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Study of stridulatory organs in the family Heteroceridae (Insecta: Coleoptera)

Исследование стридуляционных органов семейства Heteroceridae (Insecta: Coleoptera)

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KEYWORDS. Variegated mud-loving beetles; stridulatory file; plectrum; scanning electron microscope; morphology.

КЛЮЧЕВЫЕ СЛОВА. пилоусы, стридуяционный файл, плектрум, сканирующий электронный микроскоп, морфология.

ABSTRACT. The morphology of stridulatory organs for 16 species of the family Heteroceridae is described. The variegated mud-loving beetles (Heteroceridae) produce sound by abdominal-femoral movement—scraping the surface of one projection on the hindleg (the plectrum) against the surface of a series of the ridges (or ribs) on the first abdominal segment (the stridulatory file, or the pars strides). This study explores for the first-time the differences in these structures between the genera *Augyles* and *Heterocerus*. The study is based on imaging the sound-producing structures on a scanning electron microscope. The following parameters were measured: the size of the stridulatory file, the number of ridges, length of ridges, distance between ridges, width of ridges, and size of the plectrum. This study demonstrates how these parameters depend on each other in 16 species (genera *Augyles* and *Heterocerus*) and how the stridulatory organ is varied between male and female (*Augyles* genus). The stridulatory file consists of two types of ridges, which are named first-order and second-order ridges. The results of this study show that the width of the ridges and the distance between the ridges depend on the sex of the insects. Thus, the value of the distance between the ridges is greater than the width of ridges in males and vice versa in females. Future studies should focus on larger samples for each species.

РЕЗЮМЕ. Описана морфология стридуляционных органов 16 видов семейства Heteroceridae (пилоусы). Пилоусы издают звук брюшко-заднебедренным движением: трением поверхности ребра (плектрум, plectrum) на бедрах задних ног о ряда гребней (стридуляционное поле, pars strides) бедренной линии на первом сегменте брюшка. Впервые изучены различия этих структур у родов *Augyles* и *Heterocerus*. Исследование основано на визуализации звукопроизводящих структур на сканирующем электронном микроскопе. Были измерены следующие параметры: размер стридуляционного поля, количество гребней, длина гребней, расстояние между гребнями, ширина гребней и размер плектрума. На 16 видах родов *Augyles* и *Heterocerus* показано, что эти параметры зависят между собой, а также вариативность стридуляционного аппарата у самцов и самок. Ширина гребней и расстояние между ними зависят от пола. Так, значение расстояния между гребнями больше ширины гребней у самцов и меньше у самок. Будущие исследования должны быть сосредоточены на более крупной выборке для каждого вида.

Introduction

The family Heteroceridae MacLeay, 1825 (Coleoptera), or variegated mud-loving beetles, is wide-

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spread throughout the world (except Antarctica, high Arctic, highlands, deserts, and mires). The world fauna of Heteroceridae includes 6 genera and approximately 362 recent species (our data); previous estimates ranged from 320 to 370 species, because the fauna has not been revised. In general, species of the family Heteroceridae occur at temperate, subtropical, and tropical latitudes [Mascagni, 2014; Skalický, Ezer, 2014]. Heteroceridae evolved in unstable habitats of water-land ecotones and show high taxonomic diversity and abundance in riparian communities. Adults and larvae of Heteroceridae build branched networks of tunnels and chambers in a moist soft substrates, using these tunnels for feeding, egg-laying, and pupation [Ratcliffe, Fagerstorm, 1980]. If their density and the density of other stratobionts is high they give a specific micro relief for the substrate [Sazhnev, 2020]. Adults often fly toward light (LED or vapor mercury lamps) at night, which can be used as a collection method [Sazhnev, Rodionova, 2019].

Species of the family Heteroceridae prefer finely dispersed clay and sand types of soil with sufficient moisture, in which colonies are often formed with pronounced biotopic sympatry (cohabitation of species). Variegated mud-loving beetles use acoustics to communicate with each other. There are two possible ways to communicate for these beetles. The first is an acoustic channel based on stridulation, and the second is substrate vibrational communication, it is associated with the habitat [Greenfield, 2016]. Effectively transmitted vibration signals in wet sands can weaken considerably in dry sands [Aicher, Yautz, 1990]. Air or water sound waves spread in all three dimensions, but some local reduction and amplitude amplification may be caused by barriers, and also by differences in the air's impedance or animal shape and position signaling [Römer, 1993; Forrest, 1982; van Staaden, Römer, 1997].

Variegated mud-loving beetles produce sound by scraping the hindleg ridge (the plectrum) over the first abdominal ridge (the stridulatory file, or stridulitrum, or pars stridens) [Erichson, 1848; Schiödte, 1867; Gahan, Gahan, 1900]. The abdominal ridge is varied between genera; for example, for the genus *Augyles* Schiödte, 1866 it is complete; and it is incomplete for the genus *Heterocerus* Fabricius, 1792 [Aguilera et al., 1998; Stals, 2007]. The stridulatory file in males of some *Heterocerus* species (e.g., *Heterocerus heydeni* Kuwert, 1890) with a few distinct striae in the basal third (secondary ridges), smooth or with small creases in females [Litovkin et al., 2019], which may be related to sexual behavior [King, Lago, 2012; Mascagni, 2015]. The main body of work on the systematics of the family describes various characteristics, such as the male genitalia, the basic color pattern of the body, the humeral angle, types of fasciae, the anterolateral angel of the pronotum, etc. [Sazhnev, 2018; Arnett, 1963; Kiesenwetter, 1851; Kuwert, 1890; King et al., 2011]. Modern microscopy methods now allow us to investigate new features of morphology. Our research has shown differences in the structure of the hindleg ridge (the plectrum) and the stridulatory files for the genera *Augyles* and

