

Comparative analysis of male calling signals in closely related species of *Macropsis* Lewis, 1836 (Homoptera: Auchenorrhyncha: Cicadellidae: Eurymelinae: Macropsini) reveals possible ways of evolution of the signal temporal pattern

Сравнительный анализ призывных сигналов самцов близкородственных видов *Macropsis* Lewis, 1836 (Homoptera: Auchenorrhyncha: Cicadellidae: Eurymelinae: Macropsini) позволяет выявить возможные пути эволюции временного рисунка сигналов

**D.Yu. Tishechkin
Д.Ю. Тишечкин**

Department of Entomology, Faculty of Biology, M.V. Lomonosov Moscow State University, Vorobyevy Gory, Moscow 119234, Russia. E-mail: macropsis@yandex.ru

Кафедра энтомологии Биологического факультета Московского государственного университета имени М.В. Ломоносова, Воробьёвы Горы, Москва 119234, Россия.

KEY WORDS: Homoptera, Auchenorrhyncha, Cicadellidae, *Macropsis*, calling signals, variability, evolution.

КЛЮЧЕВЫЕ СЛОВА: Homoptera, Auchenorrhyncha, Cicadellidae, *Macropsis*, призывные сигналы, изменчивость, эволюция.

ABSTRACT. It is shown that, in some closely related *Macropsis* species, male calling signals consist of similar components but clearly differ in the duration of homologous parts, the shape of syllables, the presence/absence of additional components, or the presence/absence of gaps between the signal parts. In some other leafhoppers, such differences are intraspecific and can be observed in the signals of males from the same population and even in the same male. Thus, in the considered *Macropsis* species, signals were apparently formed by fixing different originally intraspecific variants in different phylogenetic lineages in the course of their divergence. This suggests that intraspecific variability can become a basis for rapid divergence of signal patterns if in different populations different pattern variants gain a selective advantage. Based on this, we can assume that male calling signals in leafhoppers can evolve not only by a gradual change in the quantitative parameters such as the signal parts duration but also through qualitative changes of temporal pattern due to adding new components, change of a syllable shape, and splitting a single signal into several parts. Since in closely related leafhopper species it is the differences in signal patterns that provide reproductive isolation, such changes can result in rapid speciation.

РЕЗЮМЕ. Показано, что у некоторых близкородственных видов *Macropsis* призывные сигналы самцов состоят из сходных компонентов, но чётко различаются по их длительности, форме серий, наличию/отсутствию дополнительных компонентов или наличию/отсутствию пауз между частями сигнала. У ряда других видов Cicadellidae подобные различия представляют собой внутривидовые вариации, наблюдающиеся в сигналах самцов из одной популяции и даже у одного и того же самца. Это позволяет предположить, что у рассмотренных видов *Macropsis* сигналы сформировались за счёт фиксации разных изначально внутривидовых вариантов в разных филогенетических линиях в процессе их дивергенции. Таким образом, внутривидовая изменчивость может стать основой для быстрой дивергенции структуры сигналов, если в разных популяциях разные варианты сигнала получают селективное преимущество. При этом у цикадовых призывные сигналы могут эволюционировать не только в результате постепенного изменения количественных параметров, например, длительности частей сигнала, но и посредством качественных изменений временного рисунка за счёт добавления новых ком-

понентов, изменения формы серий или разделения сигнала на несколько частей. Поскольку у близкородственных видов Cicadellidae репродуктивную изоляцию обеспечивают именно различия в структуре сигналов, подобные изменения могут привести к быстрому видообразованию.

Introduction

In many insect taxa, the male produces acoustic calling signals for attracting a conspecific female. Presently, it is common knowledge that, in such taxa, differences in calling signal patterns facilitate precopulatory reproductive isolation between closely related species. Thus, the trigger of divergence and, as a consequence, of speciation in such taxa can be the changes in the mating signal pattern.

In principle, speciation can occur by gradual change over long periods of time, or very rapidly or even instantaneously. In other words, it can occur through large-scale morphological and ecological divergence, or because of changes of single traits that directly affect reproductive isolation. Accordingly, in the genomes, it may appear as a deep and large-scale changes or affect just a few loci. Polygenic control of mating signal traits may suggest that signal patterns have diverged gradually, whereas single or few genes may imply that they can diverge more rapidly [Henry et al., 2002; Vedenina et al., 2006; Xu, Shaw, 2019].

Nilaparvata lugens (Stål, 1854) (Homoptera: Delphacidae) in the Pacific Region provides an example of a gradual change in the quantitative parameters of the signal in the course of divergence. The general scheme of signal temporal pattern in this species remains constant throughout its range. Populations differ only in pulse repetition frequency and these differences reflect genetic differentiation between them. The populations from Philippines and Australia were most difficult to hybridize in the laboratory and showed the greatest differences in pulse repetition frequency. However, in those crosses which were successful, there were no obvious indications of hybrid non-viability. Pulse repetition frequencies in hybrids showed values intermediate between and significantly different from both parental populations. Thus, differences in pulse repetition frequency is the only reproductive barrier on the current stage of divergence of these forms. This clearly indicates, that, first, the populations from Philippines and Australia represent an early stage of speciation process and, second, pre-mating ethological isolating mechanisms have begun to evolve in advance of post-mating ones [Claridge et al., 1985a]. Similar situation exists in Gomphocerinae (Orthoptera: Acrididae), since some grasshopper species producing different signals can be hybridised under laboratory conditions and hybrids are fertile [Vedenina, von Helversen, 2003].

Examples of geographic quantitative signal variation are also known in other taxa. The geographic variation of signals in North American orthopterans was

described by Walker [1974]. The study of molecular, acoustic, and morphological differentiation in *Gampsocleis sedakovii* (Fischer von Waldheim, 1846) (Orthoptera: Tettigoniidae) from six localities in Inner Mongolia, China showed that the structure of acoustic signals is closely related to genetic differences between populations [Zhang et al., 2015]. Booij [1982] found evidence for geographic quantitative signal variation in *Muellerianella* (Homoptera: Delphacidae) in Western Europe.

Sometimes not geographical isolation, but differences in host specialisation results in reproductive isolation and acoustic divergence. *N. lugens* can feed on cultivated and wild rices and on *Leersia hexandra* Schwartz, 1788 (Poaceae), an abundant weed often growing along the edges of rice fields. Populations of *N. lugens* from rice and *L. hexandra* distinctly differ in pulse repetition frequency, under laboratory conditions when given a choice always preferred a mate from the own population, but can hybridize in no-choice crosses. Hybrids show signal parameters intermediate between parent populations but no obvious indications of hybrid non-viability. However, no males with “hybrid” signal parameters were found in nature. This indicates a high efficiency of the reproductive barrier between the two host races and allowed to conclude that they have already reached the level of different biological species [Claridge et al., 1985b].

Thus, the *N. lugens* species complex demonstrates examples of both allopatric (geographical) and sympatric (due to host shift) speciation. Remarkably, that in both cases only quantitative signal parameters evolve.

Similarly, in closely related species of planthoppers from the genus *Ribautodelphax* Wagner, 1963 (Homoptera: Delphacidae) male calling signals have rather complex, but similar temporal patterns so that signals of different species differ mostly in quantitative parameters [den Bieman, 1986]. Male calling signals of cryptic species of *Enchenopa binotata* (Say, 1824) complex (Homoptera: Membracidae) also demonstrate only quantitative differences. A carrier frequency of a signal is the most significant for conspecific mate recognition; differences in temporal patterns are less distinct and not so much affect the choice of male by female [Cocroft et al., 2008].

Under experimental conditions, quantitative signal parameters can change quite rapidly. Artificial bidirectional selection on pulse repetition frequency in female calls of *Ribautodelphax imitans* (Ribaut, 1953) (Homoptera: Delphacidae) resulted in complete divergence in this parameter without overlap between two lines after only five generations. Male choice tests after 10 generations revealed significant preference of mates from the same line [de Winter, 1992].

