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ORIGINAL ARTICLE

Wing buzzing as a mechanism for generating vibrational signals in psyllids (Hemiptera: Psylloidea)

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Abstract Psyllids, or jumping plant lice (Hemiptera: Sternorrhyncha: Psylloidea), are a group of small phytophagous insects that include some important pests of crops worldwide. Sexual communication of psyllids occurs via vibrations transmitted through host plants, which play an important role in mate recognition and localization. The signals are species-specific and can be used to aid in psyllid taxonomy and pest control. Several hypotheses have been proposed for the mechanism that generates these vibrations, of which stridulation, that is, friction between parts of the forewing and thorax, has received the most attention. We have investigated vibrational communication in the European pear psyllid species *Cacopsylla pyrisuga* (Foerster, 1848) using laser vibrometry and high-speed video recording, to directly observe the movements associated with signal production. We describe for the first time the basic characteristics of the signals and signal emission of this species. Based on observations and analysis of the video recordings using a point-tracking algorithm, and their comparison with laser vibrometer recordings, we argue that males of *C. pyrisuga* produce the vibrations primarily by wing buzzing, that is, tremulation that does not involve friction between the wings and thorax. Comparing observed signal properties with previously published data, we predict that wing buzzing is the main mechanism of signal production in all vibrating psyllids.

Key words *Cacopsylla pyrisuga*; high-speed video recording; jumping plant lice; sexual communication; substrate-borne vibrational signals; vibrational communication

Introduction

Psyllids, or jumping plant lice (Hemiptera: Sternorrhyncha: Psylloidea), are a group of small phytophagous insects with piercing-sucking mouthparts, closely related to whiteflies, aphids and scale insects. Psyllids currently comprise about 4000 described species worldwide. They feed mainly on phloem sap and are generally host-specific (Hardy, 2018; Mauck *et al.*, 2023). Some species of psyllids are pests of crops around the world, such as citrus, potatoes, pears, apples, apricots, and others (Burckhardt, 1994). The greatest damage is caused by species that are vectors of phytopathogenic bacteria found in the phloem tissue of plants: "*Candidatus* Liberibacter" spp. and "*Ca*. Phytoplasma" spp. (Munyaneza, 2012; Grafton-Cardwell *et al.*, 2013; Jarausch *et al.*, 2019; Moreno *et al.*, 2021).

In addition to feeding, psyllids also use their host plants as a medium for sexual communication with vibrational signals transmitted through the substrate, which, together with sex pheromones and visual cues, play an important role in mate recognition and localization (Lubanga *et al.*, 2014; Civolani *et al.*, 2023). Both males and females produce species-specific vibrational signals, and the

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interaction typically involves a duet phase in which the male actively searches for the stationary female (Percy *et al.*, 2006; Lubanga *et al.*, 2014; Liao *et al.*, 2022).

Basic knowledge of insect vibrational communication has improved in recent decades, but the field remains understudied (Virant-Doberlet *et al.*, 2023). Biotremology—the study of vibrational behavior—is still considered a niche subject, but has nevertheless led to promising developments toward alternative pest control methods (Mankin *et al.*, 2011; Polajnar *et al.*, 2015), and can help with identification within complexes of cryptic species (Wells & Henry, 1998; Bluemel *et al.*, 2014; Liao & Yang, 2015). These goals are evident in recent research on psyllid vibrational communication, where mating disruption (Lujo *et al.*, 2016; Mankin *et al.*, 2016), attraction for trapping (Mankin *et al.*, 2013) and automated detection based on vibrational signals for monitoring (Mankin *et al.*, 2011) have been investigated, as well as discrimination of morphologically similar species (Yang *et al.*, 1986; Liao & Yang, 2015; Liao *et al.*, 2016).

A long-standing question about the mechanism of vibrational signal production in psyllids has remained unanswered for over half a century, as their small body size and rapid movements associated with signal production preclude direct observation. Traditional explanations focused on morphology and suggested stridulation as the most likely mechanism, with various possible pairs of scrapers and files. Among these, friction between the anal area of the forewing and the prominent axillary cords covered with scale-like denticles on the mesoscutellum and metascutellum (Taylor, 1985; Tishechkin, 2006) has received the most attention, but wing-wing, wing-leg and wing-abdomen friction have also been proposed (Lubanga *et al.*, 2014; Liao *et al.*, 2019, 2022; Avosani *et al.*, 2022). An alternative hypothesis is based on signal characteristics: Wenninger *et al.* (2009) noted that the fundamental frequency of *Diaphorina citri* signals in the range of 150–250 Hz matches the wingbeat frequency of flying insects, and proposed that the spectral properties of the signals are the result of wing buzzing which generates the fundamental frequency and its harmonics. The same idea had already been put forward by the pioneering biotremologist Ossiannilsson (1950) on the basis of his auditory impression, but he later referred to the wing-axillary cord friction hypothesis (Ossiannilsson, 1992). Wing buzzing is a special case of tremulation—an oscillatory movement of the whole body or a specific body part—that does not require specialized anatomical structures (Virant-Doberlet *et al.*, 2023), which might be more feasible in insects as small as psyllids. On the other hand, a particularly large variety of specialized vibration-generating structures is known from related, but generally larger Heteroptera (Davranoglou *et al.*, 2023; Virant-Doberlet *et al.*, 2023). Liao *et al.* (2019) tested the above stridulation hypotheses and, based on wing cutting experiments, concluded that a new hypothesis—axillary sclerite-thorax friction—is the best explanation, with wing-thorax friction being a possible complementary mechanism. They recorded the wing movements with a high-speed camera and rejected the wing buzzing hypothesis because the frequency of the wing beats was much lower than the dominant frequency of the signals in their recordings. However, due to the angle and low resolution of their video recordings, it is impossible to tell if the opposing structures are touching during signal production. To support the argument, Liao *et al.* (2019) stated that higher harmonics should be much weaker than the fundamental frequency, so the dominant frequency they recorded at the seventh harmonic is not possible without stridulation. This statement may apply to airborne sound (Arthur *et al.*, 2014), but elastic solid structures used as a medium in substrate-borne vibrational communication lead to significant frequency changes due to resonance; for example, in stink bugs (Heteroptera: Pentatomidae), the dominant frequency of signals recorded on herbaceous plants varies with the location of recording and is higher than that recorded on a nonresonant surface (Čokl *et al.*, 2005, 2009). It can be expected that the dominant harmonics would lie even higher in stiffer woody branches used by psyllids, so this may be an insufficient ground for rejecting the wing buzzing hypothesis.

