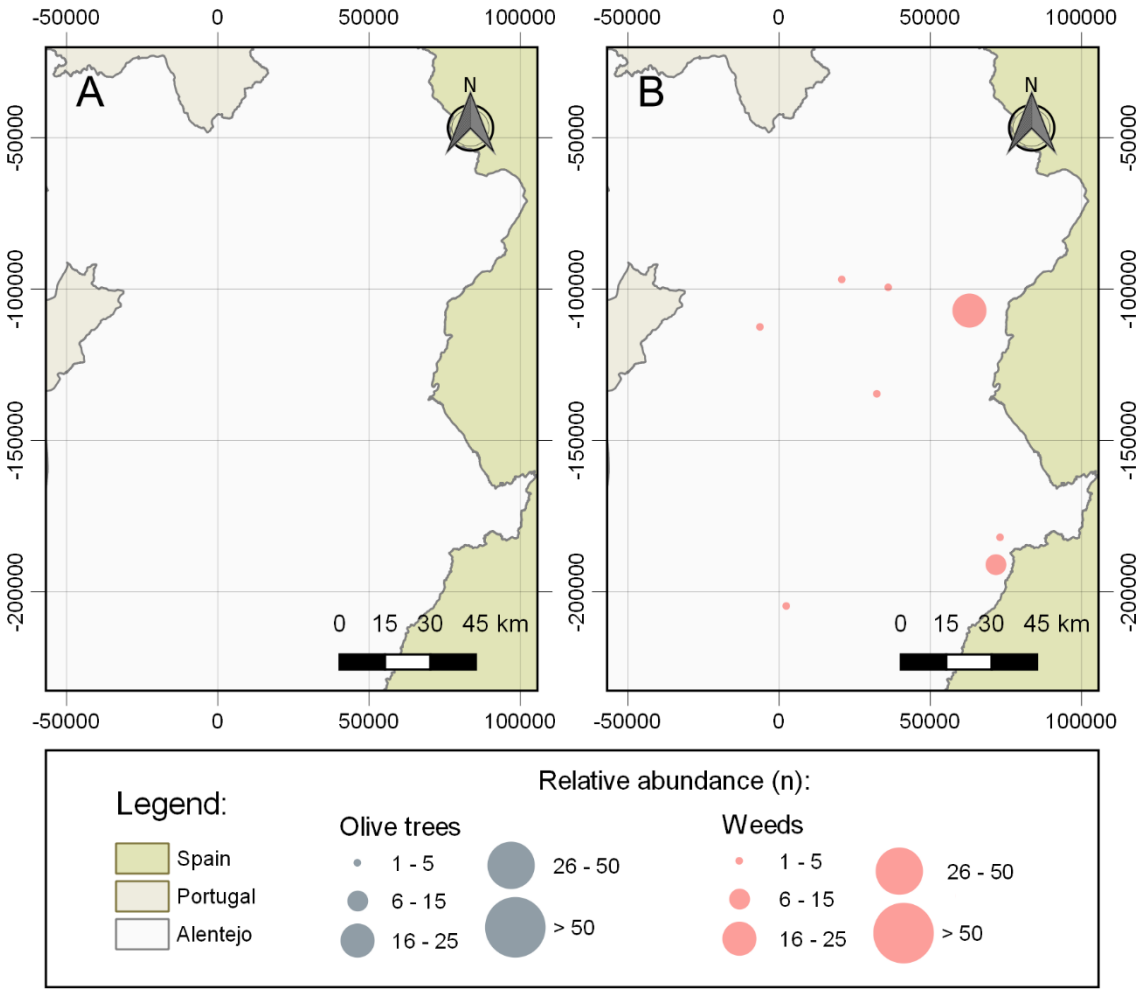

Figure A.6. Distribution of predators' abundance in the sampling sites with sorted samples. **A** – Pseudoscorpiones in olive tree samples. **B** – Pseudoscorpiones in weeds samples. This map is projected in ETRS89/PT-TM06. **Author's original.**

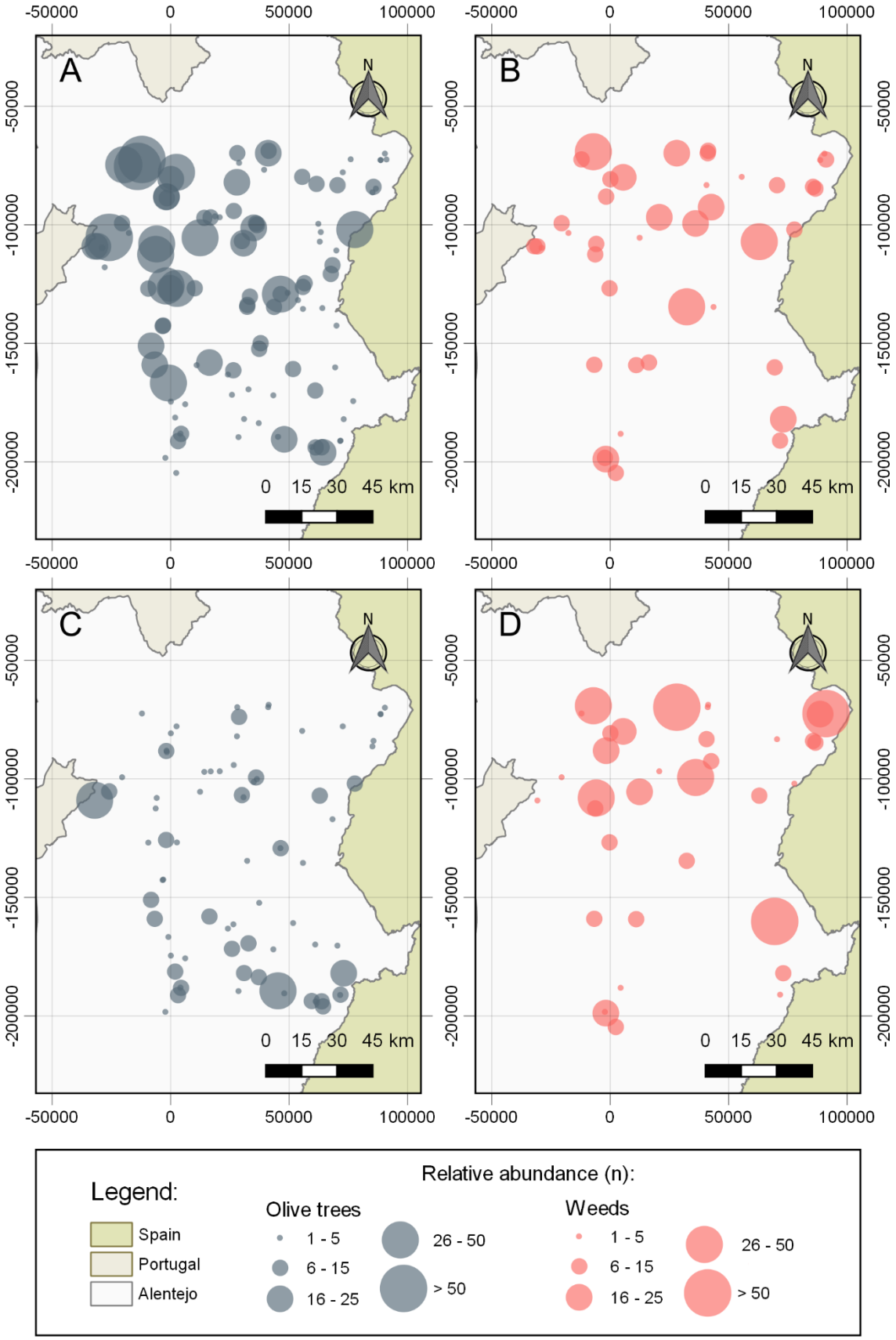

Figure A.7. Distribution of predators' abundance in the sampling sites with sorted samples. **A** – Aranea in olive tree samples. **B** – Aranea in weeds samples. **C** – Formicidae in olive tree samples. **D** – Formicidae in weeds samples. This map is projected in ETRS89/PT-TM06. **Author's original.**

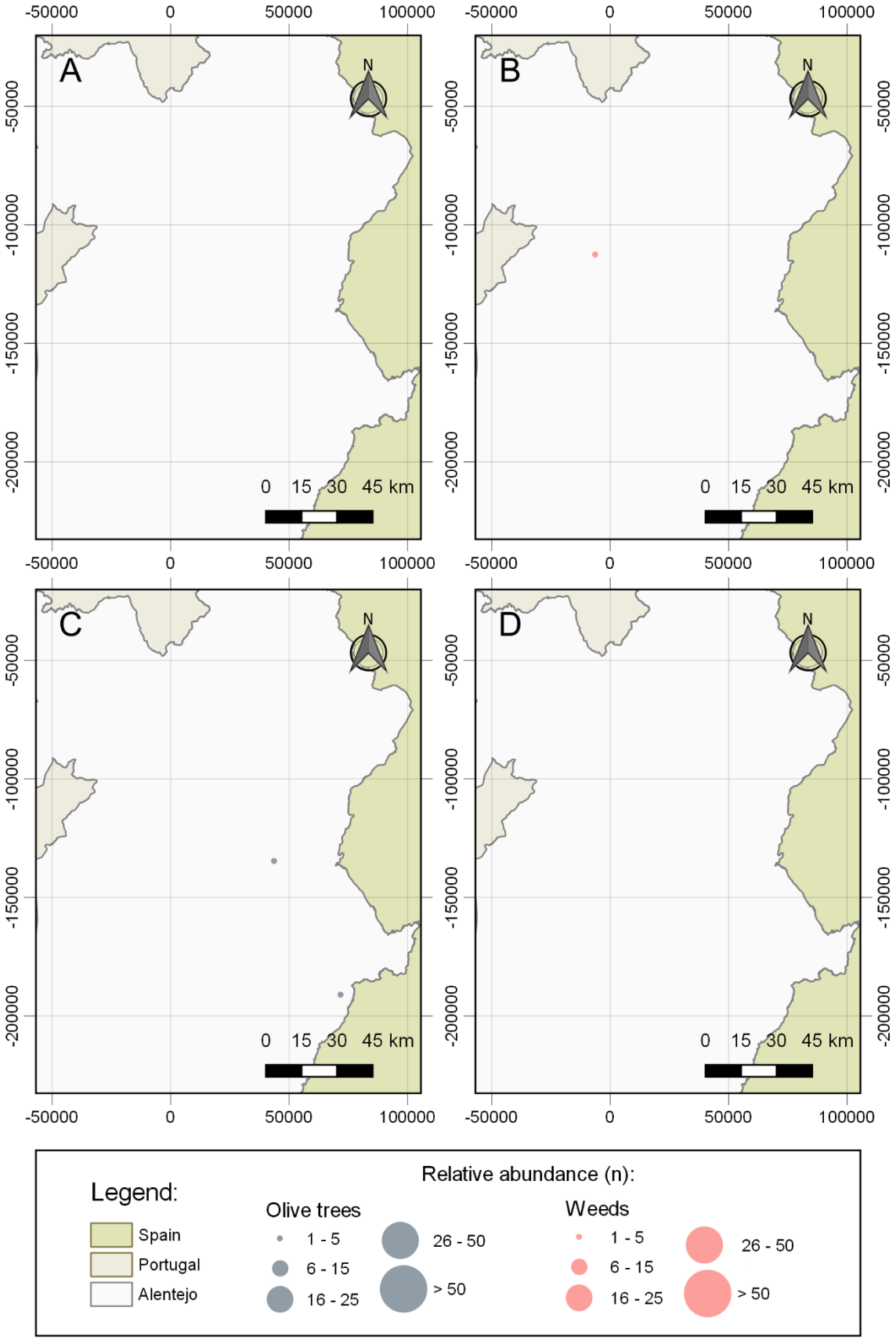

Figure A.8. Distribution of predators' abundance in the sampling sites with sorted samples. **A** – Mantodea in olive tree samples. **B** – Mantodea in weeds samples. **C** – Opiliones in olive tree samples. **D** – Opiliones in weeds samples. This map is projected in ETRS89/PT-TM06. **Author's original.**

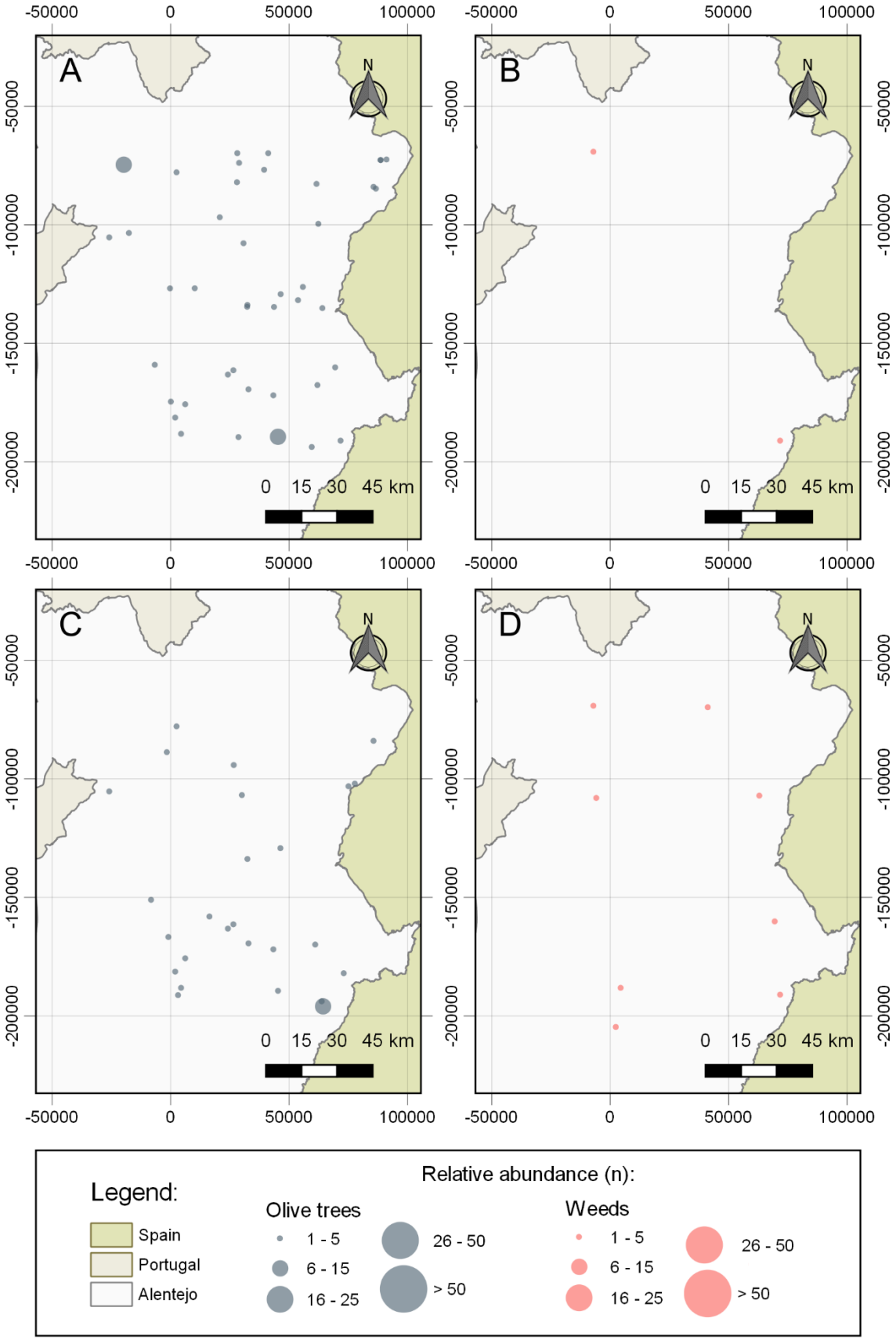

Figure A.9. Distribution of predators' abundance in the sampling sites with sorted samples. **A** – Neuroptera in olive tree samples. **B** – Neuroptera in weeds samples. **C** – Coccinellidae in olive tree samples. **D** – Coccinellidae in weeds samples. This map is projected in ETRS89/PT-TM06. **Author's original.**

Appendix 7 – Auchenorrhyncha species table

Table A.10. Number of Auchenorrhyncha adults by species according to gender, sampling site and host. [a] The number of females and males found at each site are associated to ♂ and ♀ symbols, respectively. When gender could not be determined, usually due to partial destructed individuals lacking the terminal part of the abdomen, letter "A" was used to symbolize adult. [b] Parasitized individuals are marked with and asterisk (*).