Under natural conditions, quantitative signal parameters also can evolve during rather short time span. Cave-dwelling planthoppers from the *Oliarus polyphe-mus* Fennah, 1973 species complex (Homoptera: Cixiidae) colonized lava tubes on several volcanoes on the Hawaii Island. In each cave system they formed sepa-

rate biological species differing in pulse repetition period and syllable duration in male and female signals. The age of some caves is 350–500 years, the youngest cave ages 110 years. Thus, the age of species inhabiting these caves is less than 350–500 years and is less than 110 years for the inhabitants of the youngest cave, since they could colonize a cave only after their host plant will grow on the surface above the cave and its roots on which the insects feed will penetrate into the cave [Hoch, Howarth, 1993].

Possibility of rapid qualitative changes in the signal temporal pattern may seem dubious. However, it is indirectly confirmed by hybridization experiments.

Male signals of hybrids of two species of *Nephotetix* (Homoptera: Cicadellidae: Deltocephalinae) turned out to be very variable and included elements of signals of both parental species [Claridge, 1985]. In the *Chorthippus albomarginatus* (De Geer, 1773) (Orthoptera: Acrididae: Gomphocerinae) species group novel elements and novel combinations of elements sometimes present in the songs of hybrid specimens [Vedenina, von Helversen, 2003]. In this taxon, different patterns of hybrid songs demonstrate the transition from the parental song of one species to the parental song of another one. The authors suggest that this transition probably illustrates the evolution of songs in the process of divergence and the formation of different species. Hybrid males of *Stenobothrus clavatus* Willemsse, 1979 and *S. rubicundus* (Germar, 1817) (Orthoptera: Acrididae: Gomphocerinae) from Northern Greece also produced courtship songs with intermediate features between parental songs or with completely new elements [Vedenina et al., 2012].

Our study of the signals of Cicadellidae showed that, in some species, signals demonstrate considerable variability of not only duration and repetition period of signal components, but also of the general scheme of temporal pattern [Tishechkin, 2010]. Signals of males from the same sample or even signals of the same male can differ in the syllable shape or in the presence/absence of comparatively long and elaborate components. It can be assumed that such variability can become a basis for rapid divergence of signal patterns and, as a consequence, speciation if in different populations different patterns gain a selective advantage and become genetically fixed. In this article, we illustrate this hypothesis by the examples of signals in several groups of closely related species of *Macropsis* Lewis, 1836 (Homoptera: Auchenorrhyncha: Cicadellidae: Eurymelinae: Macropsini).

Material and methods

Leafhopper vibrational calling signals were recorded by means of portable recording equipment consisting of a piezocrystal gramophone cartridge GZP-311 connected to the microphone input of a cassette recorder Elektronika-302-1 (before 2005), minidisk recorder Sony Walkman MZ-NH900 (2005–2016), or Roland R-05

wave/mp3 recorder (after 2017) via a custom-made matching amplifier. For recording, a twig of the host plant about 10–15 cm in length was attached to the cartridge by a rubber ring so that the cartridge needle touched the stem slightly. Then a nylon cage containing a male leafhopper was put on the twig. After a time, the male usually sat on the twig and started singing.

Oscillograms of signals were produced with Cool Edit Pro 2.1 software.

For elements of signal temporal pattern the following terms are used. **Pulse** is a brief elementary fragment of signal (or succession of sine waves) with rapid increase and subsequent decrease of amplitude, i.e. separated from similar fragments by amplitude minimums. Short fragments with constant temporal pattern usually repeated with regular intervals and consisting of uniform or different pulses are referred to as **syllables**. Any more or less prolonged signal with complex pattern (e.g. succession of similar or different syllables) is referred to as a **phrase**.

Data on collecting sites and temperature during recording for the signals presented on oscillograms are given in the Table. All specimens investigated are deposited in the collection of the Zoological Museum of M.V. Lomonosov Moscow State University.

Results

In the temperate zone of the Palearctic, the genus *Macropsis* includes a large number of cryptic species, almost indistinguishable by morphological traits. Typically, closely related species, despite their morphological similarity, have nothing in common in signal patterns. However, in some species, signals include similar or even almost identical components. Examples of such signals are considered below.

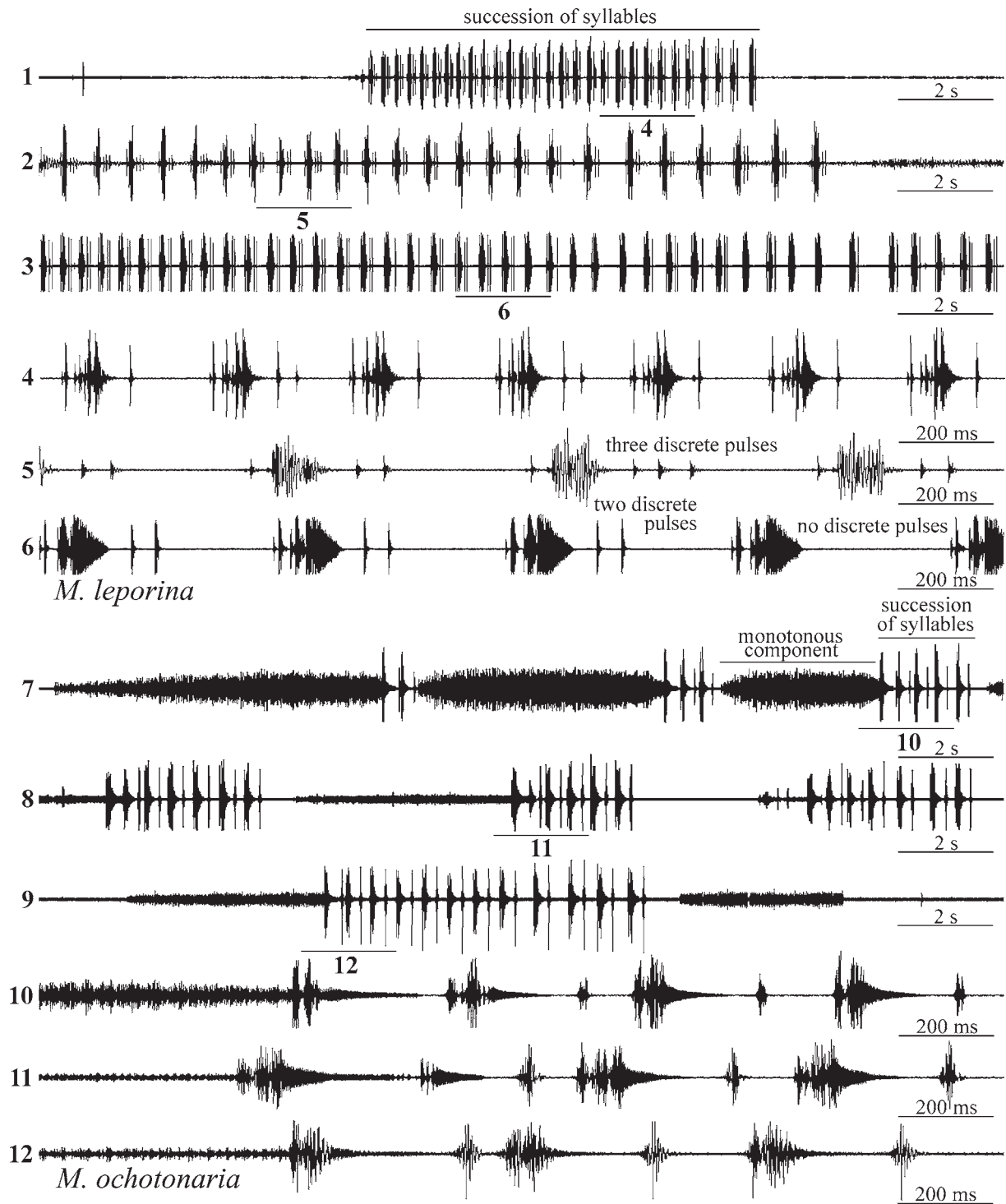
1. *M. leporina* Tishechkin, 1997 and *M. ochotonaria* Tishechkin, 1994 are two closely related species indistinguishable by morphological traits, often occurring in the same locality but dwelling on willows from different sections [Tishechkin, 1999]. In *M. leporina*, male calling signal is a long (up to 20–30 s) succession of short syllables alternating with 1–3 discrete pulses (Figs 1–6); occasionally, these pulses are absent (Fig. 6, the end of the oscillogram). In *M. ochotonaria*, calling signal is a single or repeated phrase consisting of rather low-amplitude monotonous component and of a succession of syllables alternating with single discrete pulses, almost as in *M. leporina* (Figs 7–12). Ranges of syllable repetition period in these two species completely overlap, but in *M. ochotonaria* syllable succession is usually shorter and includes no more than 10–15 syllables (Figs 7–9).