We tested the stridulation hypothesis again by using high-speed video recording equipment to directly observe the movements associated with signal production, together with laser vibrometry. We predicted contact between a scraper and a file during signal production, with the spectral properties of the signal depending on the frequency of oscillation of the moving body part and the number of teeth on the opposing file. Alternatively, if the wing buzzing hypothesis is correct, no contact would be observed between the opposing structures, and the spectral characteristics would predominantly reflect wing motion. We chose *Cacopsylla pyrisuga* (Foerster, 1848), a relatively large psyllid species (overall body length 3.5–4.2 mm) that is easy to collect in spring in central European pear orchards. It belongs to the species-rich genus *Cacopsylla* Ossiannilsson, 1970 (Psyllidae), and is known, together with several other closely related species, as a pest of pear (*Pyrus* spp.) trees (Jarausch *et al.*, 2019; Civolani *et al.*, 2023). In addition to directly affecting plant growth by sap removal and excreting large amounts of honeydew, which promotes the growth of sooty mold and can impair photosynthesis and render fruit unmarketable, *C. pyrisuga* has recently been confirmed as a vector of "*Candidatus* Phytoplasma pyri," which causes pear decline, one of the most devastating diseases of pear trees in Europe and North America (Riedle-Bauer *et al.*, 2022). In contrast to the better-studied pear psyllid species, *C. pyri* (Linnaeus, 1758) and *C. pyricola* (Foerster, 1848), for which vibrational signals have already been described (Eben *et al.*, 2015; Jocson *et al.*, 2023), those of *C. pyrisuga* are not yet known, so we also set out to describe the basic characteristics of the signals and signal emission of this species. We expected that the pattern of the male call would be similar to related species: several short repeating elements ("chirps") followed by a longer element ("trill") (Liao *et al.*, 2022).

Materials and methods

Collecting and rearing

Adult *C. pyrisuga* were collected after overwintering in early spring in pear orchards in Starý Lískovec (Brno, Czech Republic; 49.16148°N, 16.59172°E) and in the experimental pear orchards of the Biotechnical Faculty, University of Ljubljana (Ljubljana, Slovenia; 46.04856°N, 14.47235°E) in March 2022 and 2023, respectively. Specimens were collected by beating from branches of pear trees (*Pyrus communis* L.) (Horton, 1999) into a sweep net and captured with an aspirator. Males and females were then kept separate in net cages on freshly cut pear branches at room temperature until recording.

Recording of vibrational signals

The animals were placed on a cut pear tree twig (approx. 6.5 cm long free end) fixed in a plastic container filled with water and were allowed to move freely on it. The vibrations were monitored with a laser vibrometer (PDV 100, Polytec, Germany in the laboratory of the National Institute of Biology (NIB) in Ljubljana, and VH-1000-D, Ometron, UK, at Masaryk University (MU) in Brno) pointed at a small piece of reflective tape stuck on the twig. The laser output was recorded via a sound card (Sound Blaster X-Fi) and digitized by Raven Pro 1.5 software (K. Lisa Yang Center for Conservation Bioacoustics, Cornell Lab of Ornithology, Cornell University, NY, USA) at a sampling rate of 44.1 kHz at NIB, and Photon+ Dynamic Signal Analytic System (LDS-Dactron, WI, USA) at a sampling rate of 2.56 kHz at MU. As most pilot experiments did not yield any male signals, a second computer with a sound card was used to stimulate the males with a previously recorded female signal played back via a minishaker (Type 4810 10N, Brüel & Kjær, Denmark) with a sharp-tipped metal rod pressed against the substrate and fixed with Blu-Tack putty. The stimulation consisted of a loop playback of the female signal (2.75 s pulse trains consisting of seven pulses, separated by a 9.8 s silent period) set to approximately the same amplitude as the original signal. We found that the males of *C. pyrisuga* preferentially emit their signals from the base of leaf buds and from the tip of a twig. Therefore, the twig was cut distally just above a leaf bud, so that the bud's tip protruded about 1 cm above the cut (see e.g., Cp_vid01 in the online repository). The terminology used in the description of vibrational signals follows Liao *et al.* (2022); when presenting numerical values, "*N*" denotes the number of analyzed calls (complete signals), and "*n*" the number of analyzed signal elements.

Video recording and analysis

Video recordings of the males were made in the laboratory of NIB in Ljubljana at room temperature using the FASTCAM Mini AX200 high-speed camera (Photron, Japan) with a 105 mm Sigma telemacro lens (Sigma, Japan). The sampling rate was set to 6400 Hz and was controlled by a computer running the program FASTCAM Viewer 4. The vibrations associated with wing movements were recorded simultaneously with a laser vibrometer in the same way as above, and the males were stimulated in the same way as well.

Each trial started with a playback to stimulate the male. After the male had settled in a favorable position on the twig, the high-speed camera was directed from the posterior side along the body axis so that we could observe both wing motion and the dorsal body surface when the wings were raised during signal production. The bright lighting required to provide sufficient light for high-speed video recording disturbed the males in the pilot experiments. Therefore, their vibrational emissions were instead monitored with a real-time spectrogram displayed by Raven 1.5, and the video recording was triggered along with the lighting as soon as the experimenter noticed that the signal had started.

The video recordings were exported to MP4 format at 30 fps and processed with a point-tracking algorithm to obtain the track of the wing tip oscillation. No bending of the wings was observed, so the oscillating movement of the tips was used as a proxy for the movement of the entire wings. The point-tracking algorithm is based on the Persistent Independent Particles (PIPs) tracker from Harley *et al.* (2022) and Zheng *et al.* (2023), which tracks points across multiple frames. Each point was tracked independently. We found that the released

Table 1 Temporal and spectral characteristics of vibrational signals emitted by *Cacopsylla pyrisuga* based on recordings of one female and four male specimens.