Auchenorrhyncha species	Number/ gender[a,b]	Coordinates	Date	Host plant
Aphrophoridae				
Philaenus sp.	1♀	39º02'20"N; 08º20'35"W	04/11/2016	Weeds
	1♀	38º39'19"N; 09º47'41"W	25/10/2016	Weeds
	1♀	38º50'02"N; 08º21'31"W	27/10/2016	Weeds
	1♀	38º27'46"N; 08º14'19"W	10/11/2016	Olive
	1♀	38º27'20"N; 08º14'15"W	10/11/2016	Weeds
Neophilaenus campestris (Fallén, 1805)	1♀	39º02'44"N; 09º47'08"W	28/10/2016	Weeds
	1♂	39º02'20"N; 08º20'35"W	04/11/2016	Weeds
	1♀	38º46'25"N; 09º37'53"W	25/10/2016	Olive
	1♂	38º48'25"N; 09º37'53"W	25/10/2016	Olive
	1♂	38º47'46"N; 08º06'21"W	25/10/2016	Olive
	1♂*; 1♀	38º39'19"N; 09º47'41"W	25/10/2016	Weeds
	1♀	38º40'03"N; 08º40'16"W	07/11/2016	Olive
	1♀	38º27'17"N; 08º22'02"W	10/11/2016	Weeds
	1♀	38º27'17"N; 08º22'02"W	10/11/2016	Weeds
	1♂; 2♀♀	38º27'20"N; 08º14'15"W	10/11/2016	Weeds
	1♀	38º31'48"N; 08º30'27"W	07/11/2016	Olive
	1♀	38º14'40"N; 08º03'16"W	11/11/2016	Weeds
	3♂♂	38º14'04"N; 08º03'16"W	11/11/2016	Weeds
	1♂; 1♀	37º58'25"N; 08º55'03"W	31/10/2016	Weeds
Cicadellidae: Agallinae				
Agallia consobrina Curtis 1833	1♀	38º56'53"N; 09º55'51"W	28/10/2016	Weeds
Anaceratagallia laevis (Ribaut 1935)	1♀	39º00'58"N; 09º43'40"W	28/10/2016	Weeds
	1♂; 1♀	38º56'27"N; 09º52'06"W	03/11/2016	Weeds
	1♂; 2♀♀	39º02'22"N; 08º11'32"W	03/11/2016	Weeds
	1♀	39º00'40"N; 08º55'10"W	03/11/2016	Weeds
	1♂; 1♀	38º41'43"N; 09º47'59"W	25/10/2016	Weeds
Austroagallia sinuata (Mulsant & Rey, 1855)	1♀	38º54'04"N; 08º51'58"W	03/11/2016	Weeds
	2♀♀	38º14'09"N; 09º47'27"W	11/11/2016	Weeds
Agallinae 1	1♀	38º45'19"N; 08º16'15"W	27/10/2016	Olive
Cicadellidae: Deltocephalinae				
Euscelidius variegatus (Kirshbaum, 1868)	1♂	38º56'27"N; 09º52'06"W	03/11/2016	Weeds
	1♂	38º14'09"N; 09º47'27"W	11/11/2016	Weeds

Auchenorrhyncha species	Number/ gender[a,b]	Coordinates	Date	Host plant
Cicadellidae: Deltocephalinae (continuation)				
Euscelis lineolatus Brullé, 1832	2♀♀	38º14'04"N; 08º03'16"W	11/11/2016	Weeds
Goniagnathus brevis (Herrich-Schäffer, 1835)	1♀	38º27'17"N; 08º22'02"W	10/11/2016	Weeds
Exitianus capicola (Stål, 1855)	2♀♀	38º56'27"N; 09º52'06"W	03/11/2016	Weeds
	1♀	39º02'20"N; 08º20'35"W	04/11/2016	Weeds
	1♀	38º56'54"N; 08º30'29"W	04/11/2016	Weeds
	4♂♂; 3♀♀	38º27'20"N; 08º14'15"W	10/11/2016	Weeds
Orosius albicinctus Distant, 1918	1♂	38º50'02"N; 08º21'31"W	27/10/2016	Weeds
Psammotettix sp.	1♂	38º56'27"N; 09º52'06"W	03/11/2016	Weeds
	1♂; 1♀	39º00'40"N; 08º55'10"W	03/11/2016	Weeds
Deltocephalinae 1	1♀	38º31'35"N; 08º51'52"W	10/11/2016	Weeds
Deltocephalinae 2	1♀	38º27'17"N; 08º22'02"W	10/11/2016	Weeds
Deltocephalinae 3	1♀	39º00'58"N; 09º43'40"W	28/10/2016	Weeds
Deltocephalinae 4	1♂	38º56'27"N; 09º52'06"W	03/11/2016	Weeds
Deltocephalinae 5	1♀	38º01'34"N; 08º41'58"W	26/10/2016	Weeds
Deltocephalinae 6	2♀♀	38º42'06"N; 08º35'28"W	07/11/2016	Weeds
Deltocephalinae 7	1♂	38º56'27"N; 09º52'06"W	03/11/2016	Weeds
	1♂	38º56'54"N; 08º30'29"W	04/11/2016	Weeds
Deltocephalinae 8	1♀	37º52'38"N; 09º50'48"W	31/10/2016	Weeds
Deltocephalinae 9	1♀	37º56'41"N; 08º40'60"W	26/10/2016	Weeds
Cicadellidae: Idiocerinae				
Idiocerinae 1	1♀	37º57'38"N; 08º11'36"W	30/10/2016	Olive
Cicadellidae: Typhlocybinae				
Arboridia parvula (Boheman, 1845)	1♀	38º56'53"N; 09º55'51"W	28/10/2016	Weeds
	1♂	38º27'20"N; 08º14'15"W	10/11/2016	Weeds
Empoasca sp.	1♂	38º22'59"N; 09º49'43"W	11/11/2016	Olive
Frutioidia bisignata (Mulsant & Rey, 1855)	1♀	38º43'11"N; 09º34'09"W	25/10/2016	Olive
Zygina nivea (Mulsant & Rey, 1855)	1♂	38º40'47"N; 09º32'06"W	25/10/2016	Weeds
	1♂; 1♀	38º09'24"N; 08º34'27"W	15/11/2016	Olive
Zygina ordinaria (Ribaut, 1936)	1♀	38º57'51"N; 08º42'21"W	04/11/2016	Olive
	1♂; 3♀♀; 1A	38º54'04"N; 08º51'58"W	03/11/2016	Weeds
Zyginidia scutellaris (Herrich-Schäffer, 1838)	1♂	39º02'44"N; 09º47'08"W	28/10/2016	Weeds
	1♂	39º02'20"N; 08º20'35"W	04/11/2016	Weeds
	1♀	38º54'04"N; 08º51'58"W	03/11/2016	Weeds
	1♀	39º00'40"N; 08º55'10"W	03/11/2016	Weeds
	40♂♂; 39♀♀; 2A	39º00'34"N; 08º53'27"W	03/11/2016	Weeds
	1♀	38º46'20"N; 08º16'57"W	27/10/2016	Weeds
	1♂	38º42'06"N; 08º35'28"W	07/11/2016	Weeds

Auchenorrhyncha species	Number/ gender[a,b]	Coordinates	Date	Host plant
Cicadellidae: Typhlocybinae (continuation)				
Zyginidia scutellaris (Herrich-Schäffer, 1838) (continuation)	4♀♀	38º27'17"N; 08º22'02"W	10/11/2016	Weeds
	1♂	38º27'20"N; 08º14'15"W	10/11/2016	Weeds
	2♂♂; 7♀♀; 2A	38º14'04"N; 08º03'16"W	11/11/2016	Weeds
	1♀	38º13'24"N; 08º39'37"W	15/11/2016	Weeds
	1♂	37º58'25"N; 08º55'03"W	31/10/2016	Weeds
	1♂	37º52'38"N; 09º50'48"W	31/10/2016	Weeds
	1♀	37º57'38"N; 08º11'36"W	30/10/2016	Olive
Typhlocybinae 1	2♀♀	38º42'06"N; 08º35'28"W	07/11/2016	Weeds
	1♀	38º13'24"N; 08º39'37"W	15/11/2016	Weeds
Typhlocybinae 2	1♀	38º30'11"N; 08º23'59"W	10/11/2016	Olive
	2♀♀	38º14'04"N; 08º03'16"W	11/11/2016	Weeds
	3♀♀	38º13'24"N; 08º39'37"W	15/11/2016	Weeds
Typhlocybinae 3	1♀	39º02'01"N; 08º54'43"W	03/11/2016	Weeds
Typhlocybinae 4	1♀	38º40'47"N; 09º32'06"W	25/10/2016	Olive
	1♀	38º18'29"N; 09º46'22"W	11/11/2016	Olive
	1♀	38º08'31"N; 08º14'31"W	15/11/2016	Olive
Typhlocybinae 5	1♀	38º14'09"N; 09º47'27"W	11/11/2016	Weeds
Typhlocybinae 6	1♀	38º41'06"N; 09º30'52"W	25/10/2016	Weeds
Typhlocybinae 7	1♀	39º02'20"N; 08º20'35"W	04/11/2016	Weeds
Typhlocybinae 8	1♀	38º40'47"N; 09º32'06"W	25/10/2016	Weeds
Cixiidae				
Cixius nervosus (Linnaeus 1758)	1♂	38º44'11"N; 09º39'53"W	25/10/2016	Olive
Delphacidae				
Laodelphax striatella (Fallén, 1826)	1♂*	39º00'58"N; 09º43'40"W	28/10/2016	Weeds
	1♂	38º41'43"N; 09º47'59"W	25/10/2016	Weeds
Metadelphax propinqua (Fieber, 1866)	2♀♀; 1A	39º00'58"N; 09º43'40"W	28/10/2016	Weeds
	1♂	39º02'53"N; 08º20'43"W	04/11/2016	Weeds
	1♂; 1♀	39º02'20"N; 08º20'35"W	04/11/2016	Weeds
	1♂	39º00'40"N; 08º55'10"W	03/11/2016	Weeds
	1♂	39º00'34"N; 08º53'27"W	03/11/2016	Weeds
	1♂	38º43'06"N; 08º00'37"W	25/10/2016	Weeds
	4♂♂; 2♀♀; 1♂*	38º42'06"N; 08º35'28"W	07/11/2016	Weeds
	1♀	38º27'46"N; 08º14'19"W	10/11/2016	Olive
	6♂♂; 3♀♀	38º27'20"N; 08º14'15"W	10/11/2016	Weeds
	1♀	38º14'40"N; 08º03'16"W	11/11/2016	Weeds
	1♀	37º58'25"N; 08º55'03"W	31/10/2016	Weeds
	1♀	37º52'38"N; 09º50'48"W	31/10/2016	Weeds

Auchenorrhyncha species	Number/ gender[a,b]	Coordinates	Date	Host plant
Delphacidae (continuation)				
Metadelphax propinqua (Fieber, 1866) (continuation)	4♂♂; 2♀♀	37º49'29"N; 09º53'39"W	31/10/2016	Weeds
	1♀	38º01'34"N; 08º41'58"W	26/10/2016	Weeds
Issidae				
Fieberium impressum (Fieber, 1877)	2♂♂	38º55'04"N; 08º20'11"W	03/11/2016	Weeds
	1♀	38º43'11"N; 09º34'09"W	25/10/2016	Olive
Tingissus guadarramense (Melichar, 1906)	1♂	37º57'40"N; 08º22'29"W	26/10/2016	Olive
Issidae 1	1♂	38º46'20"N; 08º16'57"W	27/10/2016	Olive
Tettigometridae				
Tettigometra impressopunctata (Dufour, 1846)	1♂	38º43'06"N; 08º00'37"W	25/10/2016	Weeds
Tettigometra virescens (Panzer, 1799)	1♂	38º55'16"N; 08º34'38"W	04/11/2016	Olive

Appendix 8 – Auchenorrhyncha somatic and genital characters

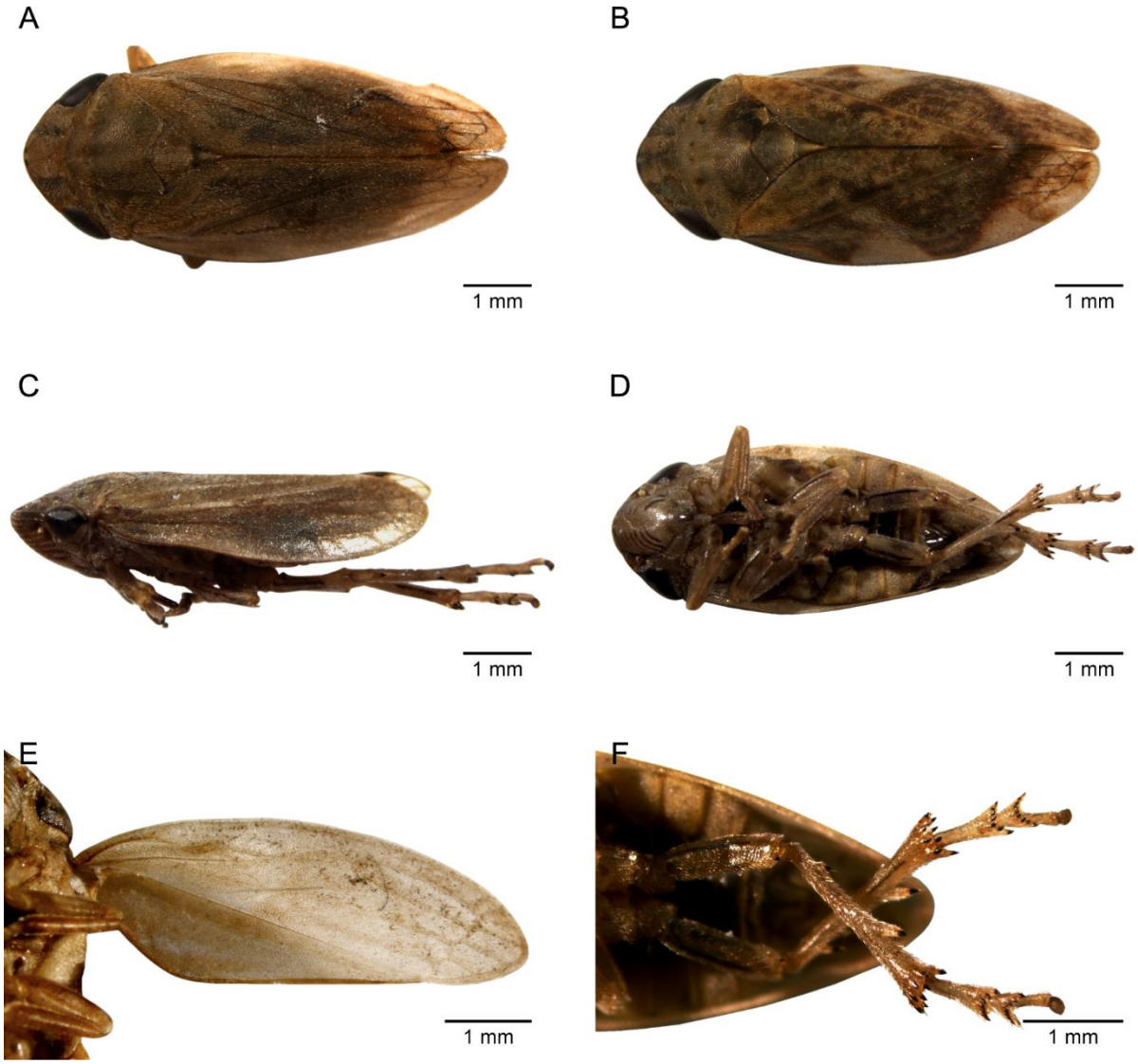

Figure A.10. *Philaenus* sp. habitus. **A** – Female in dorsal view (*populi* phenotype). **B** – Female in dorsal view (*typicus* phenotype). **C** – Female in lateral view. **D** – Female in lateral view. **E** – forewing. **F** – hindlegs. **Author's original.**

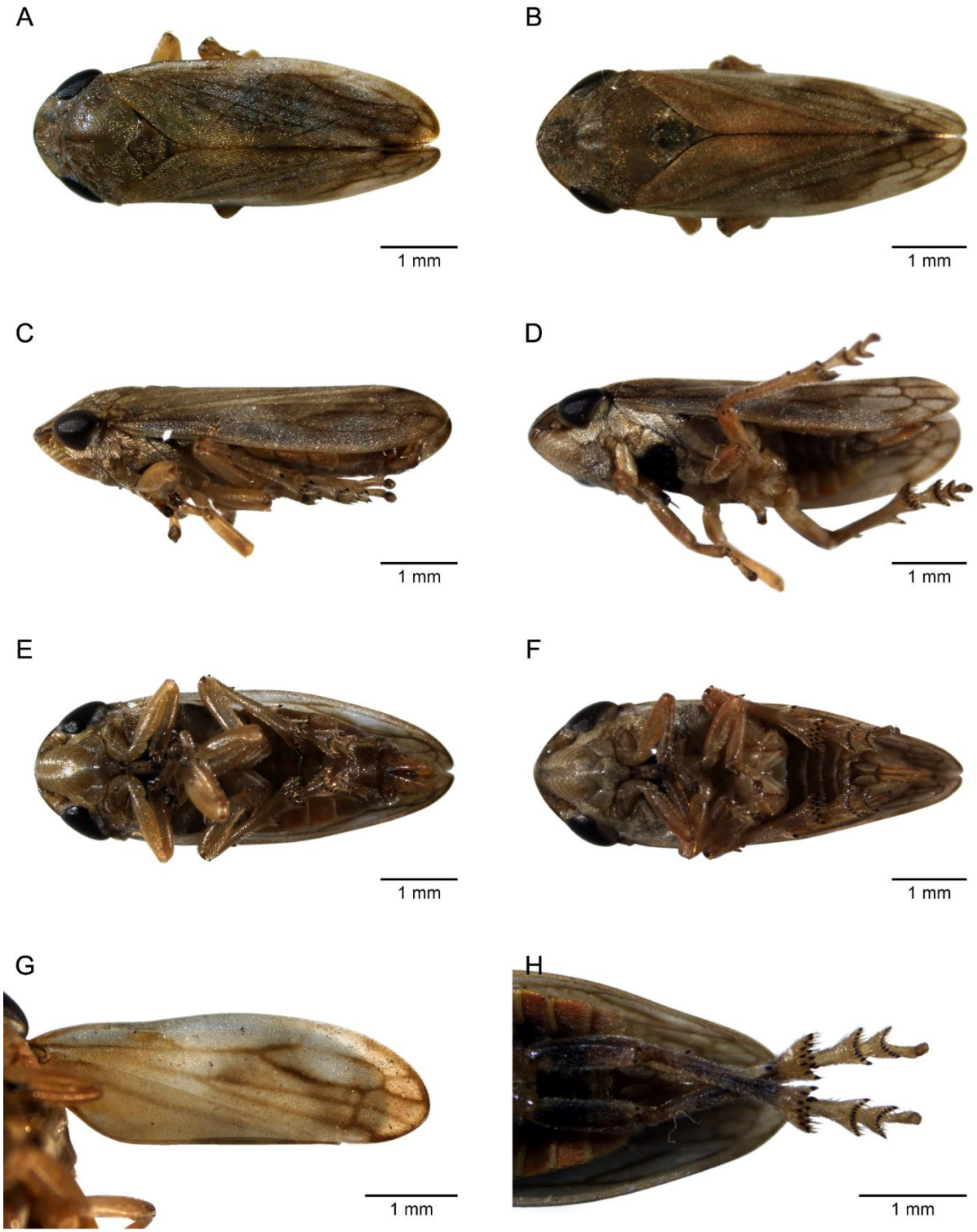

Figure A.11. *Neophilaenus campestris* (Fallén, 1805) habitus. **A** – Male in dorsal view. **B** – Female in dorsal view. **C** – Male in lateral view. **D** – Female in dorsal view. **E** – Male in lateral view. **F** – Female in lateral view. **G** – Forewing. **H** – Hindlegs. **Author's original.**