Thus, in the signal of *M. ochotonaria*, in comparison with *M. leporina*, an additional component (monotonous low-amplitude vibrations) is added, the shape of syllables slightly changes and their number decreases.

2. *M. flavida* Vilbaste, 1980, *M. multa* Tishechkin, 1997, *M. impura* (Boheman, 1847), and *M. daurica*

Table. Data for recordings of calling signals of the studied species of Cicadellidae.
Таблица. Данные о записях призывных сигналов изученных видов Cicadellidae.

Species (in the same order as on figures)	Locality	Air temperature during recording, °C
<i>Macropsis leporina</i> Tish.	Figs 1, 4: Russia, Chita Oblast, the mouth of Talacha River, ca. 12 km E of Urul'ga Village, from <i>Salix schwerinii</i> E.L. Wolf.	27
	Figs 2, 5: Russia, Primorsky Krai, env. Ryazanovka Village, 12 km SW of Slavyanka Town, from <i>S. schwerinii</i> .	22–23
	Figs 3, 6: Russia, Khabarovsk Krai, env. Obluchye Town, from <i>S. schwerinii</i> .	27
<i>M. ochotonaria</i> Tish.	Figs 7–8, 10–11: Russia, Chita Oblast, the mouth of Talacha River, ca. 12 km E of Urul'ga Village, from <i>S. miyabeana</i> Seemen.	20–25
	Figs 9, 12: Russia, Buryatia, the Bryanka River Valley, 12 km E of Onokhoi, from <i>Salix</i> sp.	24–25
<i>M. flavida</i> Vilb.	Russia, Amur Oblast, env. Kostyukovka Village, 30 km W of Svobodny Town, from <i>S. udensis</i> Trautv. et C.A. Mey.	27
<i>M. multa</i> Tish.	Russia, Irkutsk Oblast, env. Kosaya Steppe Village, ca. 22 km NW of Elantsy, from <i>S. rhamnifolia</i> Pall.	24–25
<i>M. impura</i> (Boh.)	Figs 21, 23: Russia, Volgograd Oblast, 6 km SW of Ilovlya Village, <i>S. rosmarinifolia</i> L. in sandy steppe.	31–33
	Fig. 24: Kyrgyzstan, Central Tien Shan Mts., the Dzhungal River Valley, env. Chaek Village, <i>S. rosmarinifolia</i> in the bog near the brook.	22–23
<i>M. daurica</i> Tish.	Russia, Chita Oblast, env. Nizhny Tsasuchey Village, from <i>S. nipponica</i> Franch. et Sav.	27–29
<i>M. notata</i> (Proh.)	Russia, Amur Oblast, env. Kostyukovka Village, 30 km W of Svobodny Town, from <i>S. pierotii</i> Miq.	22–24
<i>M. costalis</i> (Mats.)	Figs 32, 33, 36: Russia, Sakhalin, env. Sokol Town, from <i>S. schwerinii</i> .	25–27
	Figs 34–35, 37–38: Russia, Khabarovsk Krai, env. Obluchye Town, from <i>S. schwerinii</i> .	22–24
<i>M. milkoii</i> Tish.	Figs 39–40: Kyrgyzstan, E slope of Ferghana Mtn. Range, Urumbash River, ca. 15 km SW of Kazarman Town, from <i>S. alba</i> L.	29
	Fig. 41: Kyrgyzstan, Turkestan Mtn. Range, Lyaylyak River, env. Katran Village, from <i>S. alba</i> .	31
	Fig. 42: Kyrgyzstan, Alay Mtn. Range, Kurshab River, 10 km N of Gul'cha, from <i>S. alba</i> .	26–29
	Fig. 43: Kyrgyzstan, Chatkal Mtn. Range, env. Ala-Buka Village, from <i>S. alba</i> .	22–23
	Figs 44–47: SE Kazakhstan, Karakol River, 27 km S of Taskesken Village, from <i>Salix</i> sect. <i>Helix</i> .	24–25
<i>M. aselae</i> Tish.	Figs 48–49, 52–53: SE Kazakhstan, central part of Dzhungarsky Alatau Mtn. Range, river gorge E of Dzhansagurov Village, from <i>Salix</i> sect. <i>Helix</i> .	29
	Figs 50–51: SE Kazakhstan, the floodplain of Lepsy River 13 km S of Kolbay Village, from <i>Salix</i> sect. <i>Helix</i>	27
<i>M. megerlei</i> (Fieb.)	Russia, Moscow Oblast, Serpukhov District, env. Danki Village, from <i>Rosa</i> sp.	22–25
<i>M. ornata</i> Lindb.	Figs 58–59, 61–62: Kazakhstan, Zailiysky Alatau Mtn. Range in the env. of Almaty, from <i>Rosa spinosissima</i> L.	30
	Figs 60, 63: Kyrgyzstan, Chatkal Mtn. Range, Sary-Cheleksky Nature Reserve, env. Arkyt Village, from <i>Rosa</i> sp.	22–23
<i>Handianus fertilis</i> Mit.	Russia, Saratov Oblast, 10 km E of Ozinki Town towards Ural'sk.	26–27
<i>Aconurella diplachnis</i> Em.	Russia, Altai Mts., the Chulyshman River Valley ca. 2–3 km upstream from the mouth, from <i>Cleistogenes squarrosa</i> (Trin.) Keng.	26–27
<i>Fangamanus tripunctatus</i> (Mats.)	Russia, Primorsky Krai, W shore of the Khanka Lake, 10 km S of Tury Rog Village.	25–26
<i>Evacanthus asiaticus</i> (Osh.)	Kyrgyzstan, Chatkal Mtn. Range, Sary-Cheleksky Nature Reserve, env. Arkyt Village.	21–23
<i>Limotettix russeolus</i> (Fall.)	Russia, Moscow Oblast, Voskresensk District, env. Beloozersky Town, peat bog on the shore of the lake, from <i>Oxycoccus palustris</i> Pers.	25–27
<i>Hephathus namus</i> (H.-S.)	Figs 89, 91: Russia, Saratov Oblast, 15 km NE of Ozinki Town.	33
	Figs 90, 92: Russia, Orenburg Oblast, Guberlya River, 25 km W of Orsk.	27–28