Sex	Signal element	Parameter	Mean ± SD	*n*	*N*
Female	Chirp	Duration (s)	0.08 ± 0.01	7	1
		Repetition time (s)	0.33 ± 0.07	6	1
		Fundamental frequency (Hz)	131.4 ± 6.4	7	1
Male	Chirp	Duration (s)	0.06 ± 0.02	186	43
		Repetition time (s)	0.34 ± 0.05	129	43
	Trill	Duration (s)	1.26 ± 0.30	43	43
		Fundamental frequency (Hz)	148.3 ± 19.2	43	43

N, number of analyzed calls (complete signals); *n*, number of analyzed signal elements.

PIPs and PIPs++ models performed poorly on the task at hand, largely because the temporal span in our videos is much longer than that for which these methods were designed (i.e., $>$ 10 000 frames, while the original works are designed for $<$ 100 frames). Since we were interested in tracking specific keypoints on an object rather than general-purpose tracking, we redesigned the component that captures the appearance of these targets. We created free-variable appearance vectors, one per keypoint, and optimized these along with the rest of the model using sparse annotations in the videos. The sparse annotations were created with a custom annotation tool where tracking errors were observed.

The resulting series of video coordinates (one per point per frame) was then converted back to a sampling rate of 6400 fps. We only considered the plane of motion perpendicular to the twig, as the motion of the wings was symmetric and we assumed that any lateral force was canceled by the opposite wing. We additionally considered the vertical motion of the tip of the abdomen, where we observed a slight oscillatory motion. The Y values of all points for each frame were summed after removing the offset using the R package Seewave (Sueur *et al.*, 2008), the resulting track displayed as an oscillogram and the spectrogram computed in Raven 1.5. This track was synchronized with the vibrational track using the beginning and/or end of the trill as reference points.

Morphology

The males died shortly after the experiment, whereupon we stuck them to a slide with double-sided tape with their wings pushed aside, and photographed the dorsal side with a digital microscope (Keyence VHX7000) at 300× magnification with automated focus stacking. Finally, we measured the axillary cords and counted the scale-like denticles on the digital image to calculate the expected stridulation frequency and compare it with the measurements.

Results

Vibrational signals of Cacopsylla pyrisuga

During the period when sexually mature females were available, only one female call was recorded. It consisted of seven chirps of approximately equal duration (0.08 ± 0.01 s, $n = 7$) and spacing (pause 0.33 ± 0.07 s, $n = 6$) and resonant spectral composition (Table 1, Fig. 1A). Because of the low sampling rate of the initial recordings (2.56 kHz), higher resonances were not resolvable, but the fundamental frequency of individual female chirps was 131.4 ± 6.4 Hz ($n = 7$) and the dominant frequency corresponded variously to harmonics nos. 3–8 (i.e., 360–1020 Hz).

Males never emitted signals spontaneously and had to be stimulated with playback of the female call, as described above. In response to stimulation, males readily emitted their calls, which consisted of a short series of chirps and a continuous harmonic trill (Fig. 1B). The male calls were not precisely coordinated with the playback and could begin at any stage of the loop, including during the emission of the female signals. The male calls began with a sequence of 4–5 short chirps (median $= 4$, $n = 43$) followed by an approximately 1.3 s long trill (Table 1, Fig. 1B), also with resonant spectral composition. The dominant frequency of the male trill was usually at the first harmonic (fundamental frequency) band 157.2 ± 15.8 Hz (peak; median = 161.5 Hz, $n = 20$); numerous times at the fifth harmonic band 747.1 ± 7.1 Hz (peak; median = 742.9 Hz, $n = 14$); occasionally at the fourth harmonic band 596.2 ± 14.9 Hz (peak; median = 592.2 Hz, $n = 8$); and once at the sixth harmonic band, 925.9 Hz (peak).

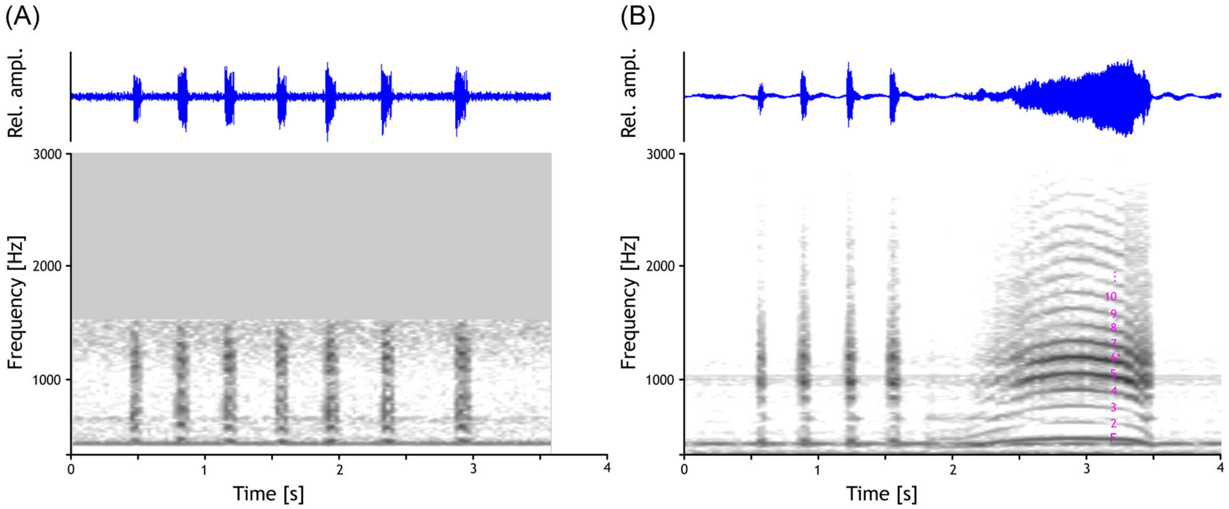

Fig. 1 Oscillograms (top) and spectrograms (bottom) of representative vibrational signals of *Cacopsylla pyrisuga*: female (A) and male (B). Labels on the male spectrogram denote harmonics F (fundamental frequency, or, the 1st harmonic) through 10th. In this signal, 6th harmonic was the dominant, denoted with an asterisk. Female signal spectrogram: Hann window, 3053 smpl, 50% overlap; male signal spectrogram: Hann window, 270 smpl, 50% overlap. Spectrogram settings differ due to the different sampling rate (2.56 kHz for the female signal recording, 44.1 kHz for the male signal recording).