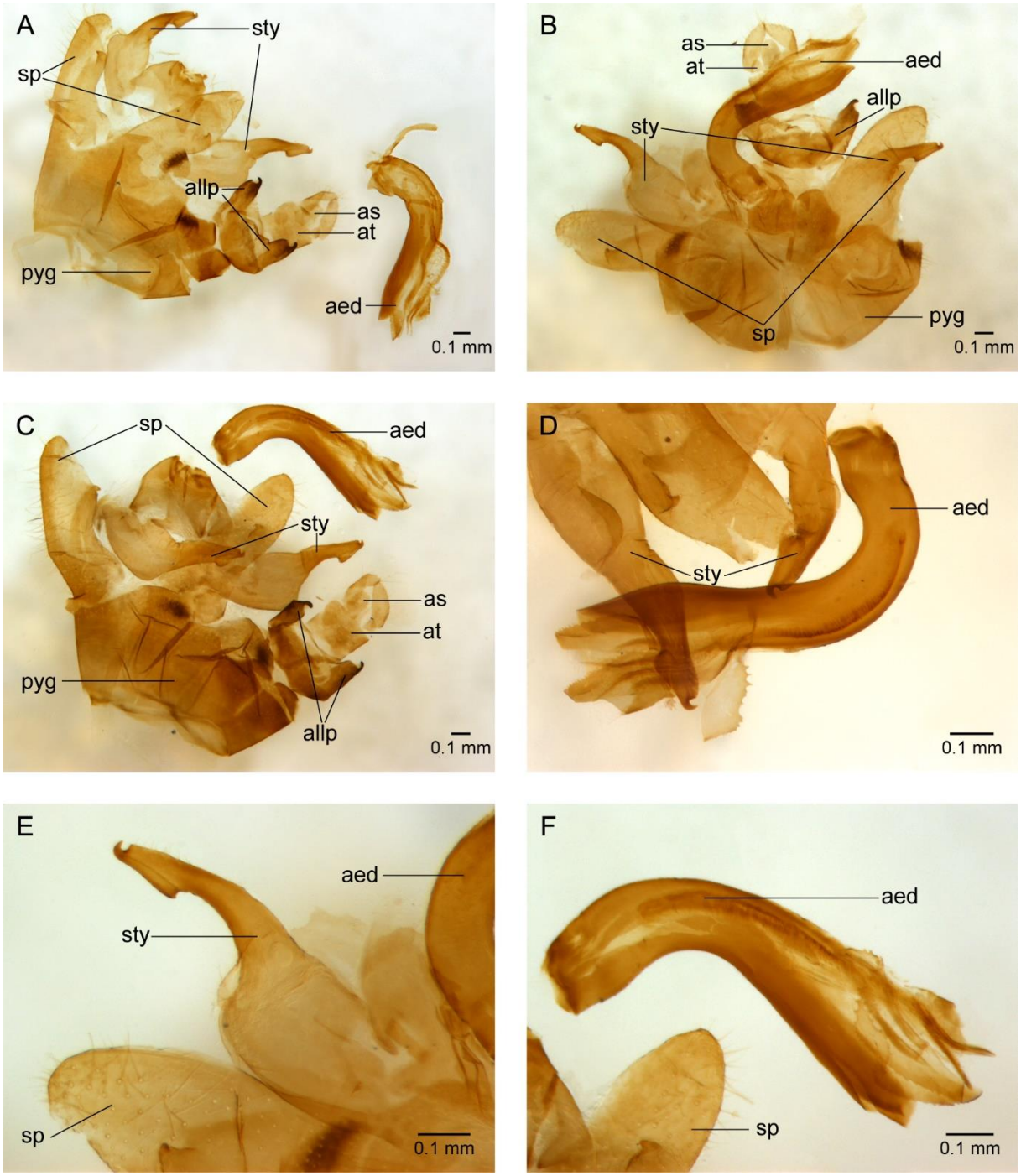

Figure A.12. *Neophilaenus campestris* (Fällen**,** 1805) genitalia. **A**-**F** – Male genital capsule from several specimens (aed = aedeagus, allp = appendage of lateral lobe of pygofer, as = anal style; at = anal tube; pyg = pygofer, sp = subgenital plate, sty = style). **Author's original.**

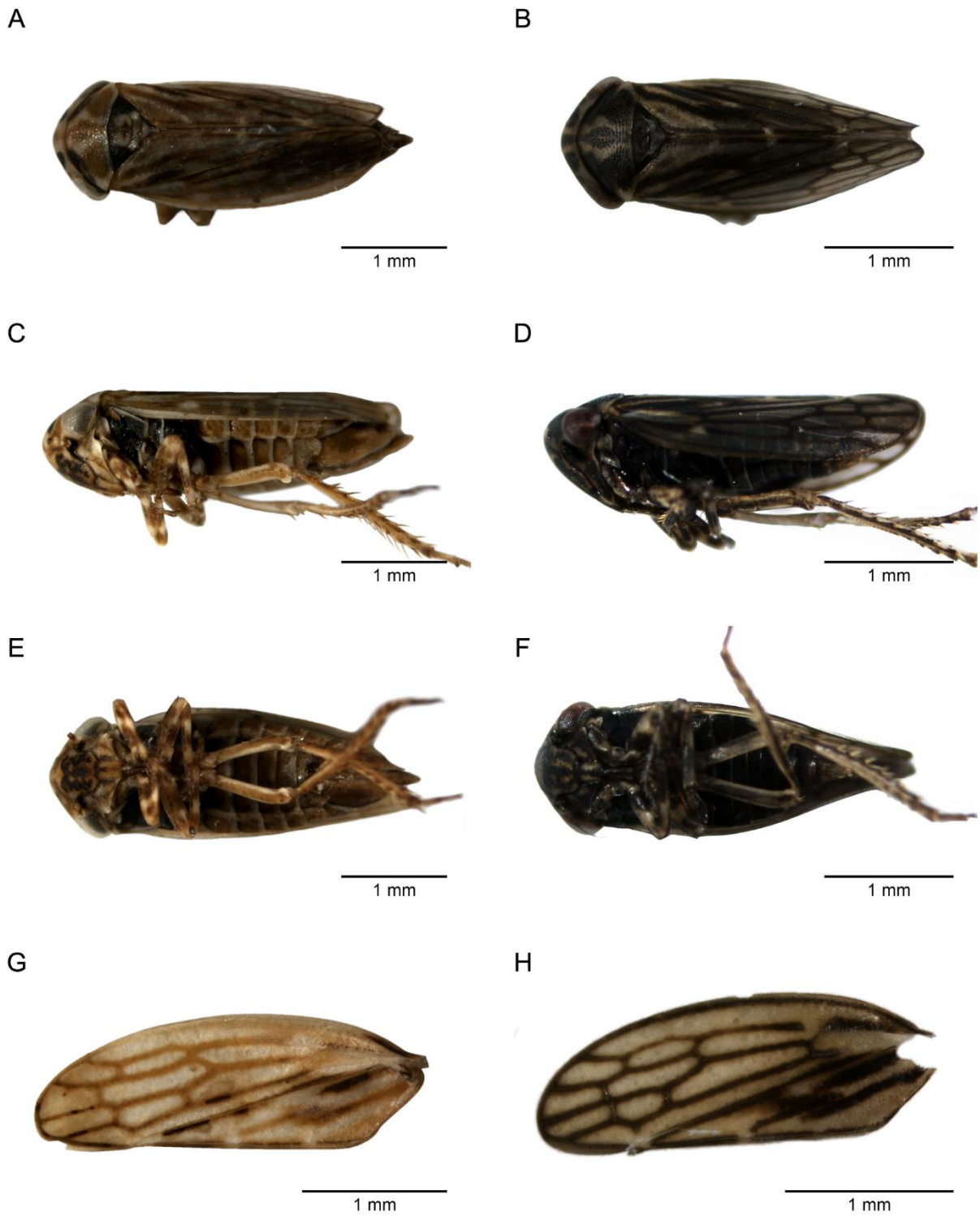

Figure A.13. *Anaceratagallia laevis* (Ribaut, 1935) habitus. **A** – Female in dorsal view. **B** – Male in dorsal view. **C** – Female in lateral view. **D** – Male in lateral view. **E** – Female in ventral view. **F** – Male in ventral view. **G** – Female forewing. **H** – Male forewing. **Author's original.**

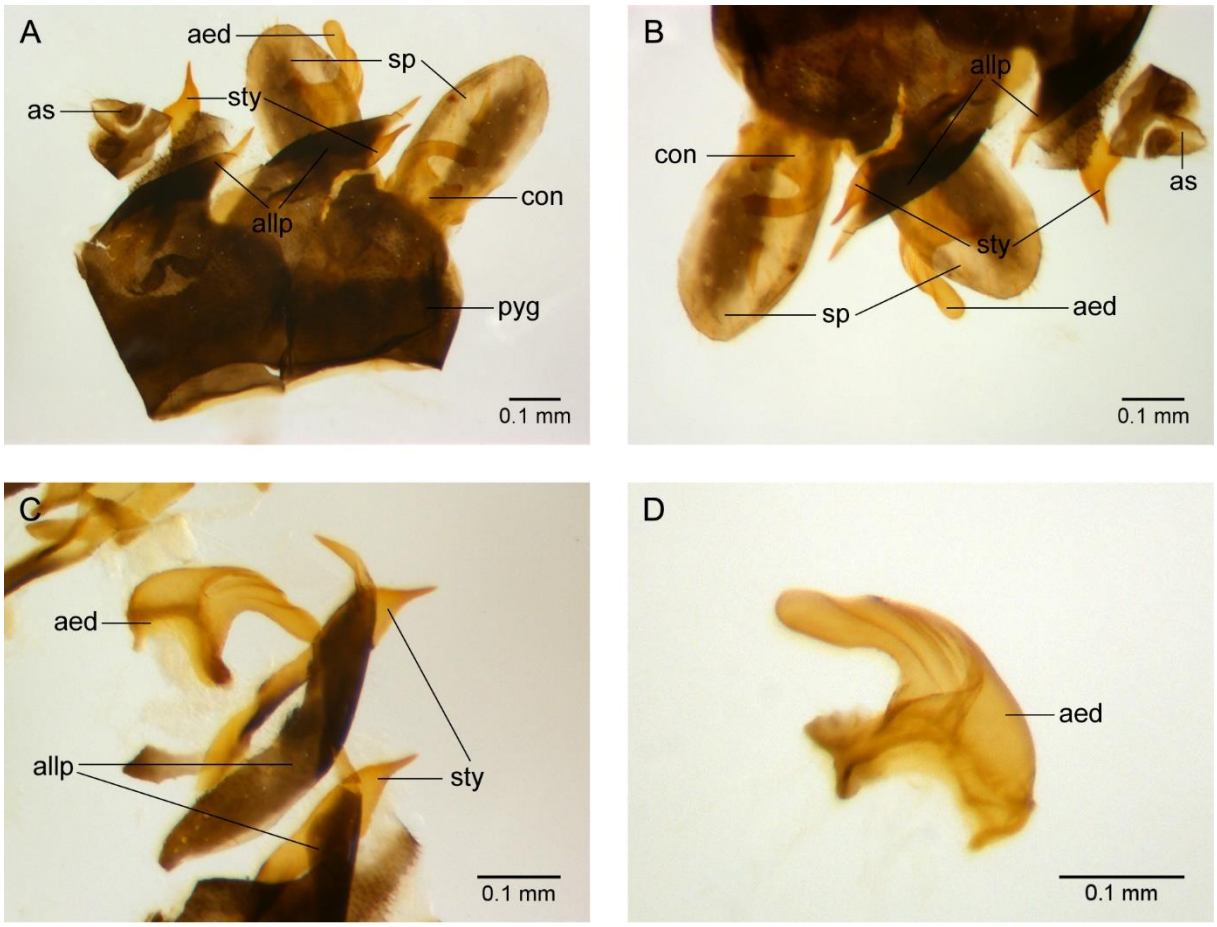

Figure A.14. *Anaceratagallia laevis* (Ribaut, 1935) genitalia. **A**-**D** – Male genital capsule from several specimens (aed = aedeagus, allp = appendage of lateral lobe of pygofer, as = anal style, con = connective; pyg = pygofer, sp = subgenital plate, sty = style). **Author's original.**

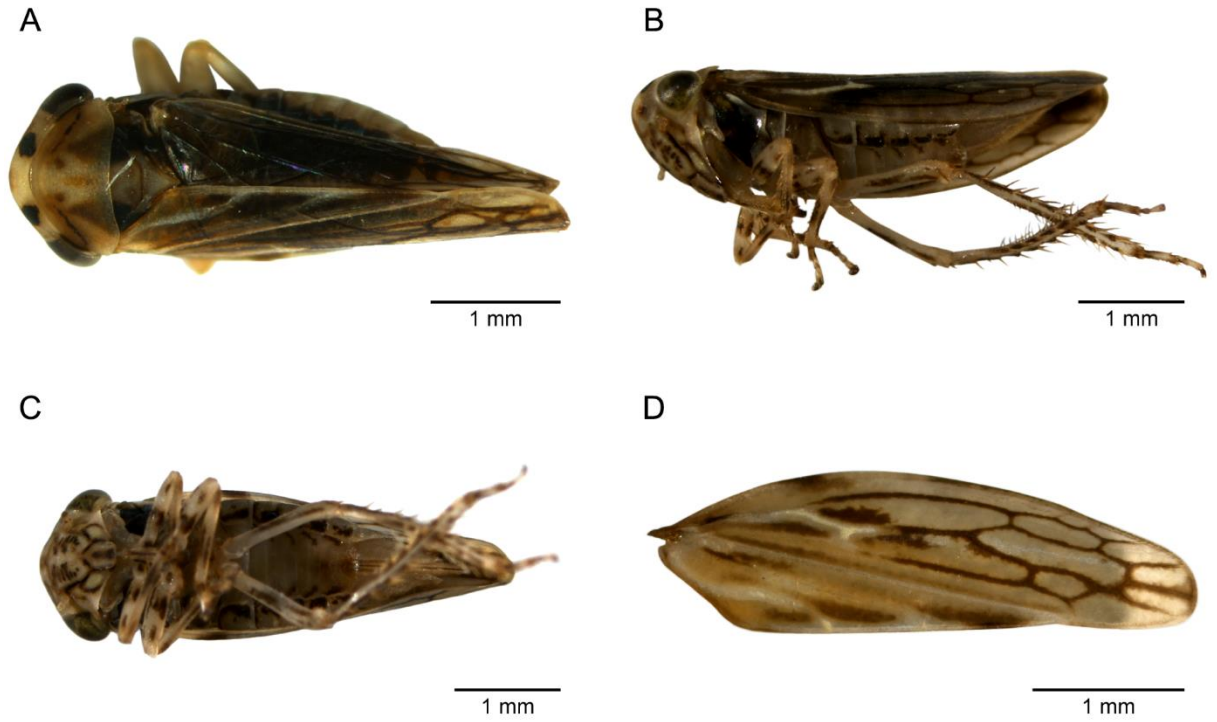

Figure A.15. *Agallia consobrina* Curtis, 1833 habitus. **A** – Female in dorsal view. **B** – Female in lateral view. **C** – Female in ventral view. **D** – Forewing. **Author's original.**

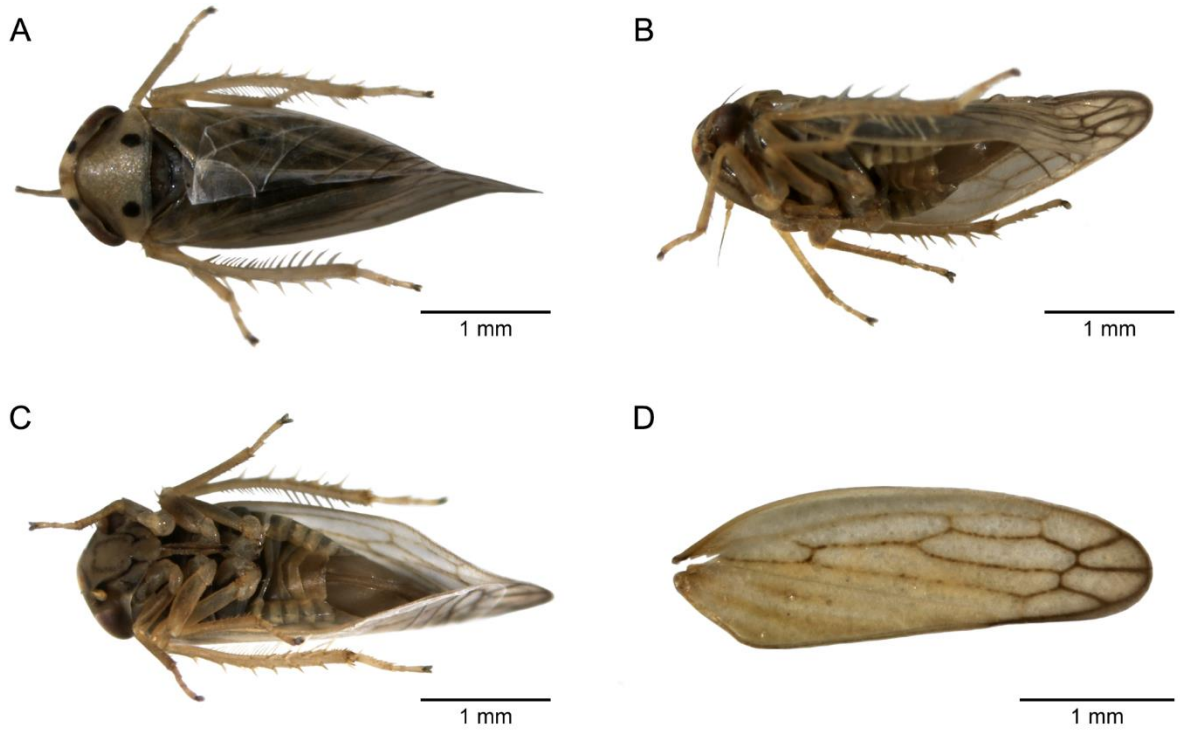

Figure A.16. *Austroagallia sinuata* (Mulsant & Rey, 1855) habitus. **A** – Female in dorsal view. **B** – Female in lateral view. **C** – Female in ventral view. **D** – Forewing. **Author's original.**