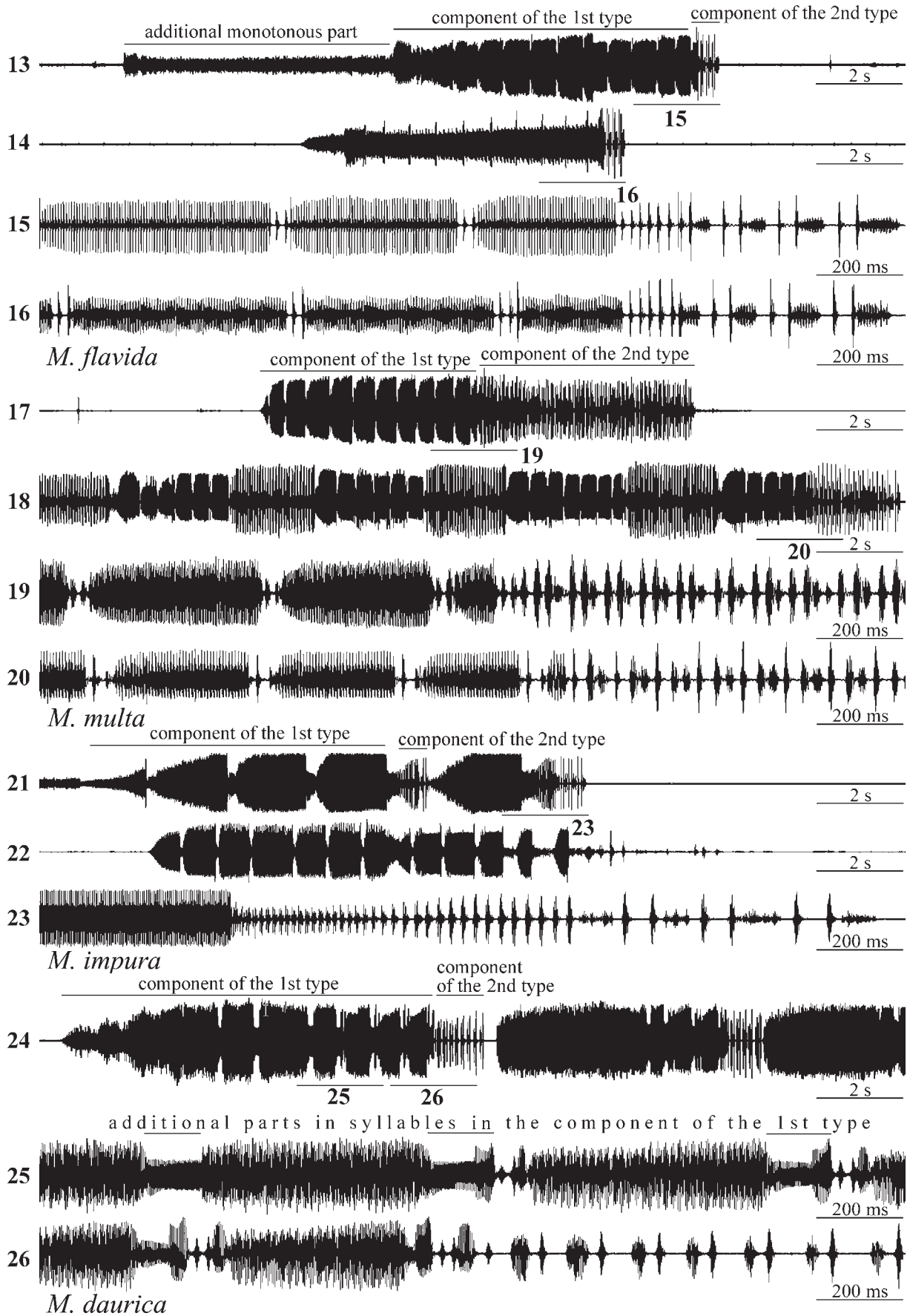


Figs 1–12. Oscillograms of male calling signals: 1–6 — *Macropsis leporina*; 7–12 — *M. ochotonaria*. Faster oscillograms of the parts of signals indicated as “4–6” and “10–12” are given under the same numbers.

Рис. 1–12. Осциллограммы призывных сигналов: 1–6 — *Macropsis leporina*; 7–12 — *M. ochotonaria*. Фрагменты сигналов, обозначенные цифрами “4–6” и “10–12”, представлены на осциллограммах под такими же номерами.

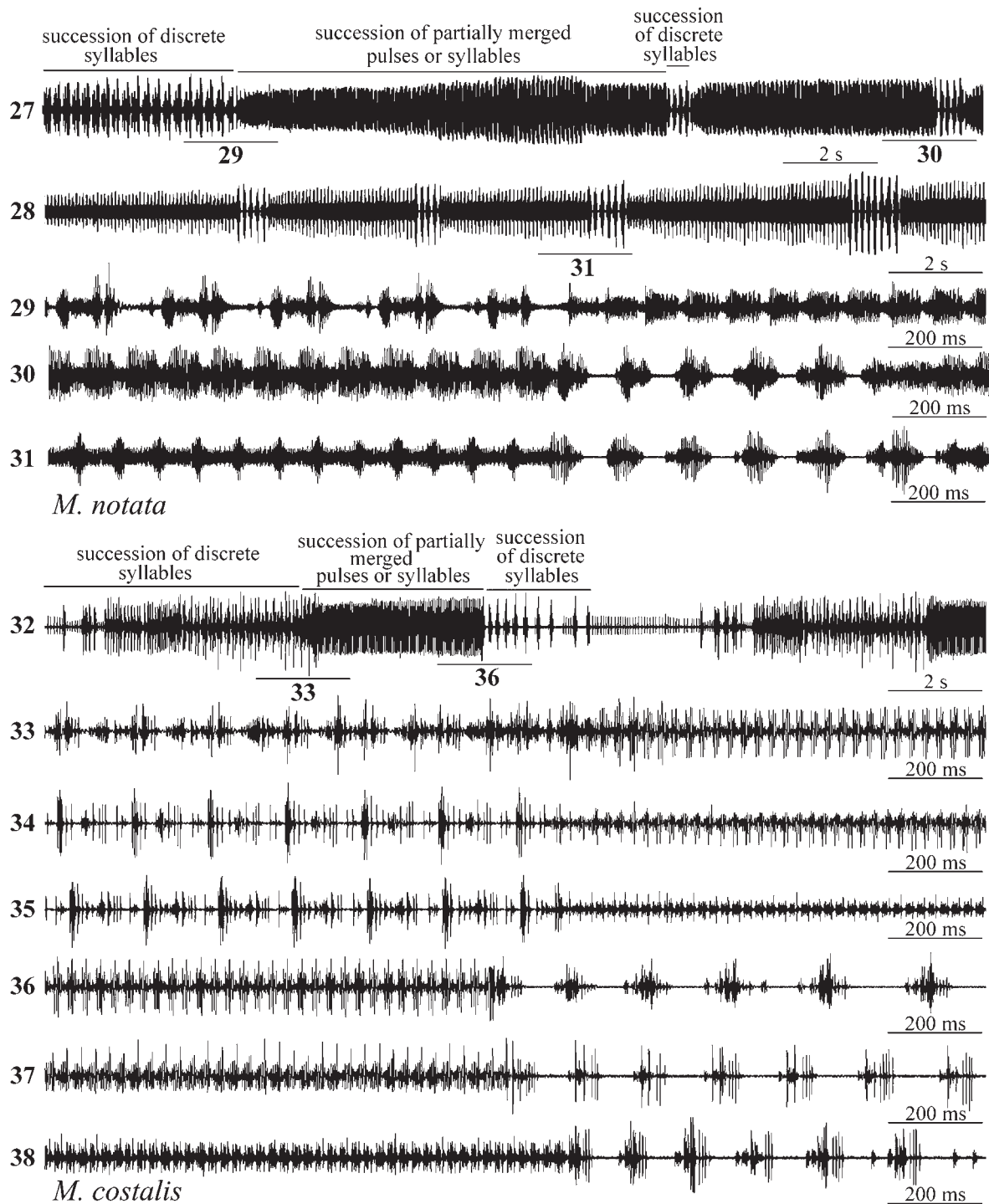
Figs 13–26. Oscillograms of male calling signals: 13–16 — *Macropsis flavida*; 17–20 — *M. multa*; 21–23 — *M. impura*; 24–26 — *M. daurica*. Faster oscillograms of the parts of signals indicated as “15–16”, “19–20”, “23”, and “25–26” are given under the same numbers.