Wing motion

We obtained two high-speed video recordings clearly showing the psyllid body structures in question, from a male emitting a signal in a favorable position (suppl. 1/Cp_vid06 and suppl. 2/Cp_vid08). These two were then processed using the PIPs algorithm. Cp_vid06 was the only one triggered early enough to observe chirps, but was partially overlapped by a female signal used for stimulation. Knowing the repetition time of playback loops, the substrate vibration waveform of the preceding female signal was subtracted from the overlapping signal to allow spectral analysis of the male signal. Both videos, as well as other, less clear videos in the series (Cp_vid01–Cp_vid05 and Cp_vid07) of a total of four males, and the associated vibration recordings are deposited in the Zenodo repository (https://doi.org/10.5281/zenodo.10000692).

The videos showed that the forewings were held rooflike over the body and flapped about the common axis during signal emission (Fig. 2). Each wing beat consisted of the anterior (costal) margin of the forewings being lifted to a nearly horizontal position and pushed back toward the body, while two modes could be distinguished in the movement of the hindwings: they either completely followed the movement of the forewings or they separated and stopped about halfway during upstroke, then were carried by the forewings during downstroke and moved with them until separation during the following upstroke. No relationship was apparent between these two modes and the characteristics of the resulting vibrations.

No contact was observed between the wing veins and the body surface, nor between the legs and the body, with all legs resting firmly on the substrate throughout the signal emission. In addition, the axillary sclerites of the wing were moved cleanly away from the thorax during the upstroke, also with no contact between the surfaces (Fig. 2).

Because the output of the laser vibrometer was proportional to the velocity component of the surface vibrations, the oscillogram of substrate vibrations could not be directly aligned with the summary oscillogram of wing and abdomen displacement from the resting position without a phase reference. However, the temporal and spectral composition of the spectrograms calculated from both tracks matched (Fig. 3), with broadband chirps followed by a frequency-modulated harmonic trill with a fundamental frequency of about 156.1 Hz. This, in turn, matched the structure of the trill with a wavelength of the main component of about 6.4 ms (1/0.0064 s = 156.3 Hz) and the duration of a whole forewing flap (41 frames at 6400 fps = 0.00641 s = = 156.0 Hz) (Fig. 2). A clean, steady portion of the trill in suppl. 2/Cp_vid084 (at about 3 min 13 s in the video, corresponding to position 2.85 s in the audio file and frame no. 5900 in the video track), was used for these comparisons.

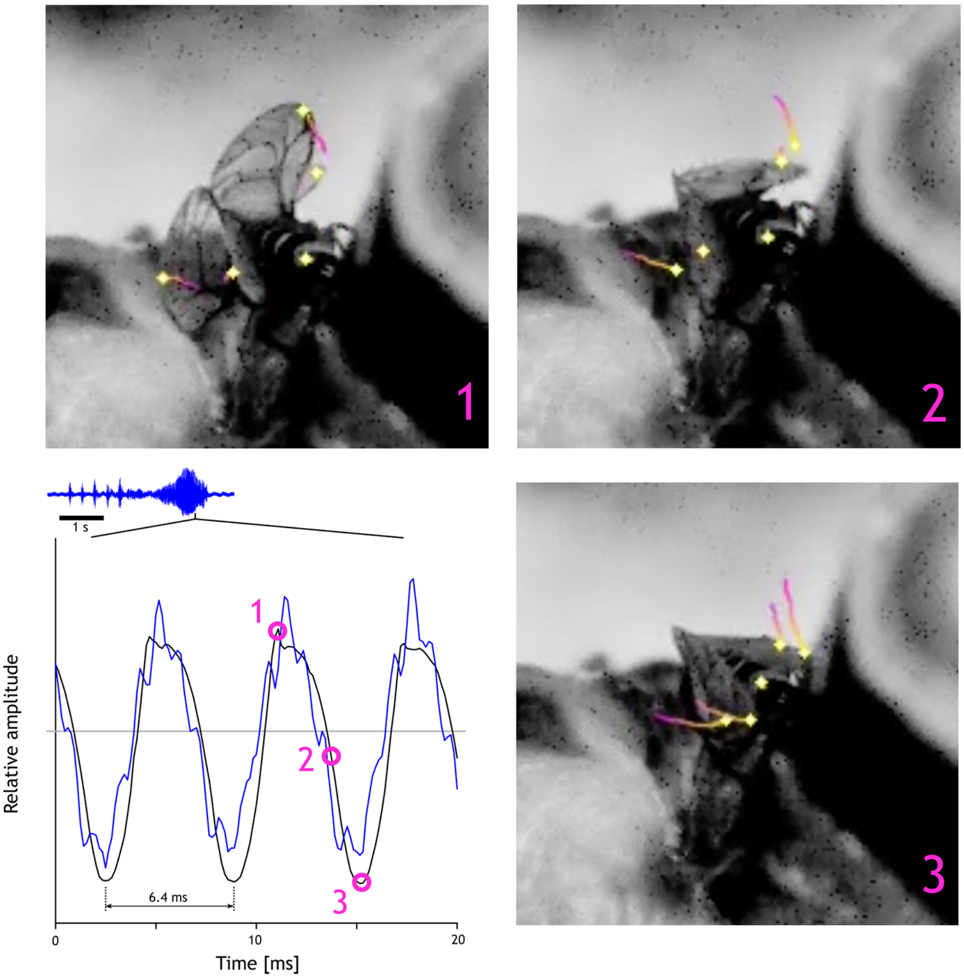

Fig. 2 Bottom left: the vibrometer track (blue) and the summary movement track (wings plus abdomen; black), with stills from the high-speed video recording (suppl. 2/Cp_vid08) showing the fully extended (1), middle (2), and resting (3) wing positions. Yellow diamonds mark the detected positions of the tracked points, with lines indicating the direction of motion. Note that the tracks were manually aligned to demonstrate matching wavelengths. However, they represent different components of the vibrations: velocity for substrate vibration (blue) and displacement for wing movement (black).