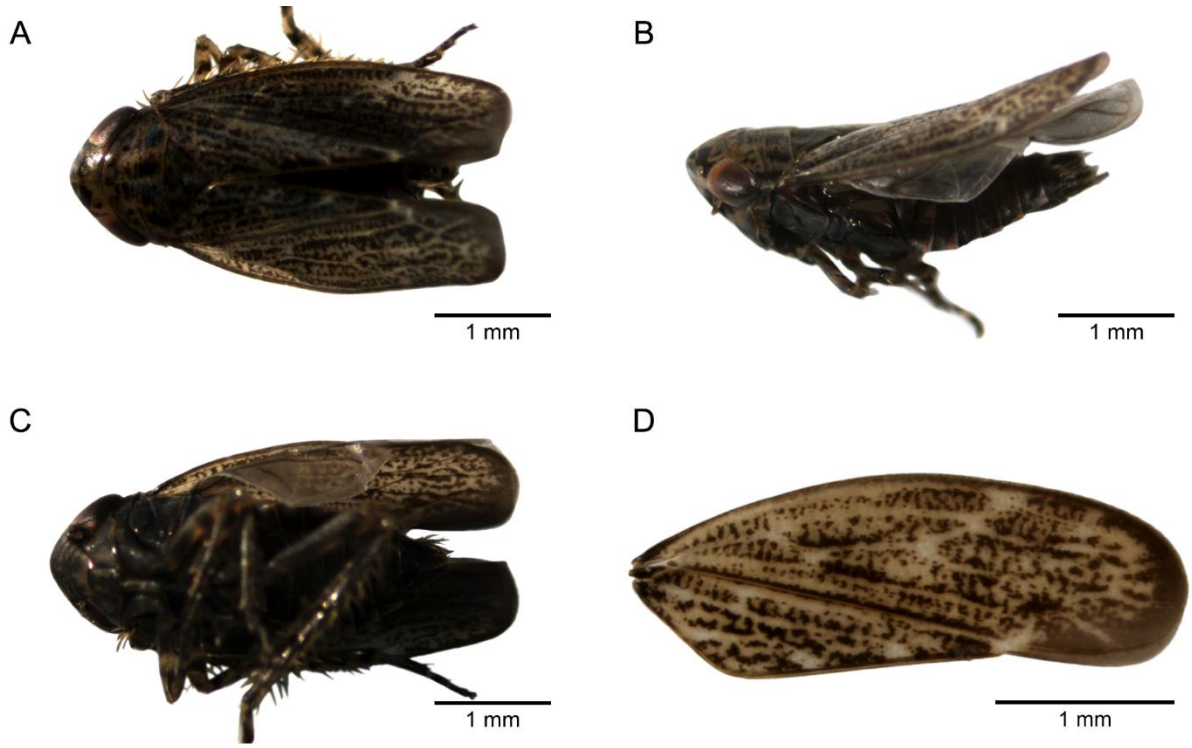

Figure A.17. *Euscelidius variegatus* (Kirschbaum, 1868) habitus. **A** – Male in dorsal view. **B** – Male in lateral view. **C** - Male in ventral view. **D** – Forewing. **Author's original.**

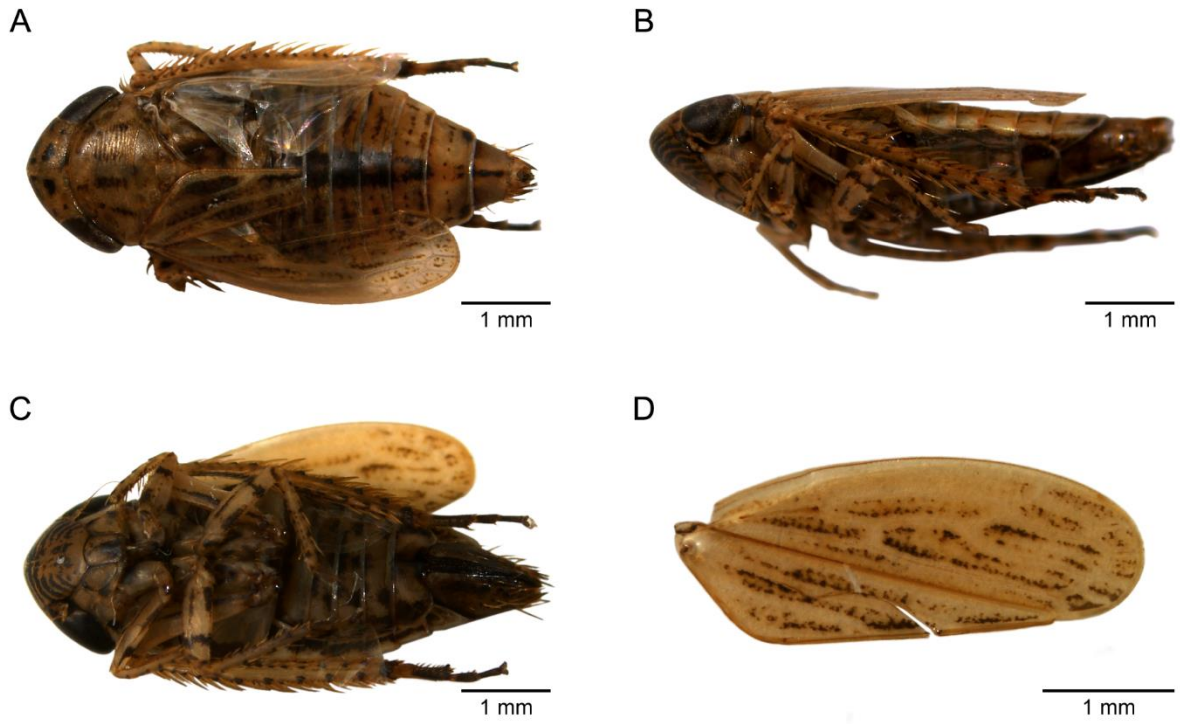

Figure A.18. *Euscelis lineolatus* Brullé, 1832 habitus. **A** – Female in dorsal view. **B** – Female in lateral view. **C** – Female in ventral view. **D** – Forewing. **Author's original.**

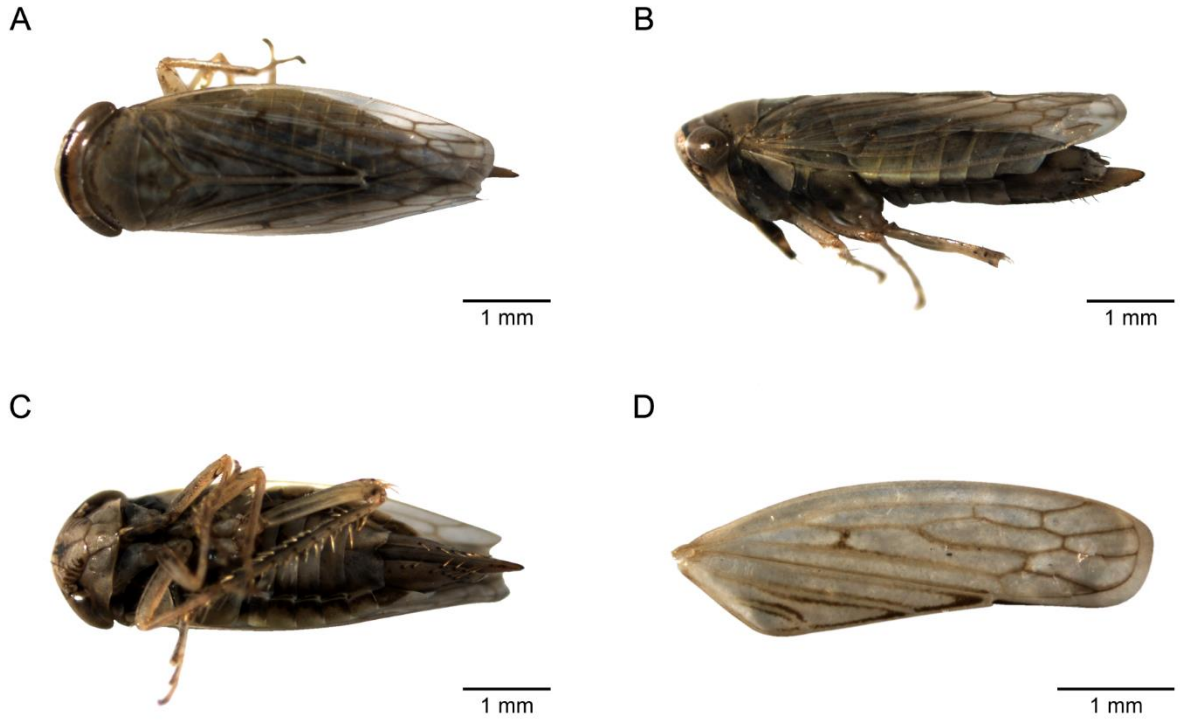

Figure A.19. *Exitianus capicola* (Stål, 1855) habitus. **A** – Female in dorsal view. **B** – Female in lateral view. **C** – Female in ventral view. **D** – Forewing. **Author's original.**

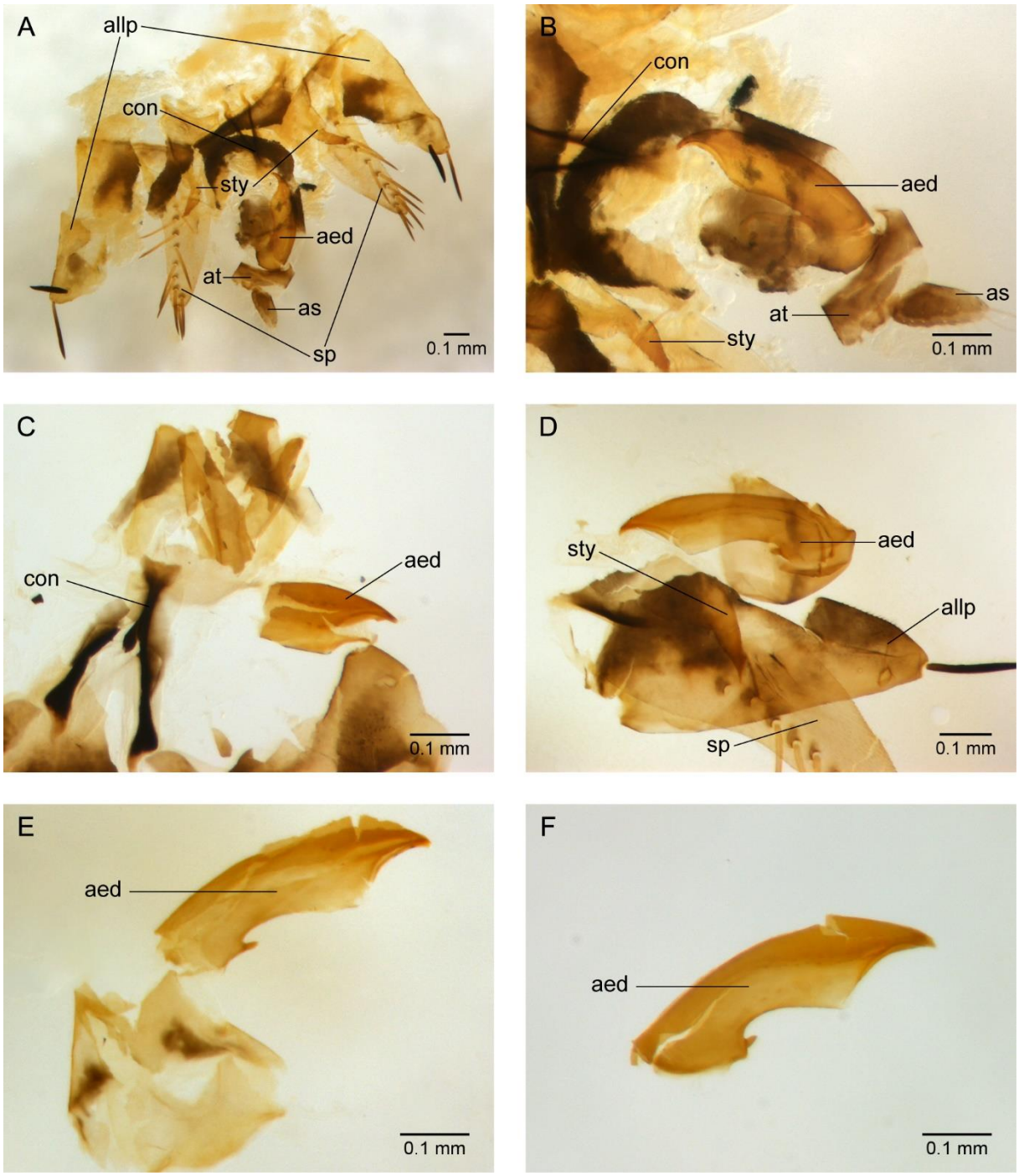

Figure A.20. *Exitianus capicola* (Stål, 1855) genitalia. **A-F** – Male genital capsule from several specimens (aed = aedeagus, allp = appendage of lateral lobe of pygofer, as = anal style; at = anal tube, con = connective, sp = subgenital plate, sty = style). **Author's original.**

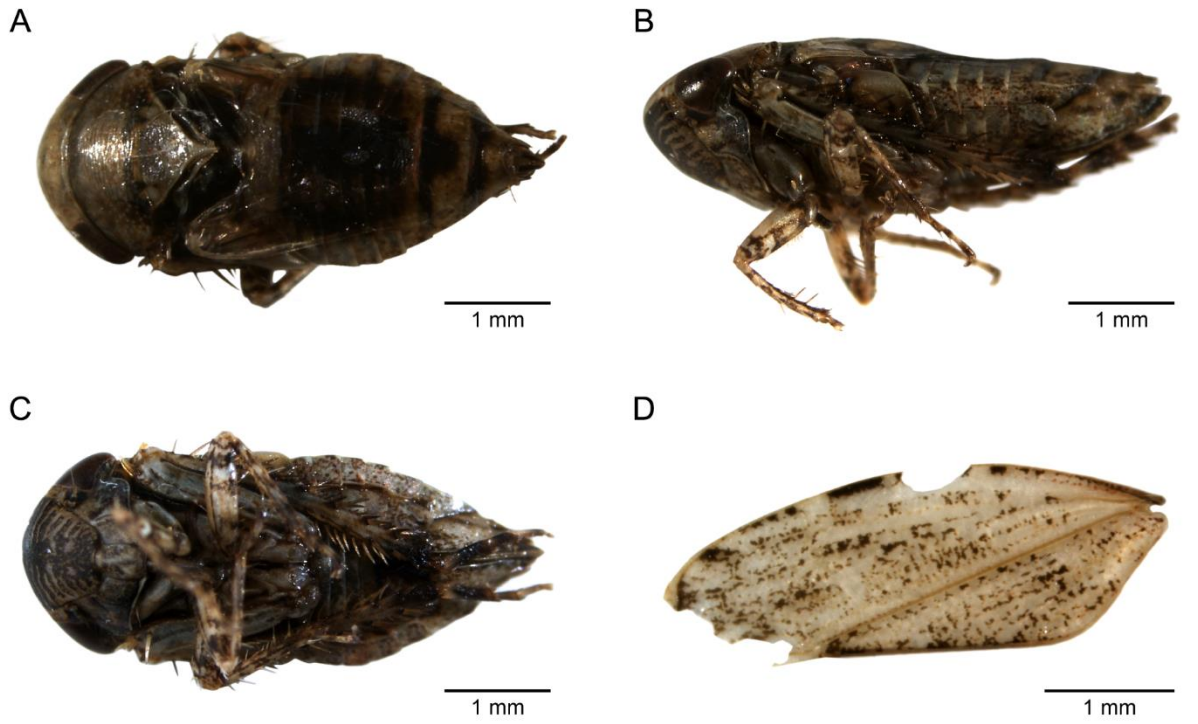

Figure A.21. *Goniagnathus brevis* (Herrich-Schäffer, 1835) habitus. **A** – Female in dorsal view. **B** – Female in lateral view. **C** – Female in ventral view. **D** – Forewing. **Author's original.**