Рис. 13–26. Осциллограммы призывных сигналов: 13–16 — *Macropsis flavida*; 17–20 — *M. multa*; 21–23 — *M. impura*; 24–26 — *M. daurica*. Фрагменты сигналов, обозначенные цифрами “15–16”, “19–20”, “23” и “25–26”, представлены на осциллограммах под такими же номерами.



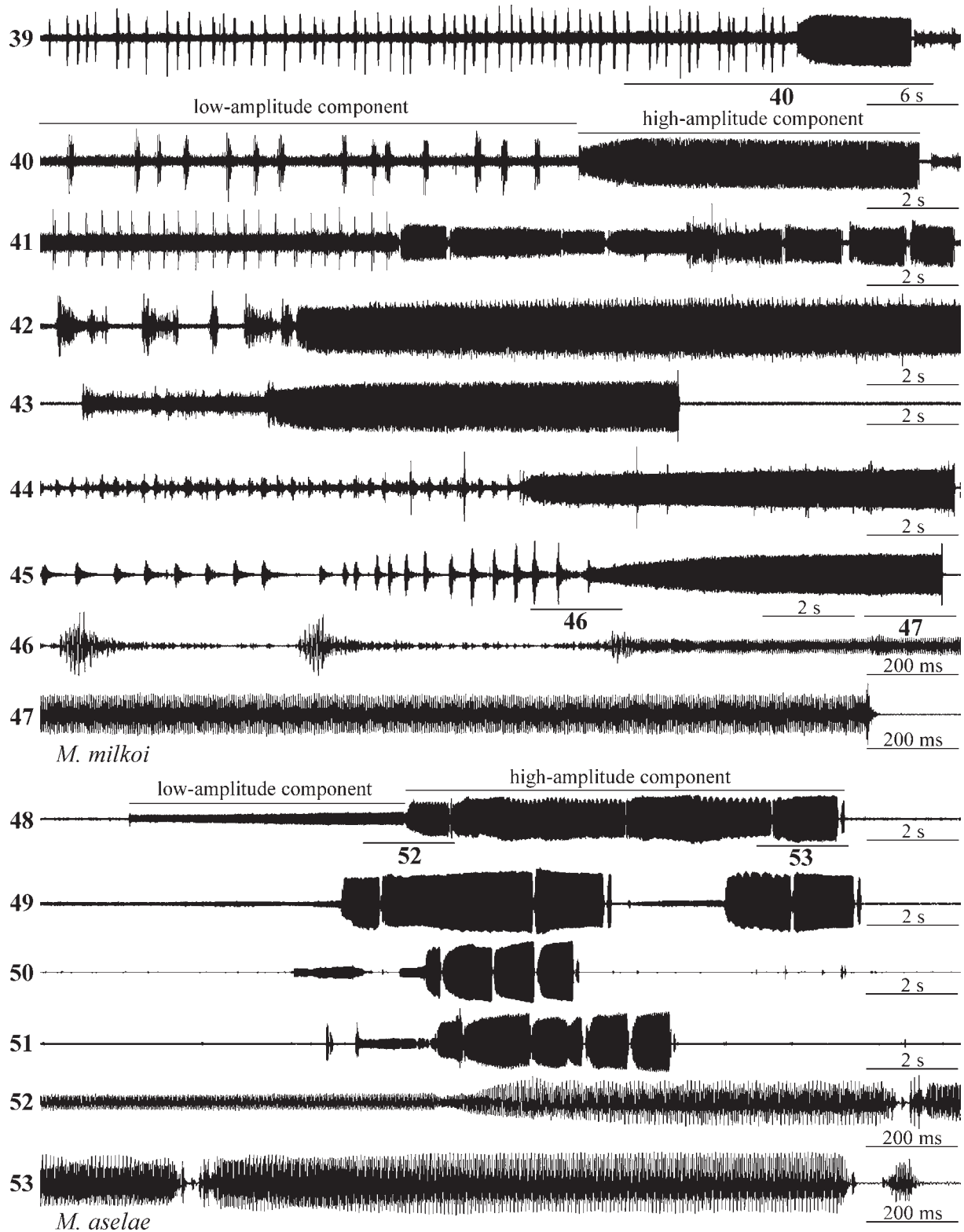
Tishechkin, 1997 belong to another group of closely related willow-dwelling species. Their relation is evidenced by the similar shape of the male 2nd abdominal apodemes and genitalia (some species are indistinguishable by these traits), and by the shape of the 2nd valvulae of ovipositor having 7–13 small additional teeth in all species [Tishechkin, 1999, 2002].

Signals of all these species share similar components of two types (Figs 13–26). Component of the first type is a succession of syllables separated by gaps with several discrete pulses. Component of the second type is a succession of discrete pulses; usually pulses with lower and higher amplitude alternate in it at least in the end.



Figs 27–38. Oscillograms of male calling signals: 27–31 — *Macropsis notata*; 32–38 — *M. costalis*. Faster oscillograms of the parts of signals indicated as “29–31”, “33”, and “36” are given under the same numbers.

Рис. 27–38. Осциллограммы призывных сигналов: 27–31 — *Macropsis notata*; 32–38 — *M. costalis*. Фрагменты сигналов, обозначенные цифрами “29–31”, “33” и “36”, представлены на осциллограммах под такими же номерами.