Morphology

There are two pairs of axillary cords on the dorsal side of the thorax of *C. pyrisuga* (Fig. 4). We counted 32 scale-like denticles in a row on the dorsal surface of the anterior (mesoscutellar) axillary cord (total length approximately 330 μm), while on the smaller posterior (metascutellar) cord (total length approximately 210 μm) the denticles were barely distinguishable; we counted 14. Considering the measured duration of wing flap and assuming that the entire putative file on the anterior axillary cord makes contact during scraping, this would correspond to a frequency of approximately 9700 Hz. The laser track (surface velocity) had a sufficient sampling rate to represent such high frequencies, but we detected no vibrational events in the oscillogram that could be associated with individual tooth strikes at this rate, nor any vibrational energy above 3 kHz in the spectrogram (Figs. 2 and 3).

Discussion

In the past century, several competing hypotheses have been proposed concerning the generation of vibratory signals in psyllids (Liao *et al.*, 2019, 2022; Avosani *et al.*, 2022). However, direct testing of these hypotheses has only become possible relatively recently, with the advent of more accessible high-speed video recording equipment

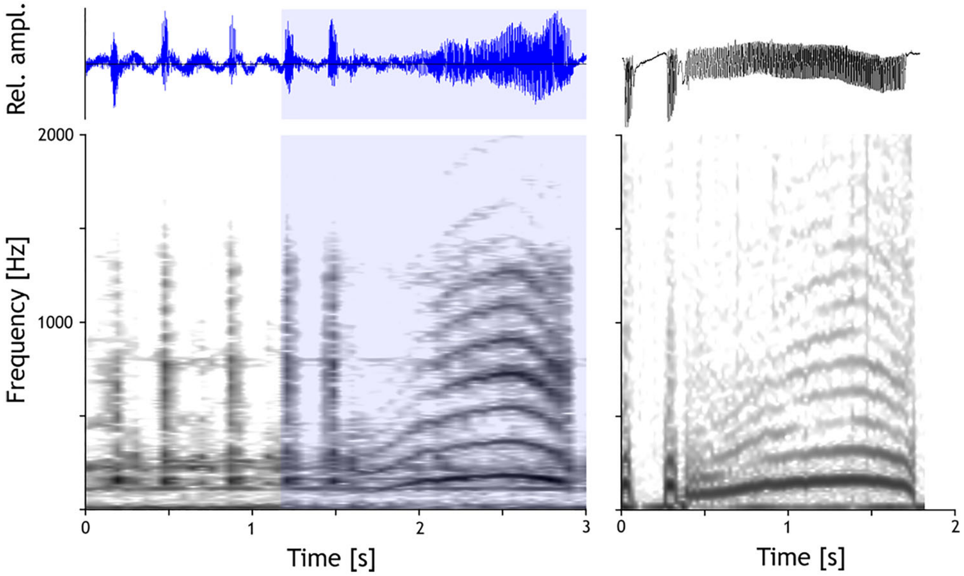

Fig. 3 Oscillograms (top) and spectrograms (bottom) of the laser track (left) and the summary movement track (wings plus abdomen) (right). The movement track is shorter because the recording could not be triggered until after the signal had begun. The shaded area of the laser track indicates the corresponding area of the movement track (length 1.82 s). Laser track spectrogram: Hann window, 3053 smpl, 50% overlap; movement track spectrogram: Hann window, 270 smpl, 50% overlap. Spectrogram settings differ due to the different sampling rate (44.1 kHz for the laser track, 6.4 kHz for the movement track).

capable of recording such small animals with sufficient resolution, as well as sensitive vibrometers.

The genus *Cacopsylla* currently includes about 460 species worldwide (Ouvrard, 2023). Vibrational signals have been described for only eight of them (Liao *et al.*, 2022; Jocson *et al.*, 2023). The pattern of male calls of *C. pyrisuga* that we recorded is similar to that of these species: a series of short, repetitive chirps followed by a longer trill (Eben *et al.*, 2015; Liao & Yang, 2015; Oppedisano *et al.*, 2020; Jocson *et al.*, 2023). The call is very similar to that of *C. picta* males, but with an even shorter trill of less than 1.5 s (compared with about 3.4 s in *C. picta*; Oppedisano *et al.*, 2020), the shortest of all known *Cacopsylla* male trills. Together with the short repetition time of the chirps (0.34 s in *C. pyrisuga* vs. 0.95 s in *C. picta*), the temporal structure seems to be species-specific, but further comparative studies are needed to confirm this. Reproduction of *C. picta* takes place on apple trees (*Malus* spp.), that is, a different host than *C. pyrisuga* (Ossiannilsson, 1992; Oppedisano *et al.*, 2020). The male calls of *C. pyri* and *C. pyricola*, which are sympatric and often occur together with *C. pyrisuga* on pear trees in Europe, differ from *C. pyrisuga* by a larger number of chirps and a much longer trill (Eben *et al.*, 2015; Jocson *et al.*, 2023). Due to the small sample size of the present study, it is not possible to definitively describe female calls or the duet sequence, but judging from the playback experiments, the mates appear to form a loose duet in the sense of Liao *et al.* (2022). A more complete description will be presented in a later paper.

In our direct observation of movements associated with signal production in *C. pyrisuga*, we found no support for the stridulation hypothesis of signal production, although we confirmed that these animals have corrugated axillary cords like all other psyllid species (Ouvrard *et al.*, 2008) and winged insects (Snodgrass, 1935). We did not observe any contact between the wings and axillary cords or any other putative scraper-file pair proposed by previous authors for Psylloidea (Lubanga *et al.*, 2014; Liao *et al.*, 2019, 2022; Avosani *et al.*, 2022), nor any vibrational events or spectral components consistent with the expected rate of tooth strikes at the observed wing-beat frequency. Instead, we found support for the wing buzzing (“wingbeat”) hypothesis proposed by Wenninger *et al.* (2009), as the frequency spectrum of oscillatory wing motion matches the spectrum of resulting substrate-borne vibrations recorded nearby. This result is also consistent with the results of Liao *et al.* (2019), although these authors derived a different hypothesis from their experiments. They found that the psyllids were still able to produce vibrations after cutting off the anal area of the forewing or cutting off the forewing except for the axillary sclerites, but the amplitude of the vibrational signal was significantly reduced compared to the control. They

Fig. 4 Dorsal view of left half of thorax and basal part of wings in male of *Cacopsylla pyrisuga* under 300× magnification showing axillary cords (length measured) with scale-like denticles.

concluded that both the anal area and the axillary sclerites play an important role in signal production by friction on the thorax. However, removing portions of the forewing would reduce drag and mass, thus producing weaker vibrations than intact wings, even with wing buzzing when no friction is involved.