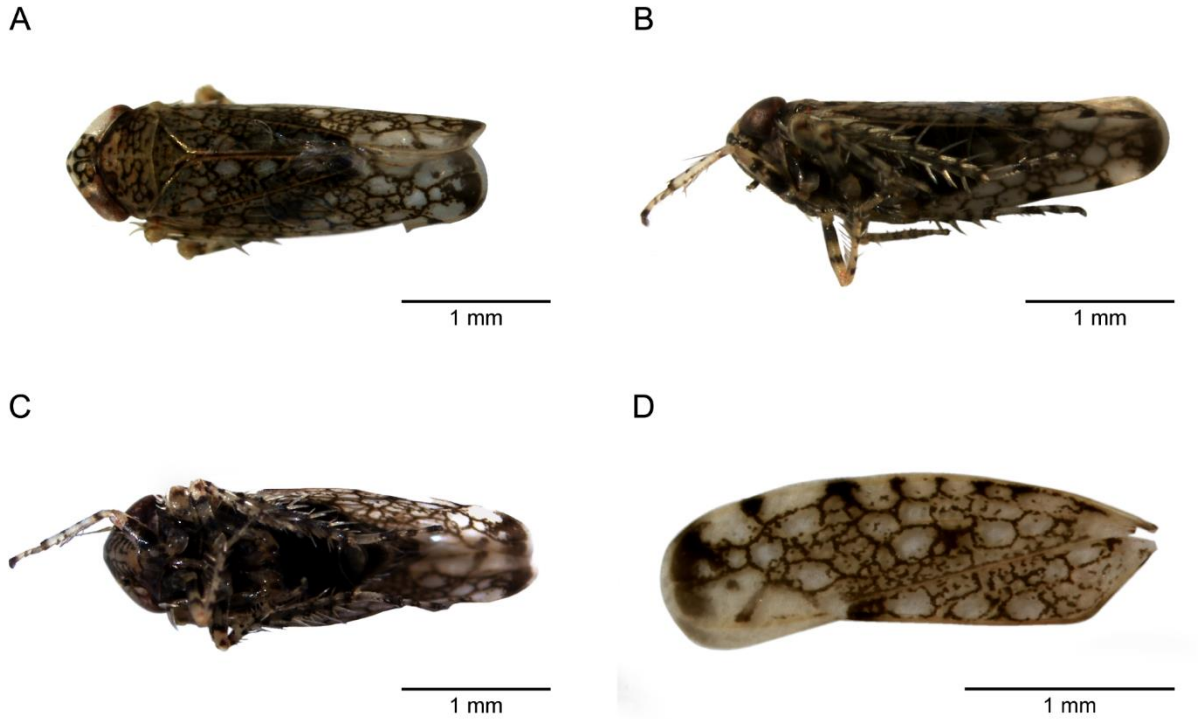

Figure A.22. *Orosius albicinctus* Distant, 1918 habitus. **A** – Male in dorsal view. **B** – Male in lateral view. **C** – Male in ventral view. **D** – Forewing. **Author's original.**

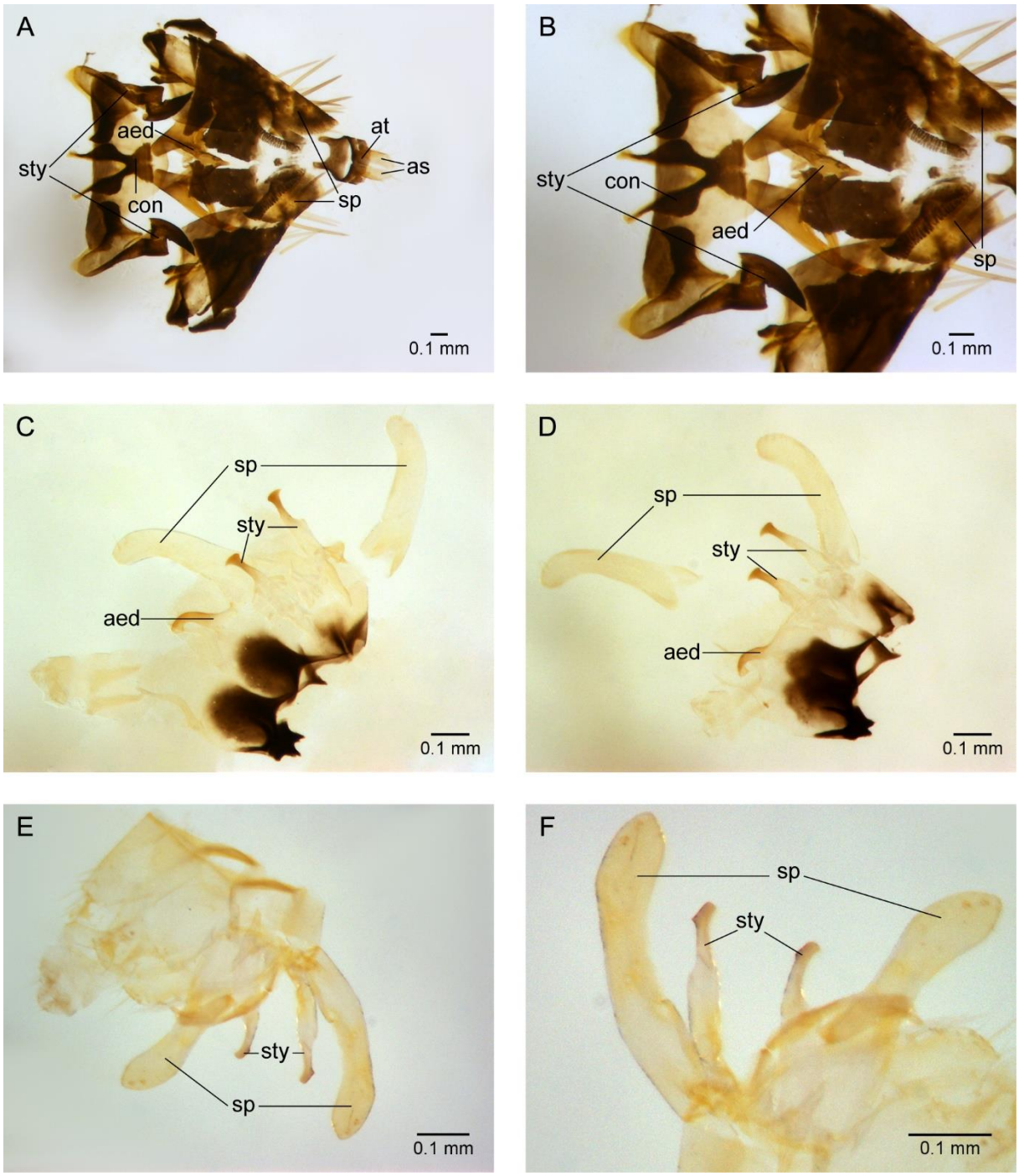

Figure A.23. Morphologic aspects of the male genitalia of three leafhopper species. **A-B** –Male genital capsule of *Orosius albicinctus* Distant, 1918. **C-D** – Male genital capsule of *Zygina nivea* (Mulsant & Rey, 1855). **E-F** – Male genital capsule of *Zygina ordinaria* (Ribaut, 1936) (aed = aedeagus; as = anal style; at = anal tube, con = connective, sp = subgenital plate, sty = style). **Author's original.**

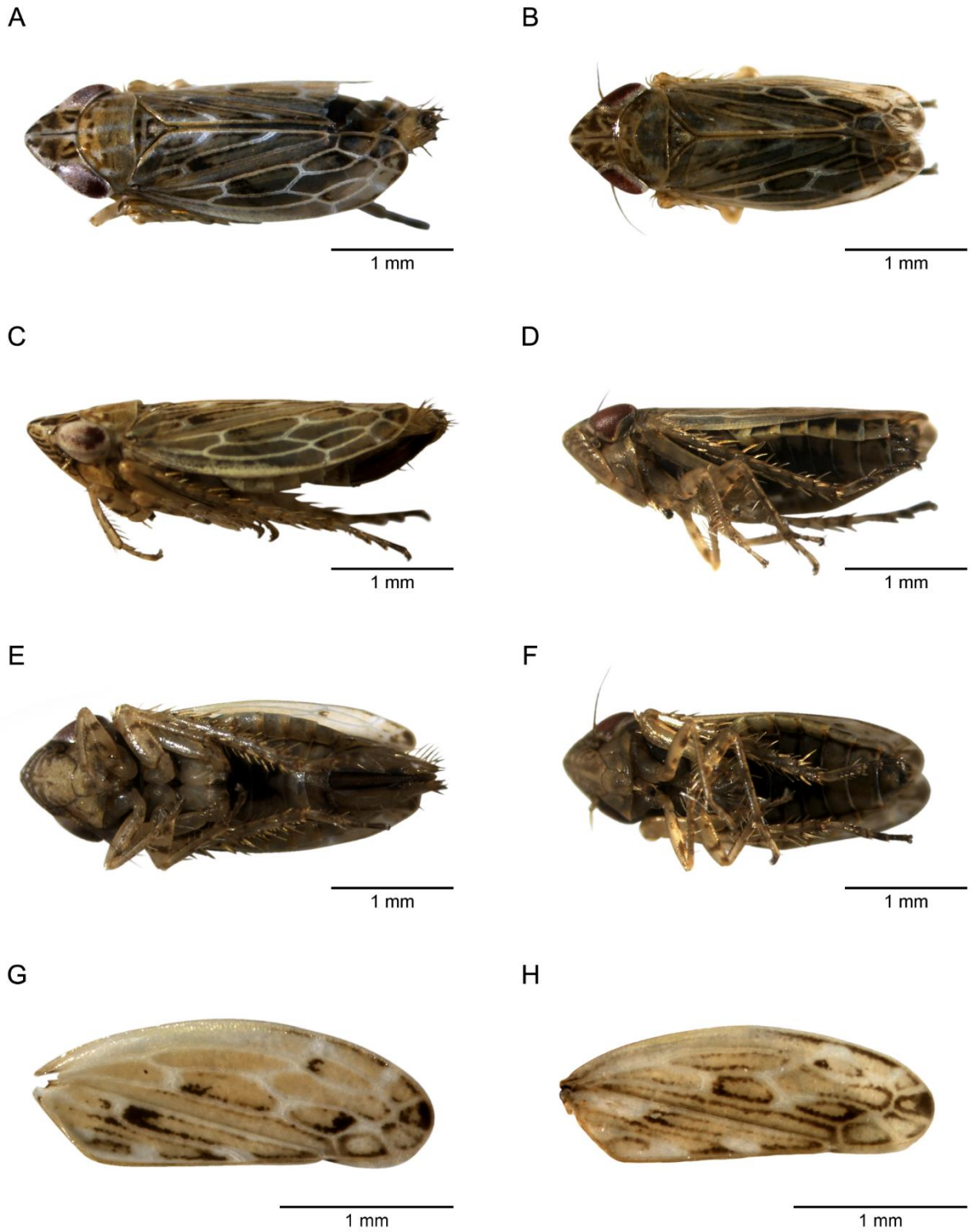

Figure A.24. *Psammotettix* sp. habitus. **A** – Female in dorsal view. **B** – Male in dorsal view. **C** – Female in lateral view. **D** – Male in lateral view. **E** – Female in ventral view. **F** – Male in ventral view. **G** – Female forewing. **H** – Male forewing. **Author's original.**

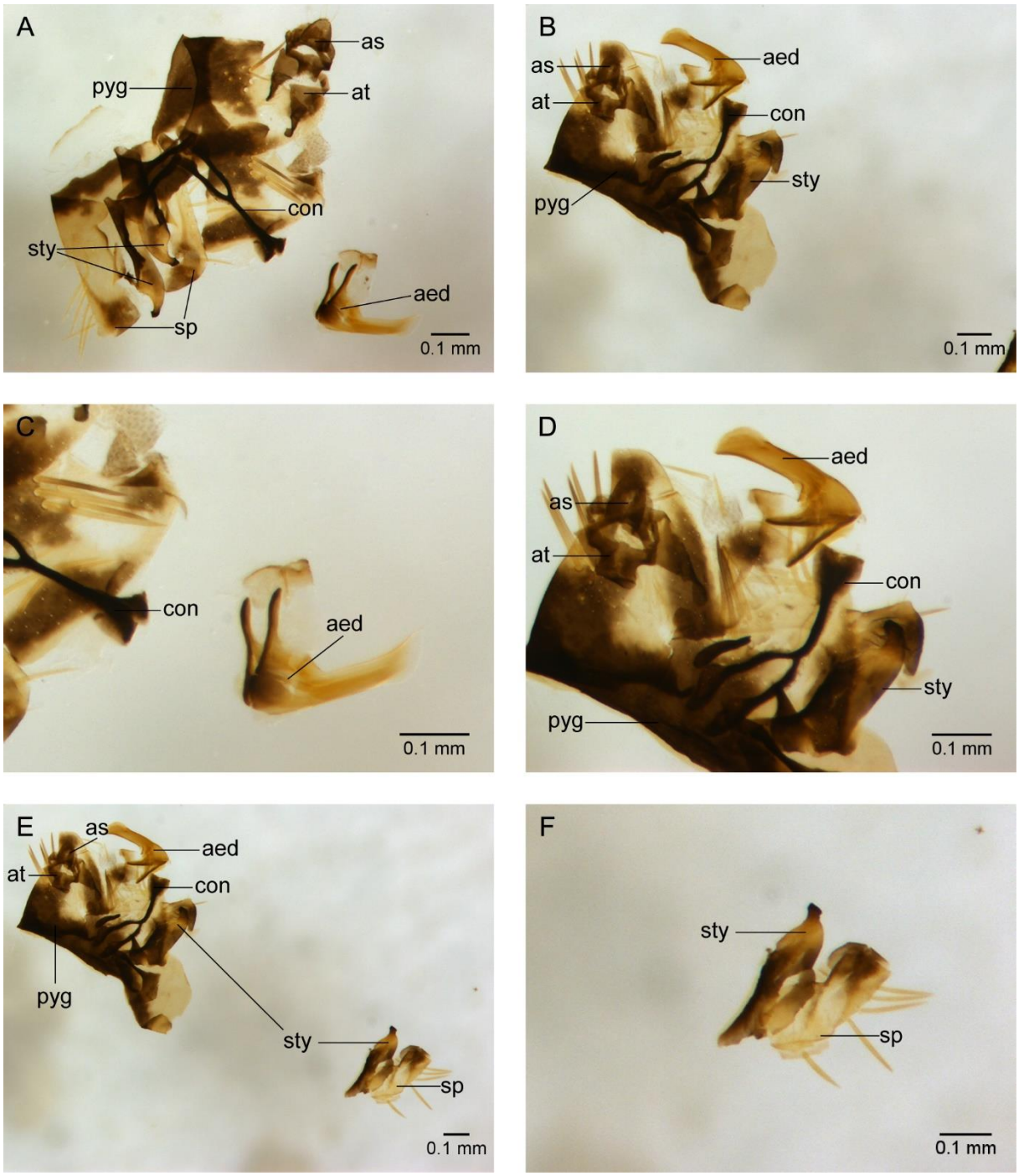

Figure A.25. *Psammotettix* sp. genitalia. **A-F** – Male genital capsule from several specimens (aed = aedeagus; as = anal style; at = anal tube, con = connective; pyg = pygofer, sp = subgenital plate, sty = style). **Author's original.**

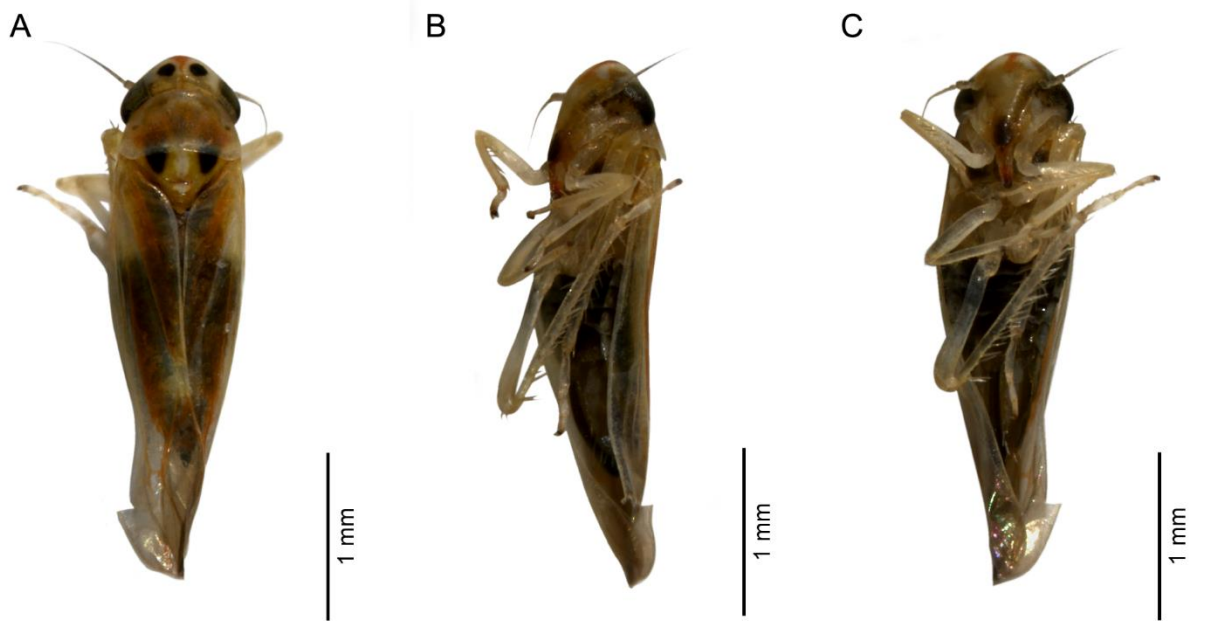

Figure A.26. *Arboridia parvula* (Boheman, 1845) habitus. **A** – Male in dorsal view. **B** – Male in lateral view. **C** – Male in ventral view. **Author's original.**