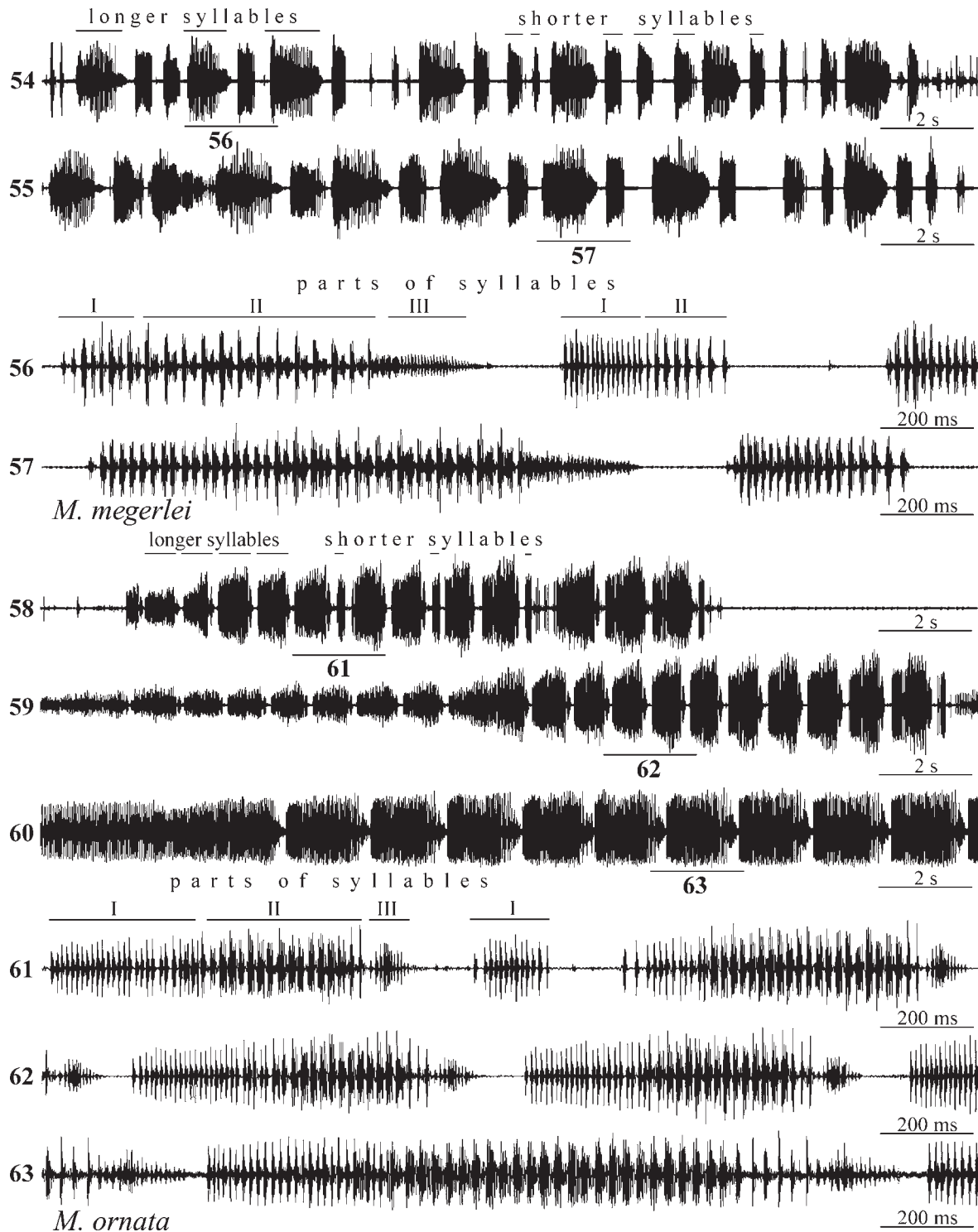


Figs 39–53. Oscillograms of male calling signals: 39–47 — *Macropsis milkoii*; 48–53 — *M. aselae*. Faster oscillograms of the parts of signals indicated as “40”, “46–47”, and “52–53” are given under the same numbers.

Рис. 39–53. Осциллограммы призывных сигналов: 39–47 — *Macropsis milkoii*; 48–53 — *M. aselae*. Фрагменты сигналов, обозначенные цифрами “40”, “46–47” и “52–53”, представлены на осциллограммах под такими же номерами.

The signal of *M. flavida* usually begins with a monotonous noise-like vibrations followed by one component of the first type and one component of the second type (Figs 13–14); in the signals of all other species the

initial monotonous part is always strongly reduced or absent (Figs 17, 21–22, 24). The signal of *M. multa* differ from signals of other species by very long component of the second type (Figs 17–20). In the signal of *M.*

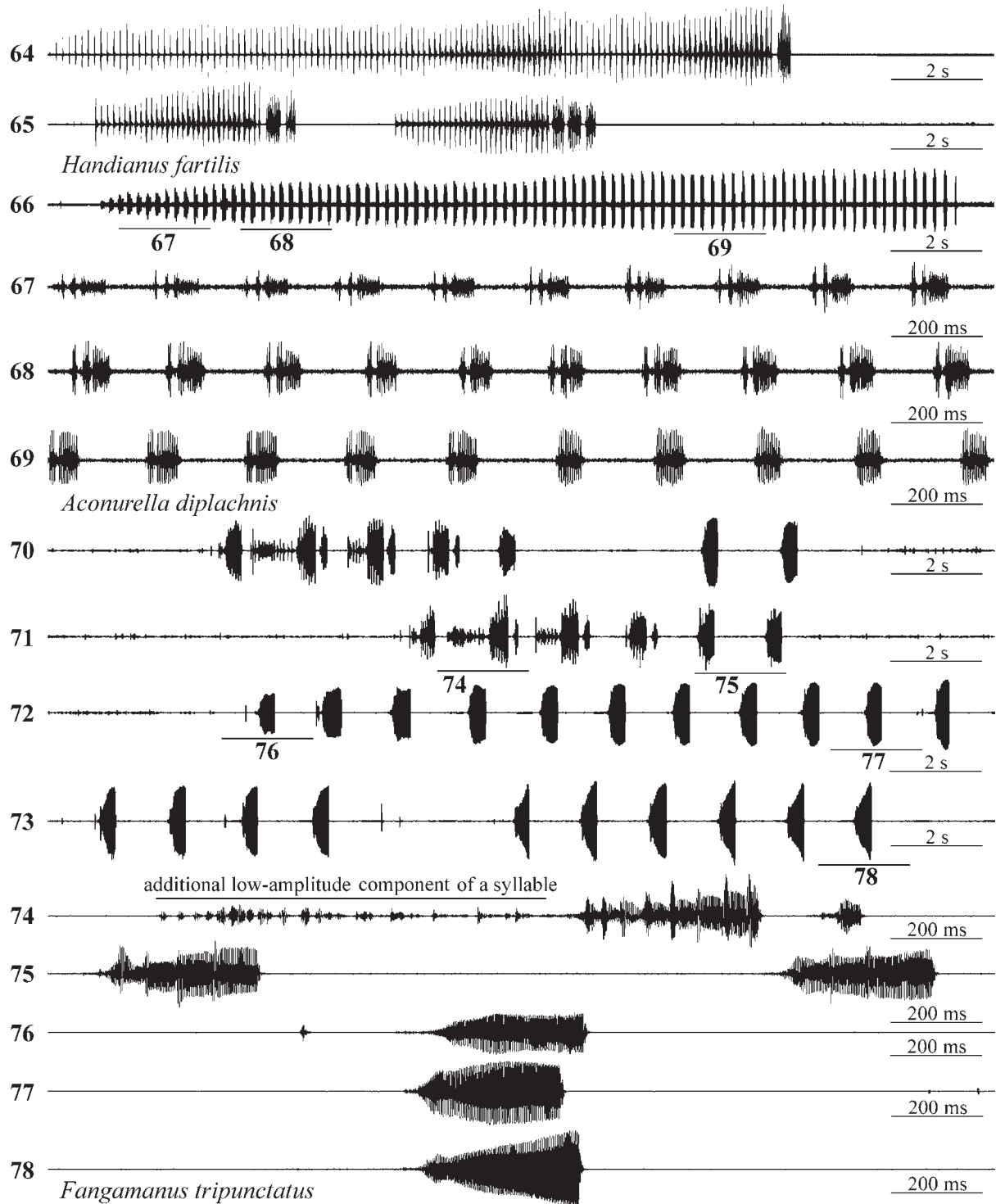


Figs 54–63. Oscillograms of male calling signals: 54–57 — *Macropsis megerlei*; 58–63 — *M. ornata*. Faster oscillograms of the parts of signals indicated as “56–57” and “61–63” are given under the same numbers.

Рис. 54–63. Осциллограммы призывных сигналов: 54–57 — *Macropsis megerlei*; 58–63 — *M. ornata*. Фрагменты сигналов, обозначенные цифрами “56–57” и “61–63”, представлены на осциллограммах под такими же номерами.

impura, the number of the second type components can vary; sometimes they are absent (Figs 21–23). In the signal of *M. daurica*, syllables in the component of the first type have an additional end part (Figs 24–26).

As a result, signals of different species in this group differ from each other, first, in the presence/absence of additional parts and, second, in the duration of homologous components.