Stridulation tends to produce broadband acoustic and/or vibrational signals with a fundamental frequency equal to the rate of individual tooth strikes, and is often associated with defensive signals in insects (Low *et al.*, 2021). Instead, the harmonic structure of the trills of *C. pyrisuga* resembles the vibrations produced by the wing buzzing of several species of stinkbugs (Hemiptera: Pentatomidae) (Čokl *et al.*, 2021). The buzzing signals of Diptera and Hymenoptera also show a certain similarity. However, the vibrations in these latter cases seem to be generated by the action of the flight muscles and the elasticity of the thorax, but largely decoupled from the wing movement, as for example in *Drosophila* spp. (Diptera: Drosophilidae) (Mazzoni *et al.*, 2013) and *Melipona seminigra* (Hymenoptera: Apidae) (Hrncir *et al.*, 2008), where higher frequencies are generated than during flight. It seems that in psyllids, the forewings are the main driver of signal generation, which was also confirmed by wing cutting experiments where signal amplitude was reduced (Liao *et al.*, 2019), in contrast with drosophilids or annoyance buzzing in stingless bees, where amputation of wings did not affect signal generation and properties (Hrncir *et al.*, 2008; Mazzoni *et al.*, 2013). The function of hindwing movements in psyllids remains unclear, but the videos suggest the possibility that uncoupling slows the forewing to make the upstroke and/or subsequent downstroke less powerful.

Regarding the issue of higher resonances sometimes becoming dominant, the influence of the substrate on the frequency of the vibrational signal is well known (Čokl, 2008; Cocroft *et al.*, 2010; Eberhard *et al.*, 2010) and is related to frequency-dependent patterns of standing waves formed in elastic solid substrates (Polajnar *et al.*, 2012). Intraspecific variations in signal frequency do not alter the responsiveness of female psyllids and may change with the position of the signaling animal or temperature, suggesting that frequency does not contain crucial information (Lubanga *et al.*, 2016; Jocson *et al.*, 2023). Unlike some other reports (e.g., Percy *et al.*, 2006; Liao *et al.*, 2019), the fundamental frequency was the dominant frequency in half of the recordings on which this study is based, but the 4th, 5th and rarely the 6th harmonic can also be dominant in *C. pyrisuga*. We therefore argue that the fundamental frequency should be the focus when we report on the characteristics of psyllid vibrational signals.

Wing buzzing also produces air movement. As has been shown in *Drosophila*, the small size makes the wings a poor impedance match to the surrounding air at low frequencies, so far-field sound is very weak and unlikely to affect behavior, whereas the effective range of near-field sound (air particle motion) is about 1/50 λ (Bennet-Clark, 1998). Psyllids perform vibrational duets and searches over distances exceeding the length of the host plant's modules, that is, many times their body size (Lubanga *et al.*, 2014), so the long-distance vibrational component is undoubtedly crucial, while near-field sound might play a role just before copulation when the male arrives near the female—if at all.

We predict that wing buzzing will in time be confirmed as the mechanism of signal production in all other vibrating Psylloidea, with any other mechanism playing at best a complementary role. Not all published descriptions of vibrational signals in the Psylloidea include spectrograms, but those we are aware of have the same mix of broadband and harmonic components with a fundamental frequency in the 100–200 Hz range and no significant vibrational components above 2 kHz, whether they are species of the same genus (i.e., *Cacopsylla*; Eben *et al.*, 2015; Liao & Yang, 2015; Oppedisano *et al.*, 2020; Jocson *et al.*, 2023), different genera of the Psyllidae (e.g., *Diaphorina* Löw, 1880; Wenninger *et al.*, 2009), or different families (e.g., Triozidae; Percy *et al.*, 2006; Liao *et al.*, 2016; Lubanga *et al.*, 2016; and Carsidaridae; Liao & Yang, 2017). Further work is needed to confirm this generalization and clarify the details. Nevertheless, we think that claims about stridulation based on morphology—that is, the presence of putative stridula—as is common in the older literature (cf. Davranoglou *et al.*, 2019; Virant-Doberlet *et al.*, 2023) should always be supported by direct behavioral evidence.

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Disclosure

The authors declare no conflicts of interest.

References

Arthur, B.J., Emr, K.S., Wyttenbach, R.A. and Hoy, R.R. (2014) Mosquito (*Aedes aegypti*) flight tones: frequency, harmonicity, spherical spreading, and phase relationships. *The Journal of the Acoustical Society of America*, 135, 933–941.

Avosani, S., Mankin, R.W., Sullivan, T.E.S., Polajnar, J., Suckling, D.M. and Mazzoni, V. (2022) Vibrational communication in psyllids. In *Biotremology: Physiology, Ecology, and Evolution* (eds. P.S.M. Hill and V. Mazzoni, N. Stritih-Peljhan, M. Virant-Doberlet & A. Wessel), pp. 529–546. Springer, Cham.

Bennet-Clark, H.C. (1998) Size and scale effects as constraints in insect sound communication. *Philosophical Transactions of the Royal Society London B*, 353, 407–419.

Bluemel, J.K., Derlink, M., Pavlovcic, P., Russo, I.R.M., King, R.A., Corbett, E. *et al.* (2014) Integrating vibrational signals, mitochondrial DNA and morphology for species determination in the genus *Aphrodes* (Hemiptera: Cicadellidae). *Systematic Entomology*, 39, 304–324.

Burckhardt, D. (1994) Psylloid pests of temperate and subtropical crop and ornamental plants (Hemiptera, Psylloidea): a review. *Trends in Agricultural Science (Entomology)*, 2, 173–186.

Civolani, S., Soroker, V., Cooper, W.R. and Horton, D.R. (2023) Diversity, biology, and management of the pear psyllids: a global look. *Annals of the Entomological Society of America*, 116, 331–357.