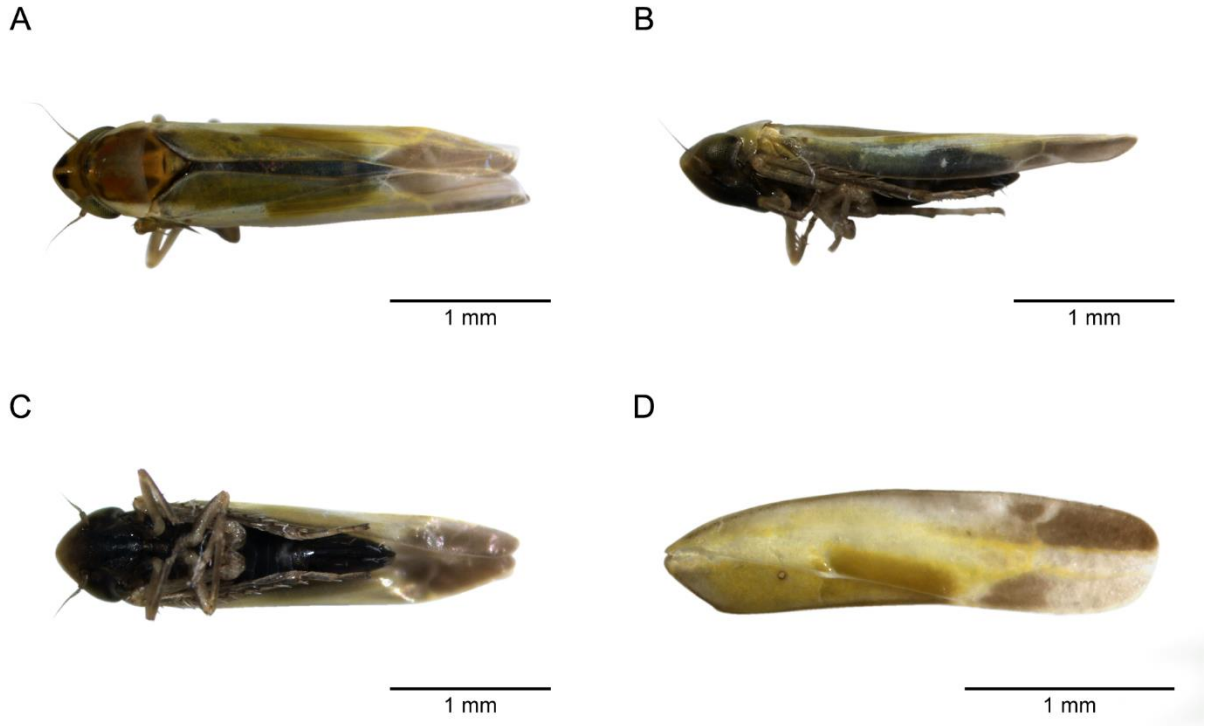

Figure A.27. *Frutioidia bisignata* (Mulsant & Rey, 1855) habitus. **A** – Female in dorsal view. **B** – Female in lateral view. **C** – Female in ventral view. **D** – Forewing. **Author's original.**

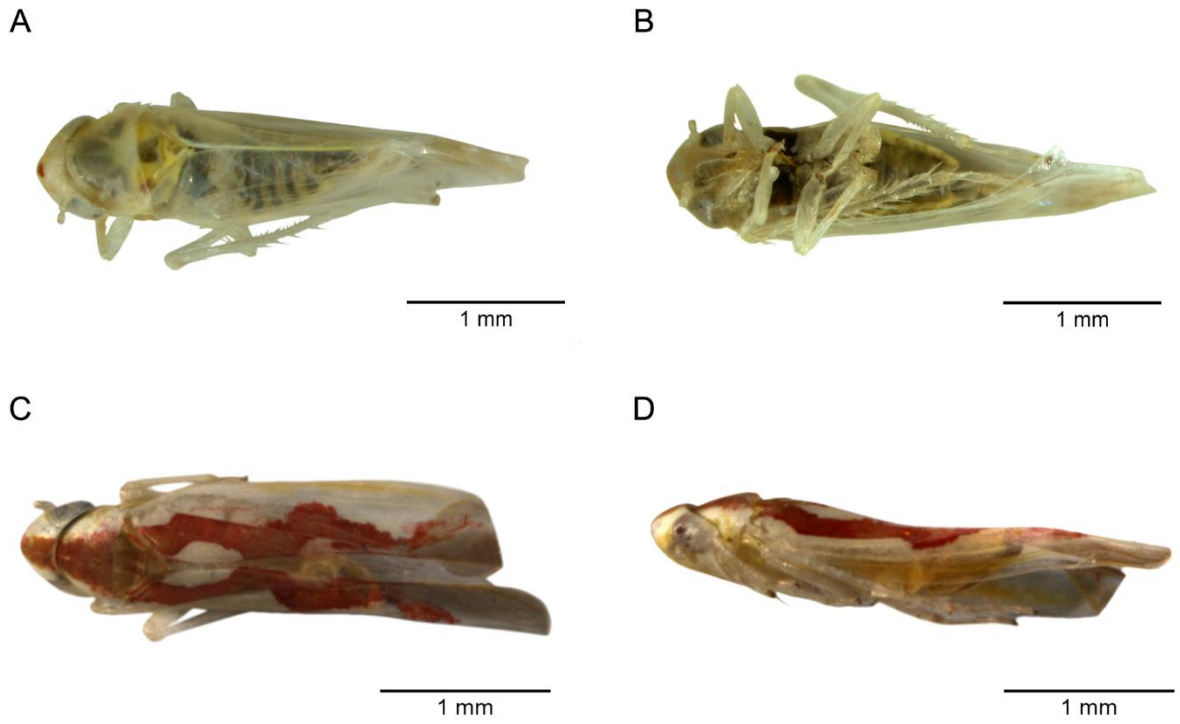

Figure A.28. *Zygina* spp. habitus. **A**-**B** – *Zygina nivea* (Mulsant & Rey, 1855) male in dorsal view (A) and in ventral view (B). **C**-**D** – *Zygina ordinaria* (Ribaut, 1936) female in dorsal view (C) and in lateral view (D). **Author's original.**

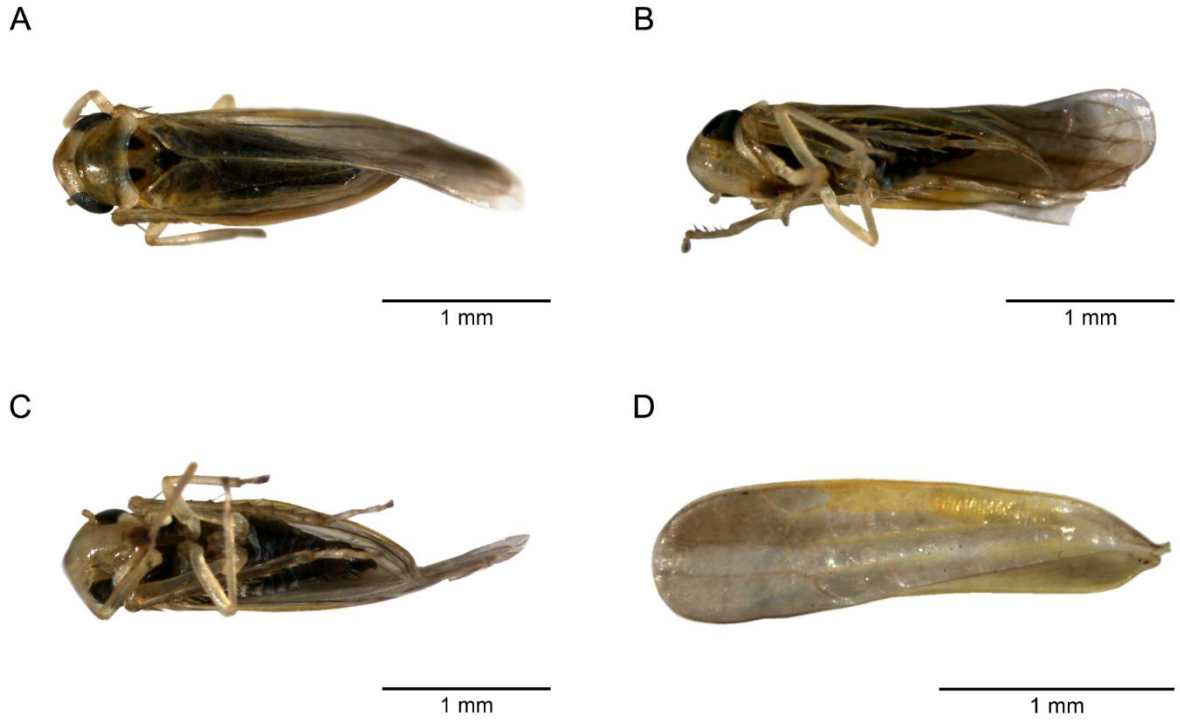

Figure A.29. *Zyginidia scutellaris* (Herrich-Schäffer, 1838) habitus. **A** – Male in dorsal view. **B** – Male in lateral view. **C** – Male in ventral view. **D** – Forewing. **Author's original.**

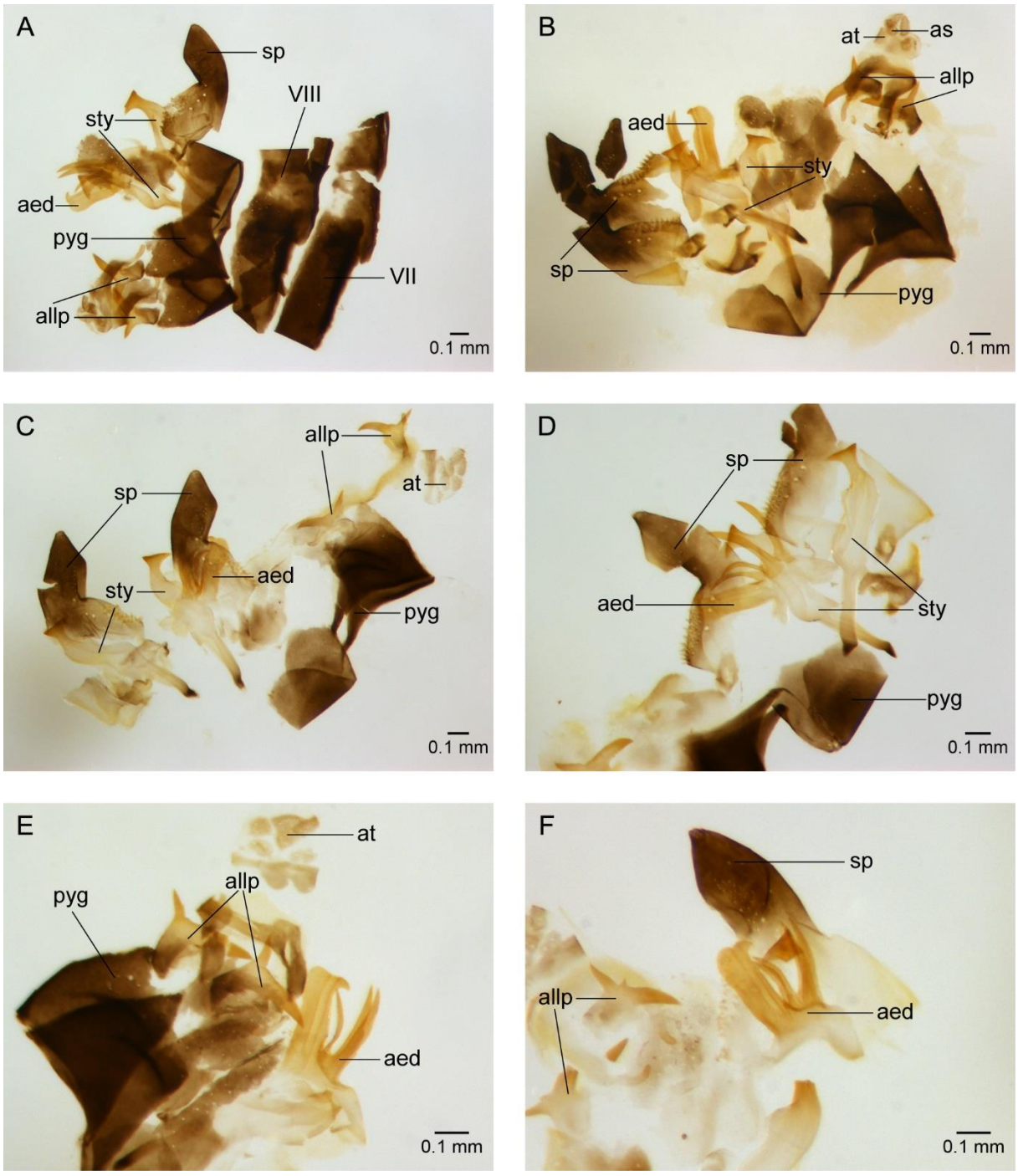

Figure A.30. *Zyginidia scutellaris* (Herrich-Schäffer, 1838) genitalia. **A-F** – Male genital capsule from several specimens (aed = aedeagus; allp = appendage of lateral lobe of pygofer; as = anal style; at = anal tube; pyg = pygofer; sp = subgenital plate, sty = style; VII = 7th segment of abdomen; VIII = 8th segment of abdomen). **Author's original.**

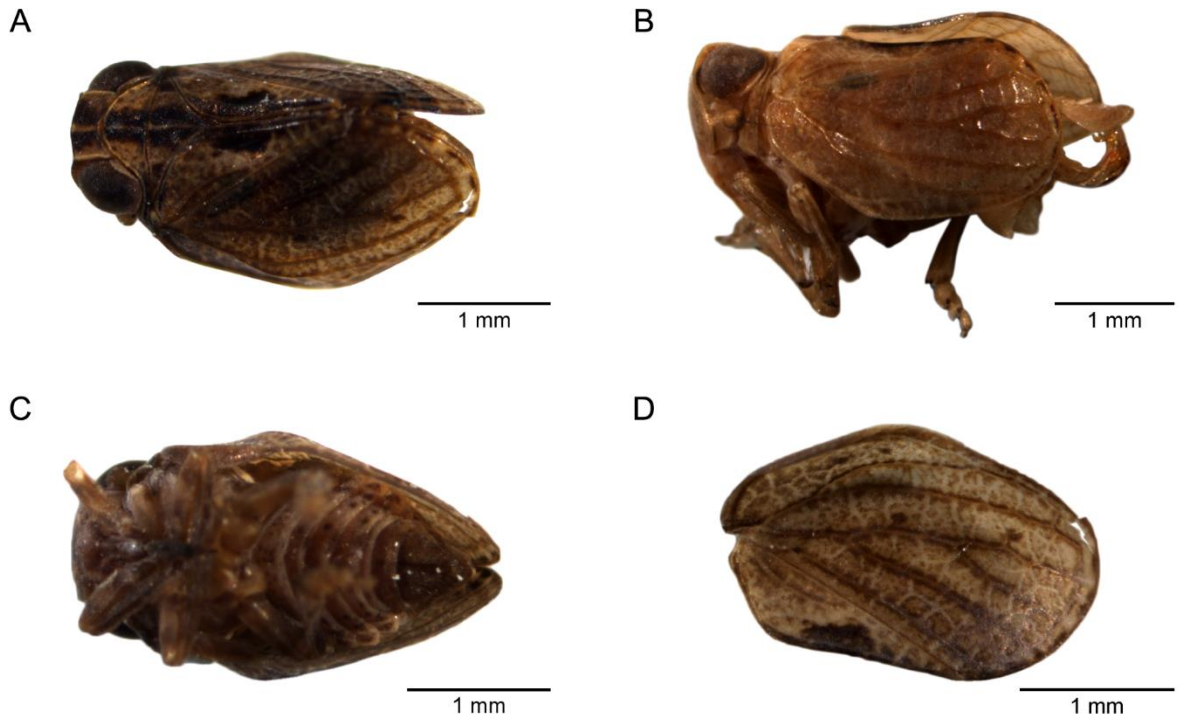

Figure A.31. *Fieberium impressum* (Fieber, 1877) habitus. **A** – Male in dorsal view. **B** – Male in lateral view. **C** – Male in ventral view (another specimen). **D** – Forewing. **Author's original.**

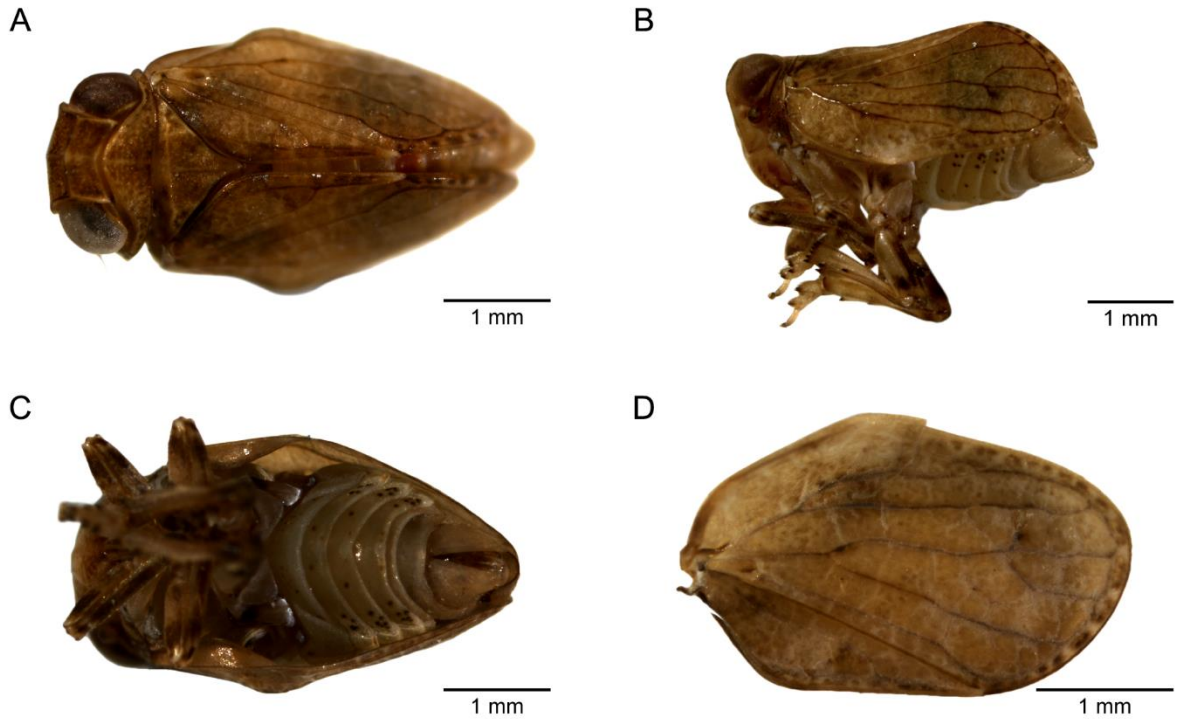

Figure A. 32. *Tingissus guadarramense* (Melichar, 1906) habitus. **A** – Male in dorsal view. **B** – Male in lateral view. **C** – Male in ventral view. **D** – Forewing. **Author's original.**