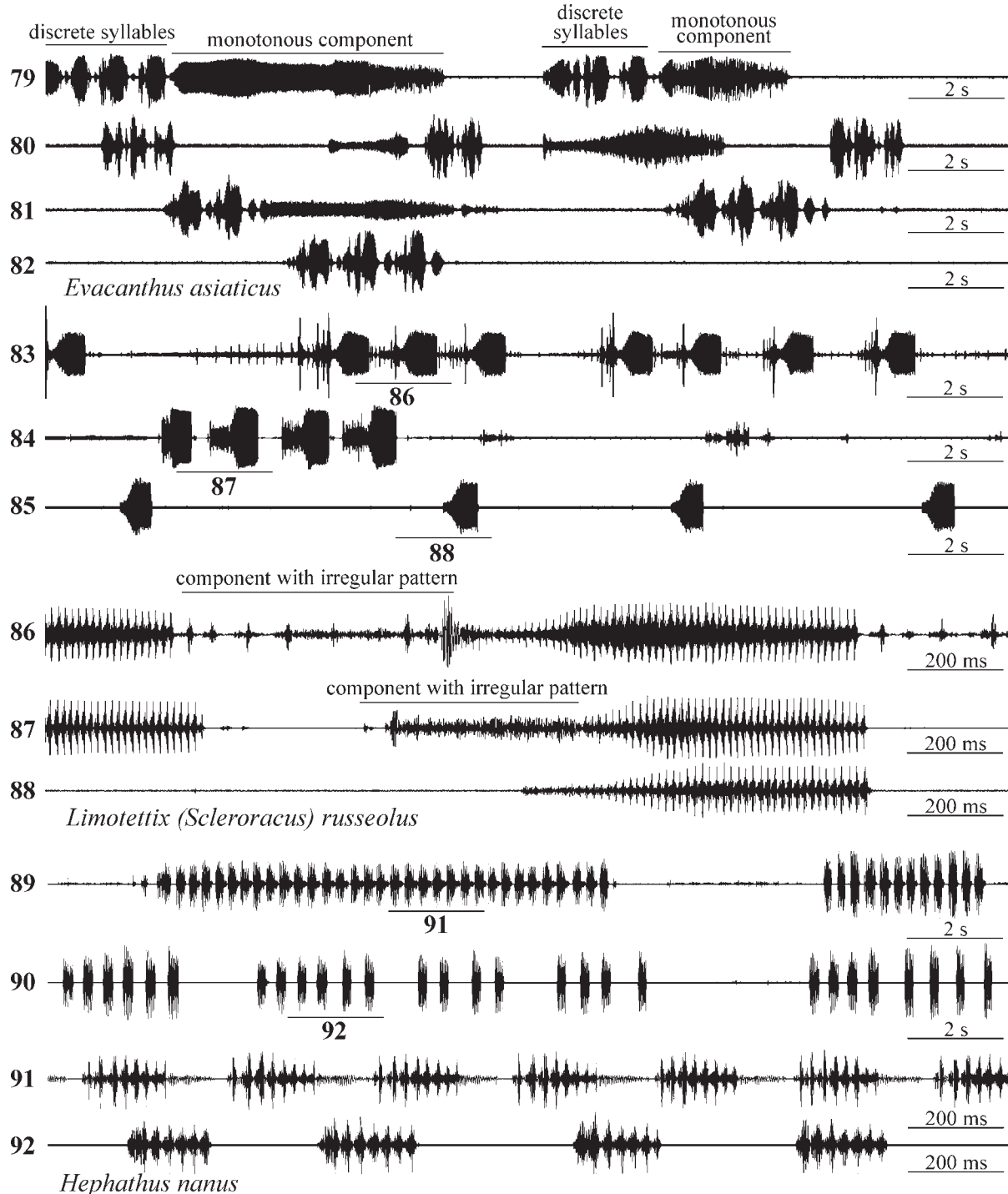


Figs 64–78. Oscillograms of male calling signals: 64–65 — *Handianus fartilis*; 66–69 — *Aconurella diplachnis*; 70–78 — *Fangamanus tripunctatus*. Faster oscillograms of the parts of signals indicated as “67–69” and “74–78” are given under the same numbers.

Рис. 64–78. Осциллограммы призывных сигналов: 64–65 — *Handianus fartilis*; 66–69 — *Aconurella diplachnis*; 70–78 — *Fangamanus tripunctatus*. Фрагменты сигналов, обозначенные цифрами “67–69” и “74–78”, представлены на осциллограммах под такими же номерами.

3. *M. costalis* (Matsumura, 1911) and *M. notata* (Prohaska, 1923) are two closely related sister-species, sympatric in the Russian Far East, but feeding on different willow species (Tishechkin, 1999). In both species, male calling signal consists of repeated phrases (Figs 27–38). The full phrase includes a succession of dis-

crete syllables, a succession of partially merged pulses or syllables, and a succession of discrete shorter syllables having somewhat another shape than in the beginning of a phrase (Figs 27, 32). In *M. notata*, the first succession of discrete syllables in a phrase is often missed (Fig. 28).



Figs 79–92. Oscillograms of male calling signals: 79–82 — *Evacanthus asiaticus*; 83–88 — *Limotettix (Scleroracus) russeolus*; 89–92 — *Hephathus nanus*. Faster oscillograms of the parts of signals indicated as “86–88” and “91–92” are given under the same numbers.

Рис. 79–92. Осциллограммы призывных сигналов: 79–82 — *Evacanthus asiaticus*; 83–88 — *Limotettix (Scleroracus) russeolus*; 89–92 — *Hephathus nanus*. Фрагменты сигналов, обозначенные цифрами “86–88” и “91–92”, представлены на осциллограммах под такими же номерами.

Thus, in both species, phrases retain the same general pattern but differ in syllable shapes in all three parts. In addition, the initial part of the phrase is often absent in *M. notata*, whereas in *M. costalis* it is always present.

4. *M. milkoii* Tishechkin, 2015 and *M. aselae* Tishechkin, 2020 are two closely related species from Central Asia. They can feed on the same species of willows, but are allopatric. *M. milkoii* is widespread in Tien Shan, Pamir-Alay, and Hissar-Darvaz Mountain systems; in Kazakhstan it was also found in the plain south of Tarbagatai Range. On the contrary, *M. aselae* so far has only been found in the foothills and low mountains of Dzhungarsky Alatau and evidently does not penetrate far from the mountains to the plain.

In both species, male calling signal is a phrase consisting of low-amplitude and high-amplitude components (Figs 39–45 and 48–51). Both components are monotonous or slightly modulated vibrations that sound like a buzzing sound to the human ear (Figs 46–47, 52–53). Durations of each component and of a phrase as a whole vary greatly, but in *M. aselae* a phrase on average is shorter and usually does not exceed 10–15 s, whereas in *M. milkoii* it sometimes lasts for more than a minute (Fig. 39). In addition, in the first component of a phrase of *M. milkoii*, short syllables usually follow among low-amplitude vibrations, and the second component is a single whole; only very rarely does it split into several fragments (Fig. 41). In *M. aselae*, syllables in the first component of a phrase are absent, and the second component always splits into several parts separated by gaps.

Accordingly, the signals of these two species differ from each other by the presence/absence of additional syllables in the first component of a phrase, by the presence/absence of gaps in the second component, and by the average duration of phrases.

5. *M. megerlei* (Fieber, 1868) and *M. ornata* Lindberg, 1926 belong to the group of species feeding on *Rosa* spp. [Tishechkin, 2015]. The range of *M. megerlei* includes Europe, Eastern Mediterranean (Turkey, Lebanon), Transcaucasia, plains of Kazakhstan, Southern Siberia eastwards as far as the Baikal Lake, and steppes of Mongolia. *M. ornata* inhabits Tien Shan, Pamir-Alay, and Hissar-Darvaz Mountains.

Calling signal of *M. megerlei* is a succession of alternating shorter and longer syllables; it can last for a minute and more and have a comparatively constant amplitude throughout its length (Figs 54–55). Calling of *M. ornata* consists of similar syllables, but gradually increases in amplitude towards the end, thus forming a phrase with a distinct beginning and end; in addition, short syllables in it are often absent (Figs 58–60). In both species longer syllables include three, obviously, homologous parts (Figs 56–57 and 61–63). Shorter syllables in *M. megerlei* consist of two parts, whereas in *M. ornata* the second part is absent.

Thus, the signal of *M. ornata* has a more regular general structure and is a distinct phrase. On the contrary, in *M. megerlei*, the signal splits into separate syllables following each other in a rather irregular order

and with more variable intervals; for this reason it cannot be referred to as a phrase. In addition, *M. ornata* has a clear tendency to reduction of short syllables in a phrase. Compared to *M. megerlei*, their number decreases, and sometimes they are completely absent; in addition, the second part is absent in them.