Cocroft, R.B., Rodríguez, R.L. and Hunt, R.E. (2010) Host shifts and signal divergence: mating signals covary with host use in a complex of specialized plant-feeding insects. *Biological Journal of the Linnean Society*, 99, 60–72.

Čokl, A. (2008) Stink bug interaction with host plants during communication. *Journal of Insect Physiology*, 54, 1113–1124.

Čokl, A., Zorović, M., Žunič, A. and Virant-Doberlet, M. (2005) Tuning of host plants with vibratory songs of *Nezara viridula* L. (Heteroptera: Pentatomidae). *The Journal of Experimental Biology*, 208, 1481–1488.

Čokl, A., Žunič, A. and Millar, J.G. (2009) Transmission of *Podisus maculiventris* tremulatory signals through plants. *Central European Journal of Biology*, 4, 585–594.

Čokl, A., Žunič Kosi, A., Blassioli Moraes, M.C., Borges, M. and Laumann, R.A. (2021) Stink bug inter-plant communication with signals produced by vibration of lifted wings. *Journal of Insect Behavior*, 34, 194–210.

Davranoglou, L.R., Mortimer, B., Taylor, G.K. and Malenovský, I. (2019) On the morphology and possible function of two putative vibroacoustic mechanisms in derbid planthoppers (Hemiptera: Fulgoromorpha: Derbidae). *Arthropod Structure & Development*, 52, 1008 80.

Davranoglou, L.R., Taylor, G.K. and Mortimer, B. (2023) Sexual selection and predation drive the repeated evolution of stridulation in Heteroptera and other arthropods. *Biological Reviews*, 98, 942–981.

Eben, A., Mühlethaler, R., Gross, J. and Hoch, H. (2015) First evidence of acoustic communication in the pear psyllid *Cacopsylla pyri* L. (Hemiptera: Psyllidae). *Journal of Pest Science*, 88, 87–95.

Eberhard, M.J.B., Lang, D., Metscher, B., Pass, G., Picker, M.D. and Wolf, H. (2010) Structure and sensory physiology of the leg scolopidial organs in Mantophasmatodea and their role in vibrational communication. *Arthropod Structure & Development*, 39, 230–241.

Grafton-Cardwell, E.E., Stelinski, L.L. and Stansly, P.A. (2013) Biology and management of Asian citrus psyllid, vector of the huanglongbing pathogens. *Annual Review of Entomology*, 58, 413–432.

Hardy, N.B. (2018) The biodiversity of Sternorrhyncha: scale insects, aphids, psyllids, and whiteflies. In *Insect Biodiversity. Science and Society. Volume II* (eds. R.G. Foottit & P.H. Adler), pp. 591–625. Wiley Blackwell, Hoboken, Chichester.

Harley, A.W., Fang, Z. and Fragkiadaki, K. (2022) Particle video revisited: Tracking through occlusions using point trajectories. In *European Conference on Computer Vision—ECCV 2022. ECCV 2022. Lecture Notes in Computer Science* (eds. S. Avidan, G. Brostow, M. Cissé, G.M. Farinella & T. Hassner), pp. 23–27. Springer, Cham.

Horton, D.R. (1999) Monitoring of pear psylla for pest management decisions and research. *Integrated Pest Management Reviews*, 4, 1–20.

Hrncir, M., Gravel, A.I., Schorkopf, D.L.P., Schmidt, V.M., Zucchi, R. and Barth, F.G. (2008) Thoracic vibrations in stingless bees (*Melipona seminigra*): resonances of the thorax influence vibrations associated with flight but not those associated with sound production. *Journal of Experimental Biology*, 211, 678–685.

Jarausch, B., Tedeschi, R., Sauvion, N., Gross, J. and Jarausch, W. (2019) Psyllid vectors. In *Phytoplasmas: Plant Pathogenic Bacteria—II* (eds. A. Bertaccini, P.H. Weintraub, G.P. Rao & N. Mori), pp. 53–78. Springer Nature, Singapore.

Jocson, D.M.I., Gonzales, M.T., Horton, D.R., Nottingham, L.B., Beers, E.H., Oeller, L.C. *et al.* (2023) Characterizing substrate-borne vibrational mating signals produced by pear psylla, *Cacopsylla pyricola* (Hemiptera: Psyllidae). *Journal of Insect Behavior*, https://doi.org/10.1007/s10905-023-09839-2.

Liao, Y.C. and Yang, M.M. (2015) Acoustic communication of three closely related psyllid species: a case study in clarifying allied species using substrate-borne signals (Hemiptera: Psyllidae: *Cacopsylla*). *Annals of the Entomological Society of America*, 108, 902–911.

Liao, Y.C. and Yang, M.M. (2017) First evidence of vibrational communication in Homotomidae (Psylloidea) and comparison of substrate-borne signals of two allied species of the genus *Macrohomotoma* Kuwayama. *Journal of Insect Behavior*, 30, 567–581.

Liao, Y.C., Huang, S.S. and Yang, M.M. (2016) Substrate-borne signals, specific recognition, and plant effects on the acoustics of two allied species of *Trioza*, with the description of a new species (Psylloidea: Triozidae). *Annals of the Entomological Society of America*, 109, 906–917.

Liao, Y.C., Wu, Z.Z. and Yang, M.M. (2019) Vibrational behavior of psyllids (Hemiptera: Psylloidea): Functional morphology and mechanisms. *PLoS ONE*, 14, e0215196.

Liao, Y.C., Percy, D.M. and Yang, M.M. (2022) Biotremology: vibrational communication of Psylloidea. *Arthropod Structure & Development*, 66, 101138.

Low, M.L., Naranjo, M. and Yack, J.E. (2021) Survival sounds in insects: diversity, function, and evolution. *Frontiers in Ecology and Evolution*, 9, 641740.

Lubanga, U.K., Guédot, C., Percy, D.M. and Steinbauer, M.J. (2014) Semiochemical and vibrational cues and signals mediating mate finding and courtship in Psylloidea (Hemiptera): a synthesis. *Insects*, 5, 577–595.

Lubanga, U.K., Peters, R.A. and Steinbauer, M.J. (2016) Substrate-borne vibrations of male psyllids vary with body size and age but females are indifferent. *Animal Behaviour*, 120, 173–182.