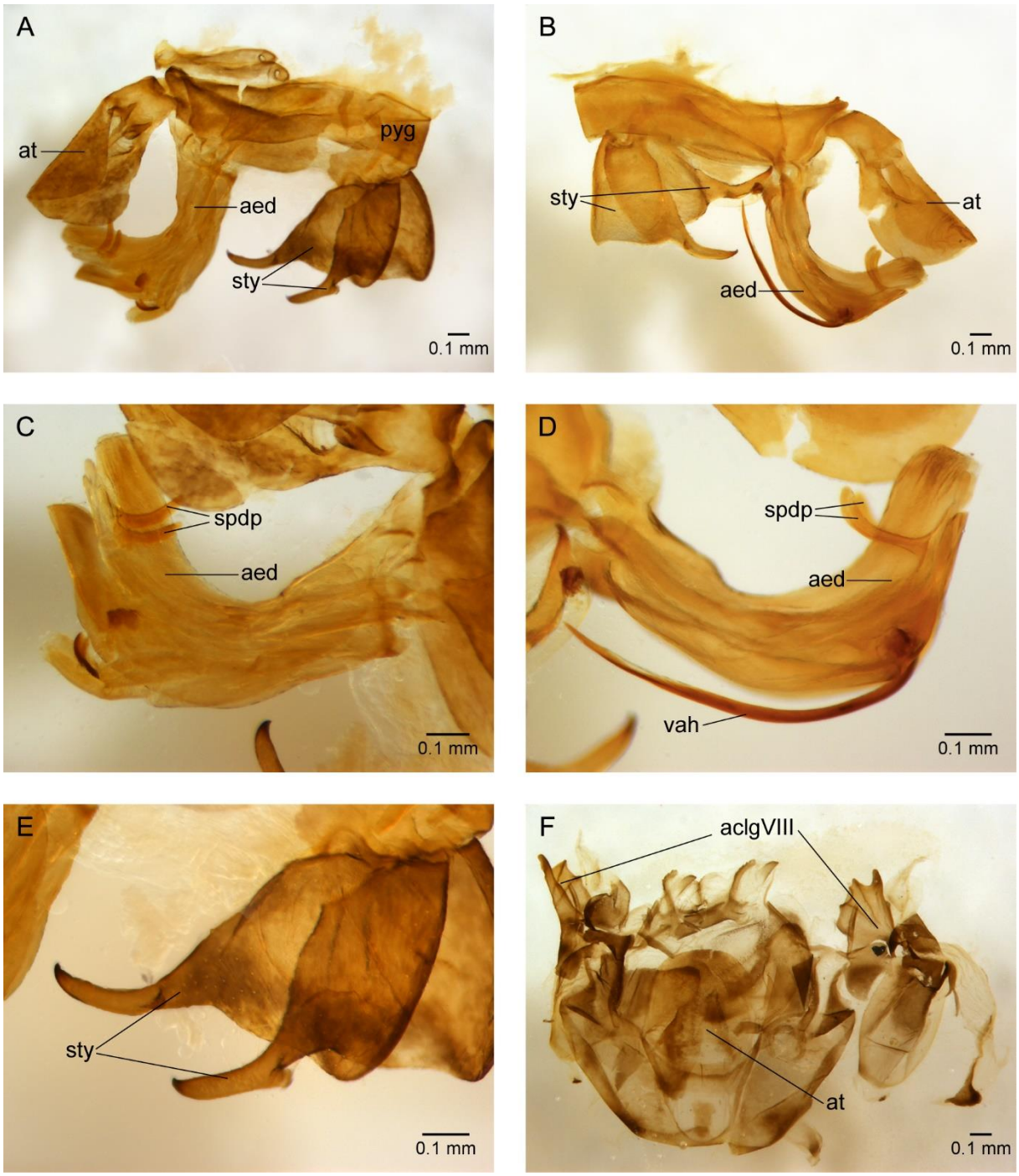

Figure A.33. *Fieberium impressum* (Fieber, 1877) genitalia. **A**-**E** – Male genital capsule from several specimens (aed = aedeagus; at = anal tube; pyg = pygofer; spdp = subapical process of dorso-lateral phallobase; sty = style; vah = ventral aedagal hooks). **F** – Female genital capsule (aclgVIII = anterior connective lamina of 8th gonapophyse; at = anal tube). **Author's original.**

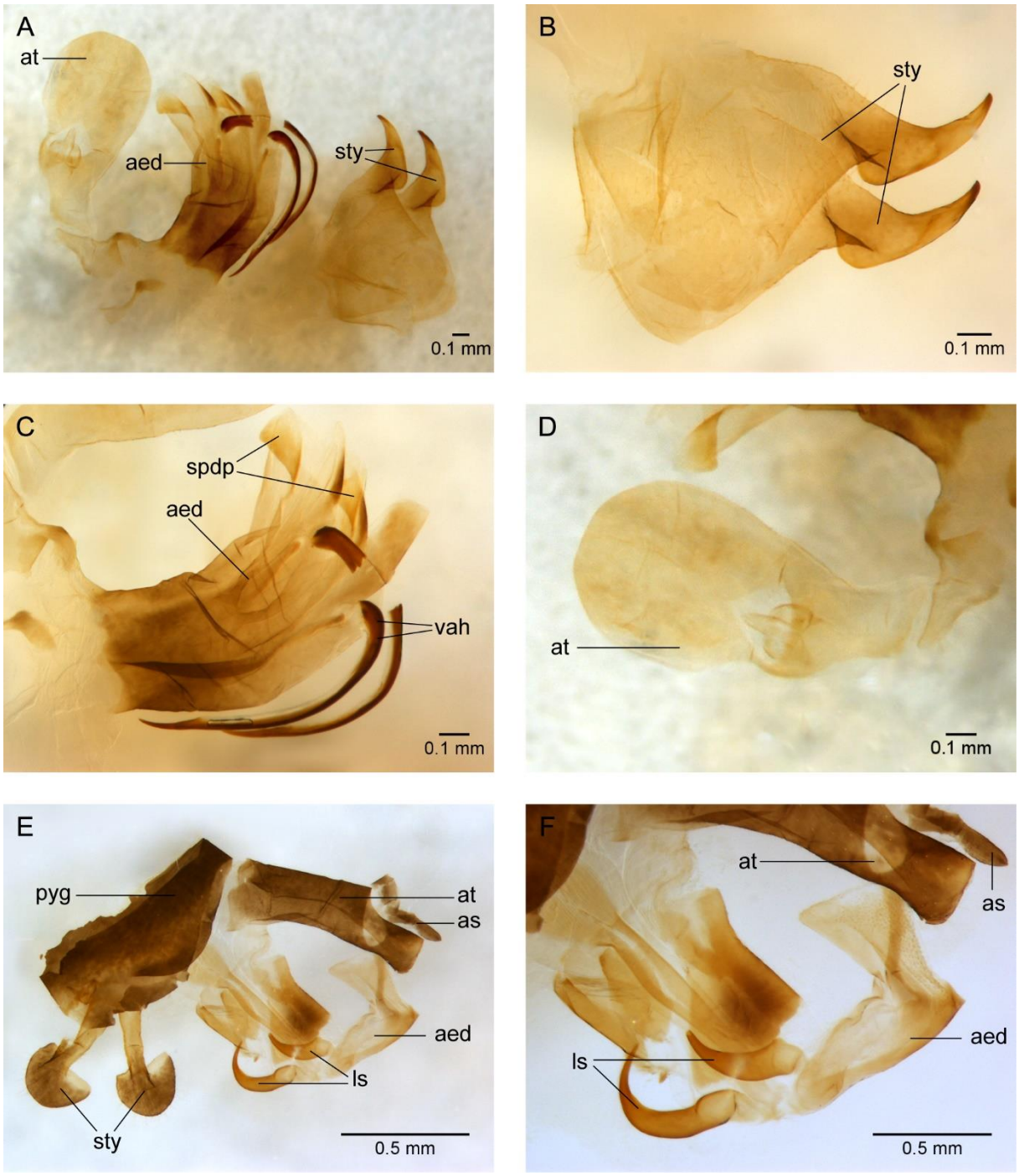

Figure A.34. Morphologic aspects of the male genitalia of two planthopper species. **A-D** –Male genital capsule of *Tingissus guadarramense* (Melichar, 1906). **E-F** – Male genital capsule of *Cixius nervosus* (Linnaeus, 1758) (aed = aedeagus; as = anal style; at = anal tube; ls = lateral spines of aedeagus; pyg = pygofer; sp = subgenital plate; spdp = subapical process of dorso-lateral phallobase; sty = style; vah = ventral aedagal hooks). **Author's original.**

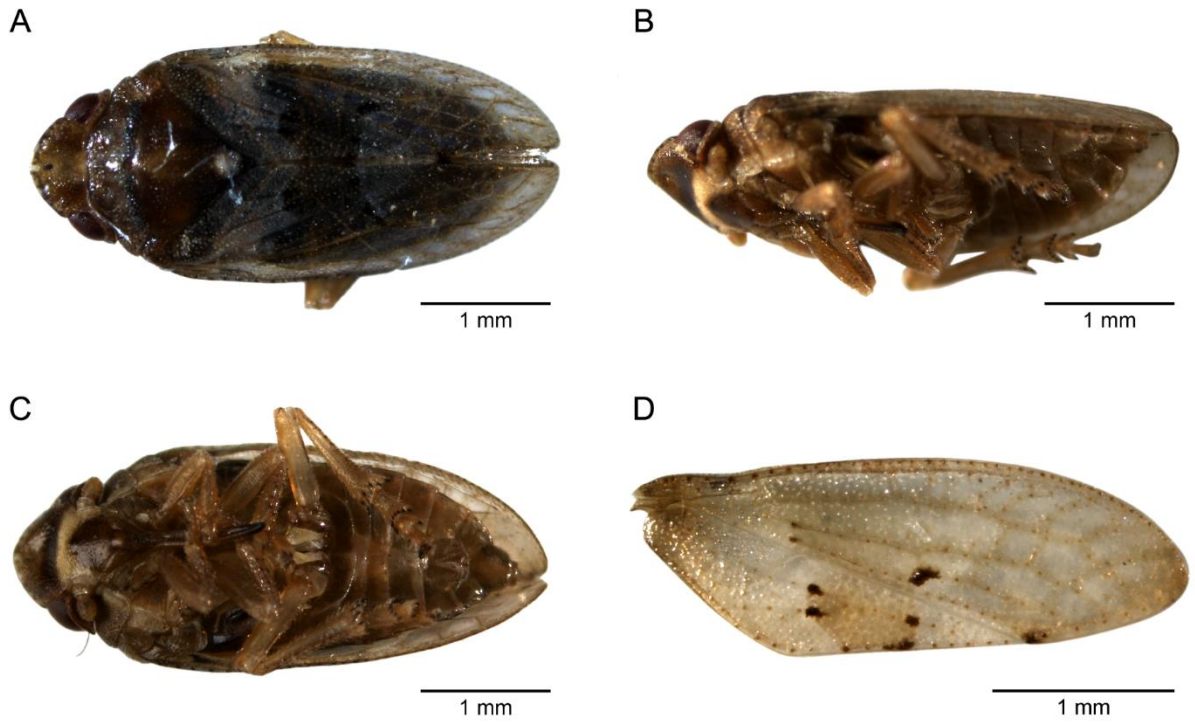

Figure A.35. *Tettigometra impressopunctata* (Dufour, 1846) habitus. **A** – Male in dorsal view. **B** – Male in lateral view. **C** – Male in ventral view. **D** – Forewing. **Author's original.**

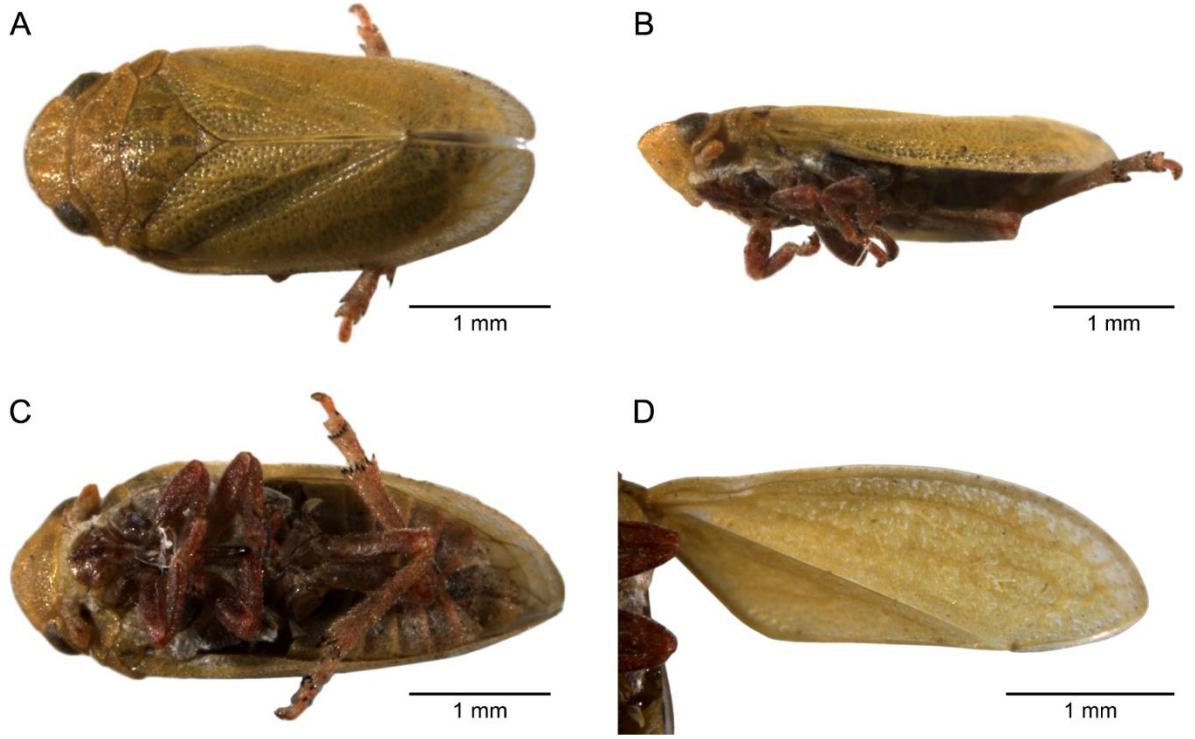

Figure A.36. *Tettigometra virescens* (Panzer, 1799) habitus. **A** – Male in dorsal view. **B** – Male in lateral view. **C** – Male in ventral view. **D** – Forewing. **Author's original.**

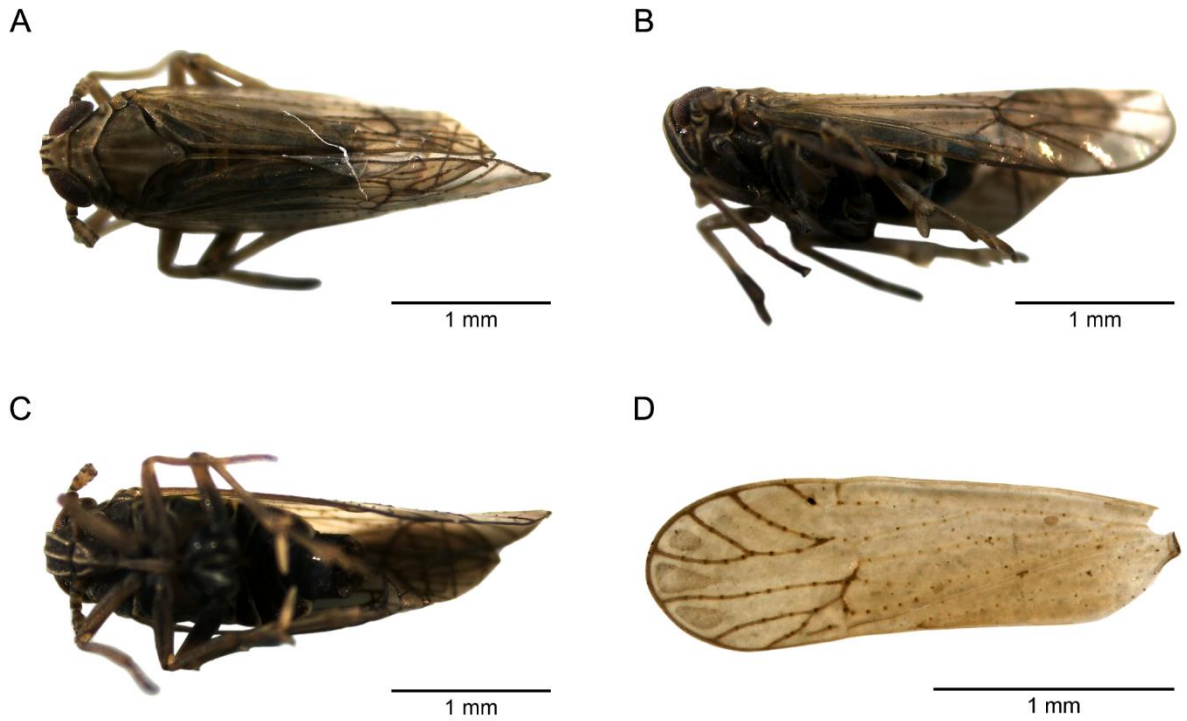

Figure A.37. *Metadelphax propinqua* (Fieber, 1866) habitus. **A** – Male in dorsal view. **B** – Male in lateral view. **C** – Male in ventral view. **D** – Forewing. **Author's original.**