Discussion

As in all insects, the signals of the studied species exhibit some variability. The duration of phrases or their components varies to some extent in almost all species (for example, Figs 21–22, 48–51). The ratio of the amplitudes of different parts of the phrase is sometimes significantly different (Figs 7–8, 33–35). In some species, syllable shape also vary (Figs 4–6, 15–16, 30–31). In some species, the male produces certain component of a signal only occasionally, so sometimes it is absent. For example, the *M. notata* signal often lacks an initial sequence of discrete syllables (cf. Figs 27 and 28) and the *M. ornata* signal usually lacks short syllables (cf. Figs 58 and 59–60).

Despite this, signals of species under consideration always distinctly differ from each other in temporal pattern. These differences can be classified into several categories.

1. Considerable difference in the duration of the homologous parts of a signal.
2. Difference in a syllable shape.
3. Presence/absence of additional components.
4. Splitting the signal or its components into several parts in one of two sister-species.

At the same time, in other leafhoppers (Homoptera: Cicadellidae), such differences sometimes are intraspecific and can be observed between the signals of males from the same population or even in the same male [Tishechkin, 2010 and below].

Intraspecific signal variability in *Handianus fartilis* Mitjæev, 1975 (Figs 64–65) is basically the same as difference between signals of *M. flavida*, *M. impura*, and *M. daurica* on the one hand and *M. multa* on the other (Figs 13–14, 21–22, 24 and 17–18). In some signals of *H. fartilis*, the first part is many times longer than in others. Similarly, the main difference between signals of *M. multa* and three other species is that the end part of the signal in *M. multa* is much longer than in others. Signals of *M. milkoii* and *M. aselae* also differ in average durations approximately at the same level as different signals of *H. fartilis*. Similarly, signals of *M. leporina* and *M. ochotonaria* differ in a duration of a succession of syllables (Figs 1–3 and 7–9).

A gradual change of a syllable shape from the beginning to the end of a signal is observed in many species of Cicadellidae; a typical example is *Aconurella diplachnis* Emeljanov, 1964 (Figs 66–69). In some other species, the shape of syllables within a signal is more or less constant, whereas different signals distinctly differ in this trait; *Fangamanus tripunctatus* (Matsumura, 1915) can be cited as an example (Figs 70–78). It must be

emphasized that the oscillograms on Figs 70–78 show the signals of one male recorded during the period of about half an hour.

Differences in a syllable shape between *M. ochotonaria* and *M. leporina* (Figs 4–6 and 10–12) and between *M. notata* and *M. costalis* (Figs 29–31 and 33–38) generally are the same as between syllables from the beginning and from the end of a signal of *A. diplachnis* and between syllables in different signals of *F. tripunctatus*. In *M. leporina*, pulses in syllables are usually separated by distinct gaps (Fig. 4), although sometimes they merge with each other (Fig. 5). In *M. ochotonaria*, pulses in syllables are always partially merged (Figs 10–12). In *M. costalis*, syllables usually consist of discrete pulses (Figs 34–35, 37–38); only occasionally they partially merge with each other (Figs 33, 36). On the contrary, in *M. notata*, pulses in syllables are always merged and indistinct (Figs 29–31). Similarly, the syllables at the beginning of the *A. diplachnis* signal consist of several pulses, but short discrete pulses at the beginning of each syllable gradually reduce towards the end of a phrase (Figs 67–69). In *F. tripunctatus*, some syllables consist of distinct although partially merged pulses (Figs 74–75), while others are almost uniform fragments (Figs 76–78).

The appearing/reduction of an additional component of a signal is also typical of many leafhopper species. For example, in *F. tripunctatus*, additional low-amplitude component sometimes precedes the main part of a syllable (Figs 70–71, 74). In *Evacanthus asiaticus* Oshanin, 1871, a phrase normally consists of 2–4 discrete syllables followed by monotonous component (Fig. 79). However, sometimes this component is strongly reduced (Fig. 80) or absent (Figs 81, the end part of the oscillogram, 82). In *Limotettix (Scleroracus) russeolus* (Fallén, 1826) the phrase consists of a component with irregular pattern followed by a train of pulses (Figs 83–84, 86–87), but sometimes a male misses the irregular component (Figs 85, 88).

Similar differences are observed between the signals of the considered *Macropsis* species. In *M. ochotonaria*, compared to *M. leporina*, additional monotonous component is added to a succession of syllables (cf. Figs 1–3 and 7–9). The presence of this component is a species-specific trait of *M. ochotonaria* and is the main difference from the signal of *M. leporina*. At the same time, in *E. asiaticus*, the presence/absence of a similar component is observed in different signals of the same male. Signals of *M. daurica* differ from the signals of related species by the presence of an additional part in the end of syllables (Fig. 25). One of the differences between *M. milkoi* and *M. aselae* is the presence of additional syllables in the first component of a phrase in the signal of *M. milkoi*. *M. ornata* differs from *M. megerlei*, inter alia, by the absence of a second part in shorter syllables and by the reduction of the number of these syllables; in some phrases they are completely absent.

Splitting the signal into separate discrete fragments was described earlier for *Hephathus nanus* (Herrich-

Schäffer, 1835) [Tishechkin, 2010 and Figs 89–92]. Similarly, in *L. (S.) russeolus*, phrases consisting of two parts usually follow one after another with short gaps (Figs 83–84, 86–87) thus forming a single signal, whereas phrases consisting only of a sequence of pulses are separated by pauses up to 5–10 s (Figs 85, 88). Similarly, in *M. ornata*, syllables form a distinct phrase, whereas the calling signal of *M. megerlei* is rather a succession of syllables without distinct beginning and end.

Thus, within each of the five *Macropsis* groups considered above, the nature of interspecific differences between signal patterns is the same as intraspecific differences in some other leafhoppers. This suggests that, in related *Macropsis* species, signals consisting of similar components, apparently, were formed by fixing different originally intraspecific variants in different phylogenetic lineages in the course of their divergence and a subsequent speciation. It should be added that the considered *Macropsis* species differ in host specialization and/or morphological traits and colouration and do not produce signals with intermediate characters. This proves that they are different biological species and not intraspecific forms.

The driving force of such signal divergence is obscure and can be different in each particular case. A study of the cryptic lacewing species (Neuroptera: Chrysopidae) suggested that the rapid divergence of their signal patterns up to the level of good species is caused by sexual selection [Henry, 1985]. Results from hybridizing of two species of *Chrysopa* (Neuroptera: Chrysopidae) support a key role for random mutation in species divergence, impacting mate choice immediately. It was supposed, that in this case substitution of a single allele will change the song in a major way, affecting mate choice of both males and females [Henry et al., 2002]. This confirms the hypothesis that single-gene control of signal traits may result in a rapid change of signal pattern and in a speciation.

On the other hand, for populations of *Nilaparvata lugens* from rice and *Leersia hexandra* no evidence were found that male or female signal divergence has been driven by diverging female or male preferences, respectively. Thus, these data are not consistent with sexual selection. Drift is the most likely explanation although some features of the comparison between the rice- and *Leersia*-feeding populations suggest the involvement of natural selection [Butlin, 1996].

Underlying genetic mechanism of signal variability in species studied is also unknown. Differences in signal patterns between different *Macropsis* species are obviously genetically fixed. Differences between songs of different males from the same population can also be explained by genetic differences between them. However, differences between signals of the same male (as on Figs 70–78) evidently has no relation to genetic polymorphism.

Anyway, the above examples support the assumption that male calling signals in leafhoppers can evolve not only by a gradual change in the quantitative parameters such as signal parts duration, but also through

qualitative changes of temporal pattern due to adding/reduction of rather large components, change of a syllable shape, or by splitting the signal or its components into several parts. Since it is the differences in the signal pattern that provide reproductive isolation, such changes can result in a rapid speciation. This can well occur if any signal trait is controlled by only one or few genes; this possibility is also confirmed by experimental data on the change of the signal pattern in hybrids. Morphological traits do not necessarily have to be involved in this process; if they remain unchanged, cryptic species are evolved.

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