Lujo, S., Hartman, E., Norton, K., Pregmon, E.A., Rohde, B.B. and Mankin, R.W. (2016) Disrupting mating behavior of *Diaphorina citri* (Liviidae). *Journal of Economic Entomology*, 109, 2373–2379.

Mankin, R.W., Hagstrum, D.W., Smith, M.T., Roda, A.L. and Kairo, M.T.K. (2011) Perspective and promise: a century of insect acoustic detection and monitoring. *American Entomologist*, 57, 30–44.

Mankin, R.W., Rohde, B.B., Mcneill, S.A., Paris, T.M., Zagvazdina, N.I. and Greenfeder, S. (2013) *Diaphorina citri* (Hemiptera: Liviidae) responses to microcontroller-buzzer communication signals of potential use in vibration traps. *Florida Entomologist*, 96, 1546–1555.

Mankin, R.W., Rohde, B. and McNeill, S. (2016) Vibrational duetting mimics to trap and disrupt mating of the devastating Asian citrus psyllid insect pest. *Proceedings of Meetings on Acoustics*, 25, 010006.

Mauck, K.E., Gebiola, M. and Percy, D.M. (2023) The hidden secrets of Psylloidea: biology, behavior, symbionts, and ecology. *Annual Review of Entomology*, 69, 277–302.

Mazzoni, V., Anfora, G. and Virant-Doberlet, M. (2013) Substrate vibrations during courtship in three *Drosophila* species. *PLoS ONE*, 8, e80708.

Moreno, A., Miranda, M.P. and Fereres, A. (2021) Psyllids as major vectors of plant pathogens. *Entomologia Generalis*, 41, 419–438.

Munyaneza, J.F. (2012) Zebra chip disease of potato: biology, epidemiology, and management. *American Journal of Potato Research*, 89, 329–350.

Oppedisano, T., Polajnar, J., Kostanjšek, R., De Cristofaro, A., Ioriatti, C., Virant-Doberlet, M. *et al.* (2020) Substrate-borne vibrational communication in the vector of apple proliferation disease *Cacopsylla picta* (Hemiptera: Psyllidae). *Journal of Economic Entomology*, 113, 596–603.

Ossiannilsson, F. (1950) Sound-production in Psyllids (Hem. Hom.). *Opuscula Entomologica*, 15, 202.

Ossiannilsson, F. (1992) *The Psylloidea (Homoptera) of Fennoscandia and Denmark*. Brill, Leiden—New York—Köln.

Ouvrard, D. (2023) Psyl'list—The World Psylloidea Database. https://flow.hemiptera-databases.org/psyllist/ (accessed on 8 October 2023)

Ouvrard, D., Burckhardt, D., Soulier-Perkins, A. and Bourgoin, T. (2008) Comparative morphological assessment and phylogenetic significance of the wing base articulation in Psylloidea (Insecta, Hemiptera, Sternorrhyncha). *Zoomorphology*, 127, 37–47.

Percy, D.M., Taylor, G.S. and Kennedy, M. (2006) Psyllid communication: acoustic diversity, mate recognition and phylogenetic signal. *Invertebrate Systematics*, 20, 431–445.

Polajnar, J., Svenšek, D. and Čokl, A. (2012) Resonance in herbaceous plant stems as a factor in vibrational communication of pentatomid bugs (Heteroptera: Pentatomidae). *Journal of the Royal Society Interface*, 9, 73.

Polajnar, J., Eriksson, A., Lucchi, A., Anfora, G., Virant-Doberlet, M. and Mazzoni, V. (2015) Manipulating behaviour with substrate-borne vibrations—potential for insect pest control. *Pest Management Science*, 71, 15–23.

Riedle-Bauer, M., Paleskic, C., Schönhuber, C., Staples, M. and Brader, G. (2022) Vector transmission and epidemiology of "*Candidatus* Phytoplasma pyri" in Austria and identification of *Cacopsylla pyrisuga* as new pathogen vector. *Journal of Plant Diseases and Protection*, 129, 375–386.

Snodgrass, R.E. (1935) *Principles of Insect Morphology*. McGraw-Hill, New York.

Sueur, J., Aubin, T. and Simonis, C. (2008) Seewave: a free modular tool for sound analysis and synthesis. *Bioacoustics*, 18, 213–226.

Taylor, K.L. (1985) A possible stridulatory organ in some Psylloidea (Homoptera). *Australian Journal of Entomology*, 24, 77–80.

Tishechkin, D.Y. (2006) On the structure of stridulatory organs in jumping plant lice (Homoptera: Psyllinea). *Russian Entomological Journal*, 15, 335–340.

Virant-Doberlet, M., Stritih-Peljhan, N., Žunič-Kosi, A. and Polajnar, J. (2023) Functional diversity of vibrational signalling systems in insects. *Annual Review of Entomology*, 68, 191–210.

Wells, M.M. and Henry, C.S. (1998) Songs, reproductive isolation, and speciation in cryptic species of insects. In *Endless Forms: Species and Speciation* (eds. D.J. Howard & S.H. Berlocher), pp. 217–233. Oxford University Press, Oxford.

Wenninger, E.J., Hall, D.G. and Mankin, R.W. (2009) Vibrational communication between the sexes in *Diaphorina citri* (Hemiptera: Psyllidae). *Annals of the Entomological Society of America*, 102, 547–555.

Yang, M.M., Yang, C.T. and Chao, J.T. (1986) Reproductive isolation and taxonomy of two Taiwanese *Paurocephala* species (Homoptera: Psylloidea). *Taiwan Museum Special Publication Series*, 6, 177–203.

Zheng, Y., Harley, A.W., Shen, B., Wetzstein, G. and Guibas, L.J. (2023) Pointodyssey: a large-scale synthetic dataset for long-term point tracking. *Proceedings of the IEEE/CVF International Conference on Computer Vision (ICCV)*, 19855–19865.

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Supporting Information

The two processed videos (Cp_vid06 and Cp_vid08) are available as supplementary files to this paper. All recordings and additional supporting information can be accessed online in the dataset openly deposited in Zenodo repository [https://doi.org/10.5281/zenodo.10000692].