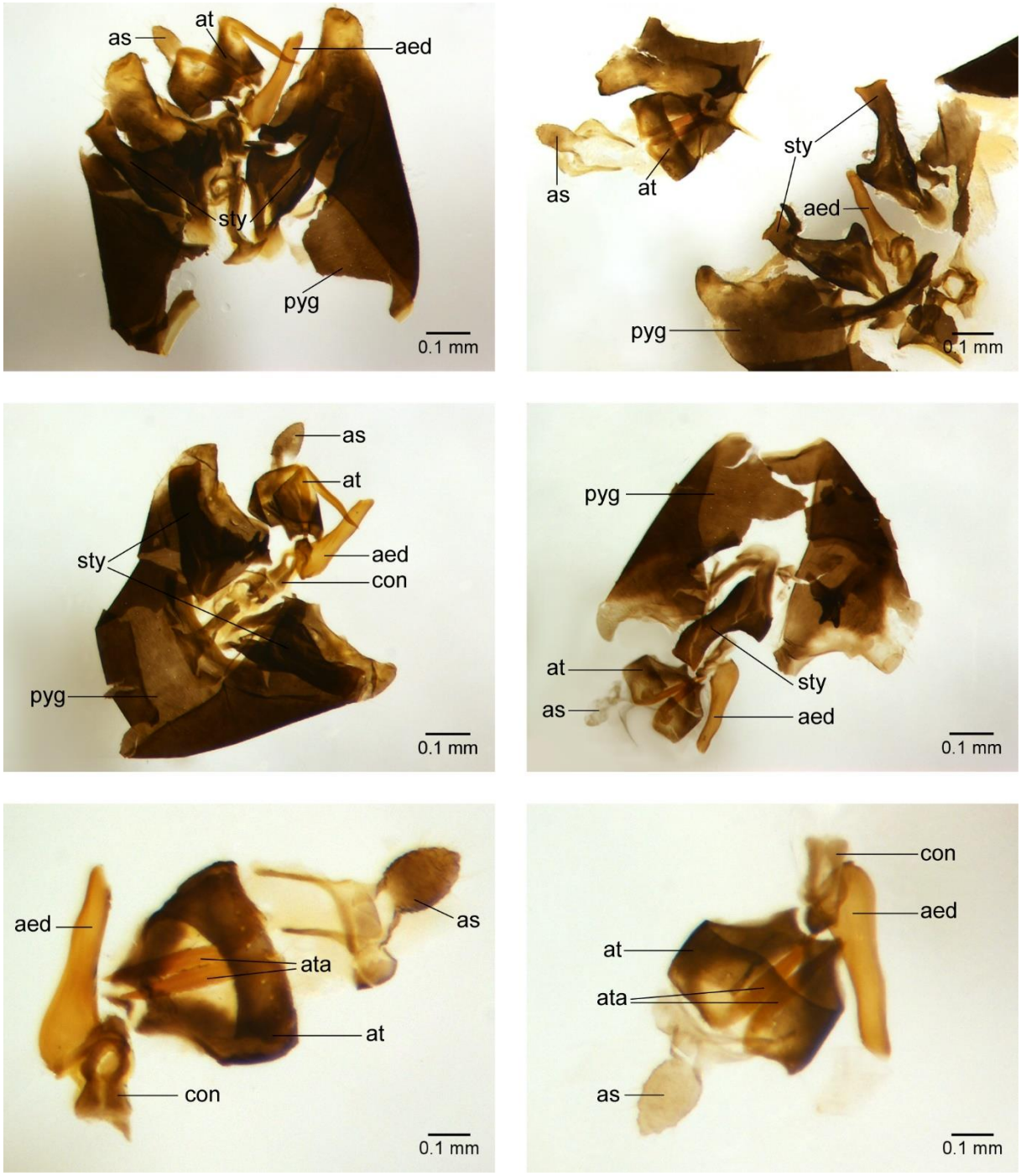

Figure A.38. *Metadelphax propinqua* (Fieber, 1877) genitalia. **A-F** – Male genital capsule from several specimens (aed = aedeagus; as = anal style; at = anal tube; ata = anal tube appendages; con = connective; pyg = pygofer; sty = style). **Author's original.**

Appendix 9 – Draws from somatic and genital characters

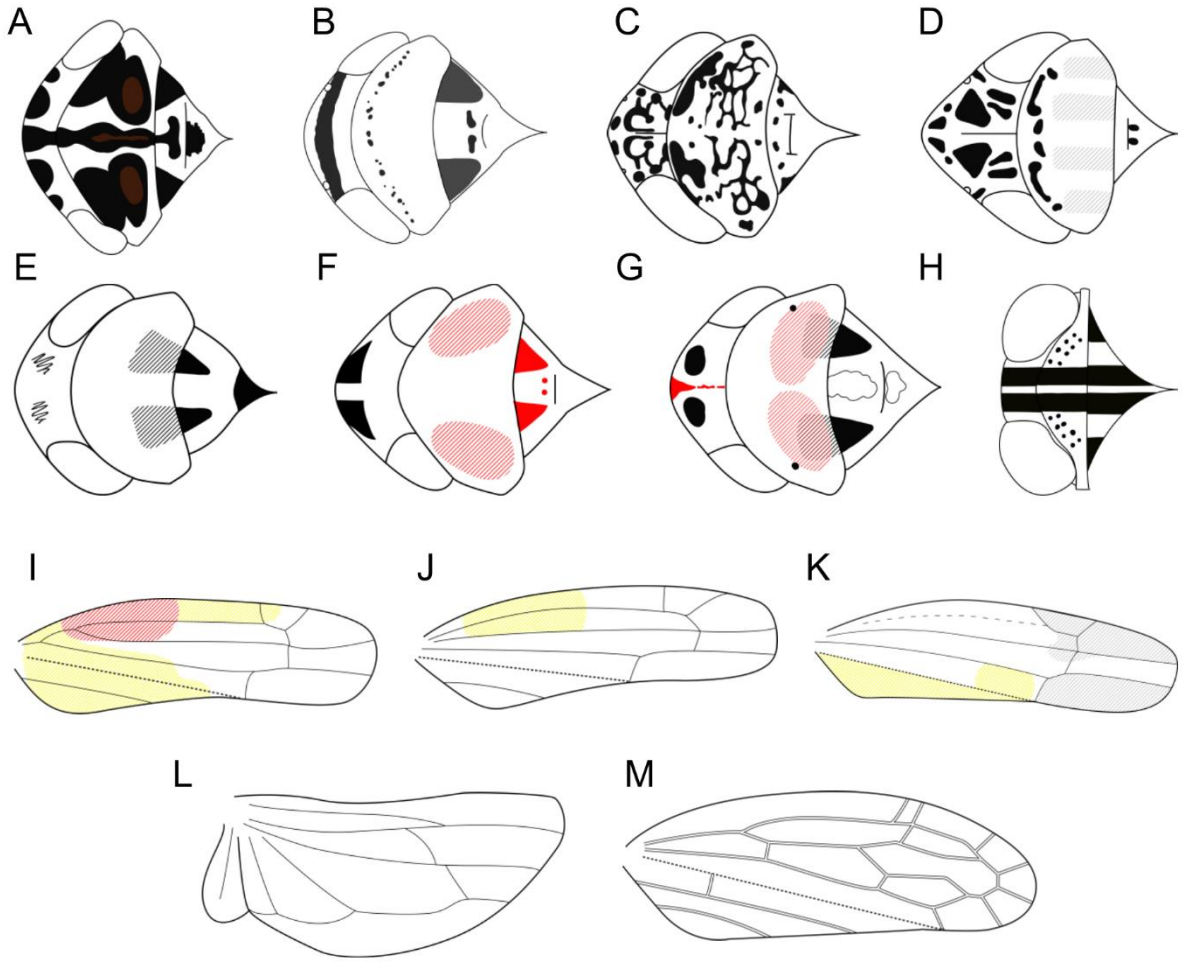

Figure A.39. Somatic characters from some of the collected Auchenorrhyncha species. **A** – *Anaceratagallia laevis* (Ribaut, 1935). vertex, pronotum and scutellum. **B** – *Exitianus capicola* (Stål, 1855) vertex, pronotum and scutellum. **C** – *Orosius albicinctus* Distant, 1918 vertex, pronotum and scutellum. **D** – *Psammotettix* sp. vertex, pronotum and scutellum. **E** – *Zyginidia scutellaris* (Herrich-Schäffer, 1838) vertex, pronotum and scutellum. **F** – *Frutioidia bisignata* (Mulsant & Rey, 1855) vertex, pronotum and scutellum. **G** – *Arboridia parvula* (Boheman, 1845) vertex, pronotum and scutellum. **H** – *Fieberium impressum*. (Fieber, 1877) vertex, pronotum and scutellum. **I-J** – *Zyginidia scutellaris* forewings (different specimens). **K-L** – *Frutioidia bisignata* forewing (K) and hindwing (L). **M** – *Psammotettix* sp. forewing. **Author's original.**

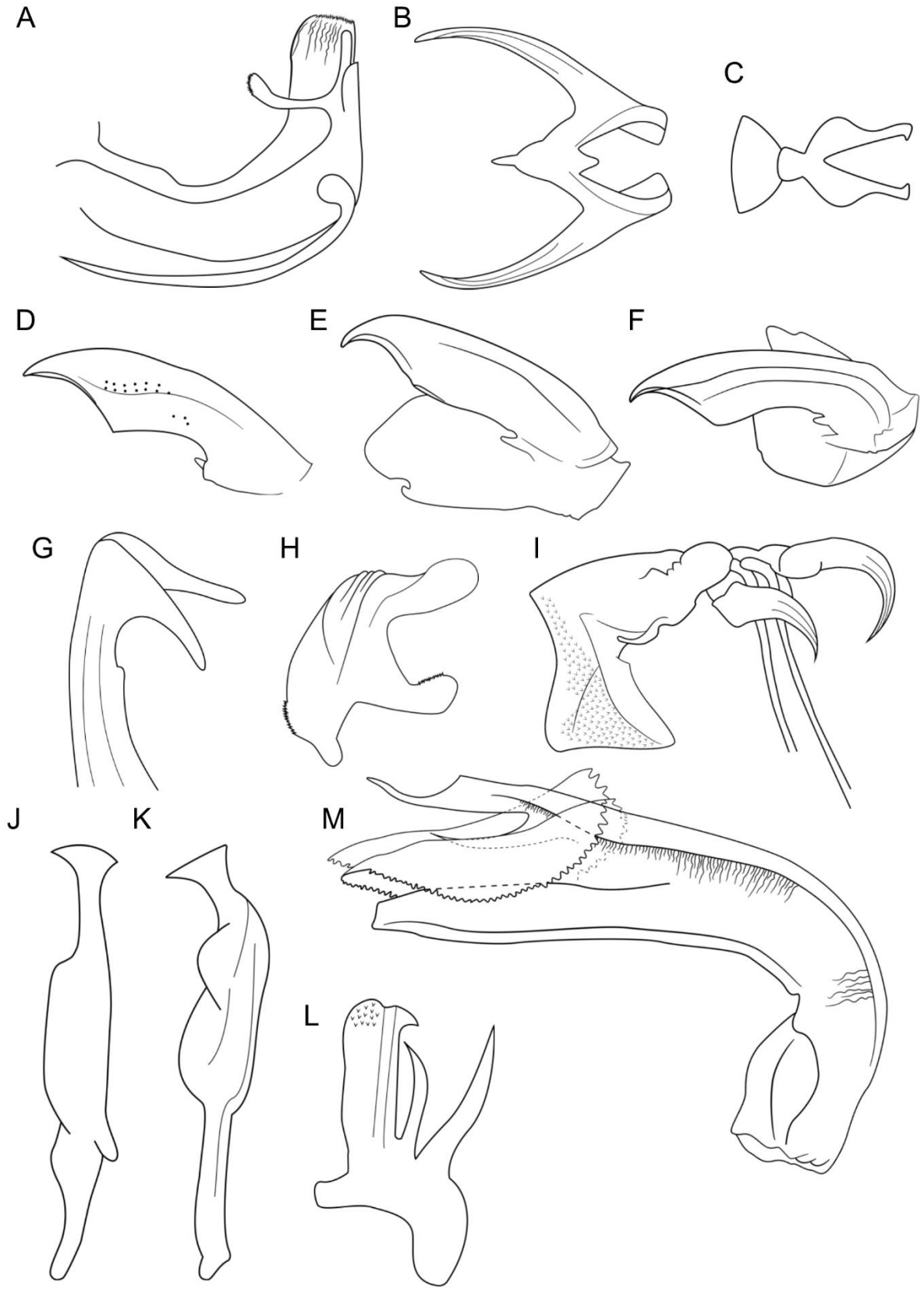

Figure A.40. Genital characters from some of the collected Auchenorrhyncha species. **A** – *Fieberium impressum* (Fieber, 1877) aedeagus. **B**-**C** – *Orosius albicinctus* Distant, 1918 aedeagus (B) and connective (C). **D**-**F** – *Exitianus capicola* (Stål, 1855) aedeagus (several specimens). **G** – *Psammotettix* sp. aedeagus. **H** – *Anaceratagallia laevis* (Ribaut, 1935) aedeagus. **I** – *Cixius nervosus* (Linnaeus, 1758) aedeagus. **J** – *Zygina nivea* (Mulsant & Rey, 1855) style. **K**-**L** *Zyginida scutellaris* (Herrich-Schäffer, 1838) style (K) and aedeagus (L). **M** – *Neophilaenus campestris* (Fallén, 1805) aedeagus. **Author's original**.

Appendix 10 – Spittlebug distribution maps

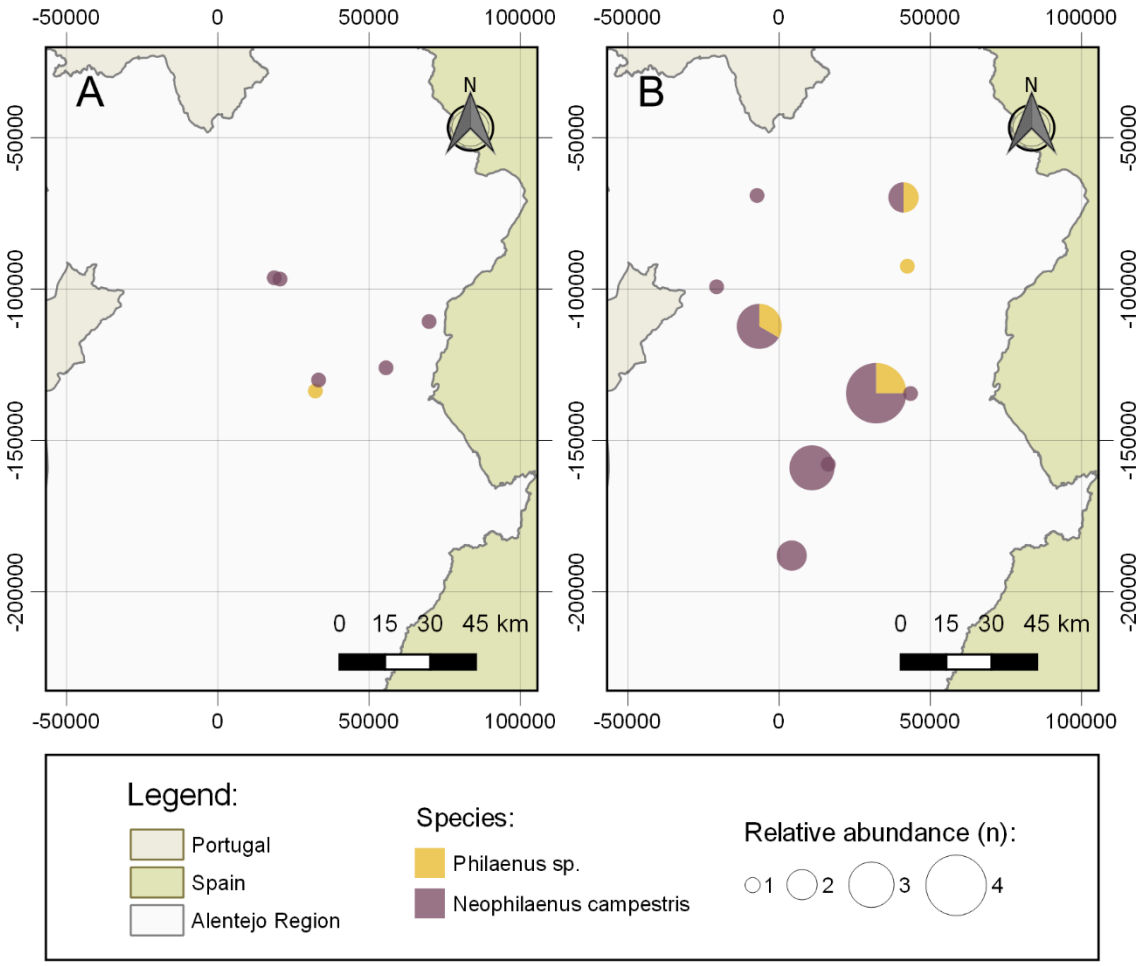

Figure A.41. Distribution of spittlebugs' abundance in the sampling sites with sorted samples by species. **A** – Olive tree samples. **B** – Weeds samples. This map is projected in ETRS89/PT-TM06. **Author's original.**