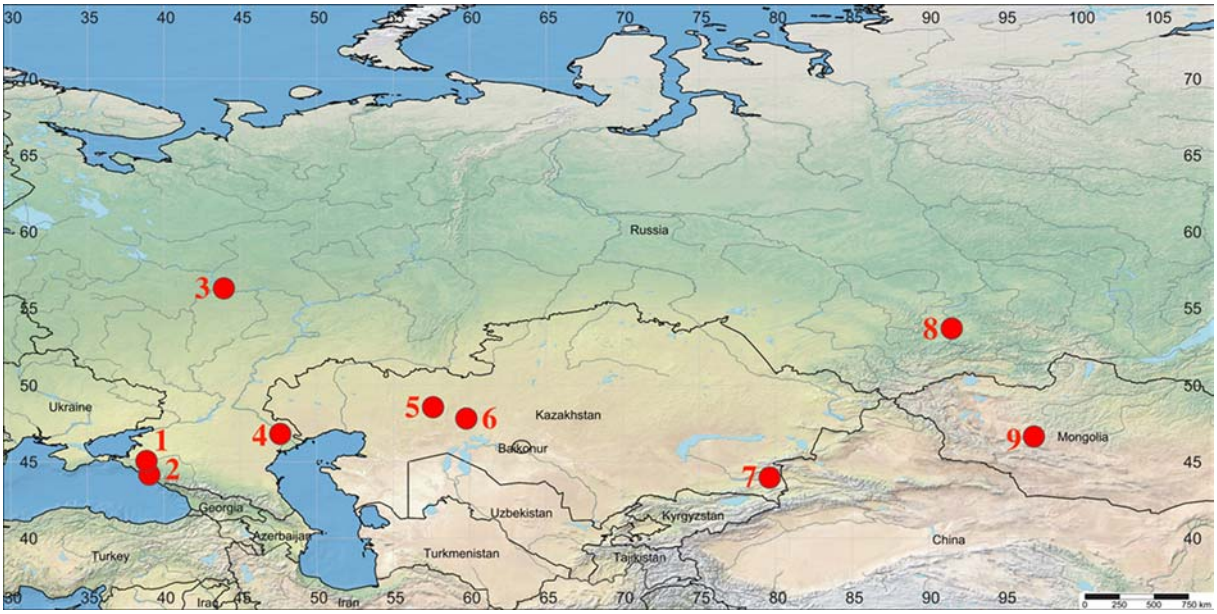


Fig. 1. The localities of the samples: 1 — Krasnodar (Krasnodar Krai, Russia); 2 — Agoy vill. (Krasnodar Krai, Russia); 3 — Nizhny Novgorod (Nizhny Novgorod Oblast, Russia); 4 — Zamyany vill. (Astrakhan Oblast, Russia); 5 — Zhagabulak vill. (Aktobe Region, Kazakhstan); 6 — Shalkar vill. (Aktobe Region, Kazakhstan); 7 — Koktal vill. and Altyn-Emel National Park (Almaty Region, Kazakhstan); 8 — Abakan (Republic of Khakassia, Russia); 9 — Taishir Reservoir (Taishir, Mongolia).

Рис. 1. Места сбора образцов: 1 — Краснодар (Краснодарский край, Россия); 2 — с. Агой. (Краснодарский край, Россия); 3 — Нижний Новгород (Нижегородская область, Россия); 4 — с. Замьяны (Астраханская область, Россия); 5 — село Жагабулак (Актюбинская область, Казахстан); 6 — село Шалкар (Актюбинская область, Казахстан); 7 — село Коктал и Национальный парк Алтын-Эмель (Алматинская область, Казахстан); 8 — Абакан (Республика Хакасия, Россия); 9 — Тайширское водохранилище (Тайшир, Монголия).

Heterocerus for the first time. The structure of the stridulatory apparatus can vary in these genera and shows differences between species and between sexes.

Materials and methods

The beetles were collected on wet substrates on riverbanks in the East Palearctic region: Russia, Kazakhstan, and Mongolia. All beetles were fixed in 70° ethanol. The location of the collecting sites and the species are described in Table 1 and shown in Fig. 1. The individuals of *Augyles* and *Heterocerus* were identified using external morphology and structure of the male genitalia [Mascagni, 2014; Skalický, Ezer, 2014]. All specimens were taken from a collection of the Laboratory of Ecology of Aquatic Invertebrates, Papanin Institute for Biology of Inland Waters, Russian Academy of Sciences (IBIW RAS). The map was created using the SimpleMappr online service (www.simplemappr.net).

To image the sound-producing structures in males and females, the beetles were prepared for scanning electron microscopy by separating the abdomen and the posterior leg from the rest of the body. The samples were mounted on aluminum stubs and coated with palladium (Ion coater IB-5, Eiko Engineering Co., Ltd., Japan) before imaging using a variable pressure scanning electron microscope (SEM) (Hitachi TM-1000, Hitachi, Japan) at the Zoological Institute, Russian Academy of Sciences. Part of the photography for analysis was made by A.A. Prokin at Tyumen State University in 2017.

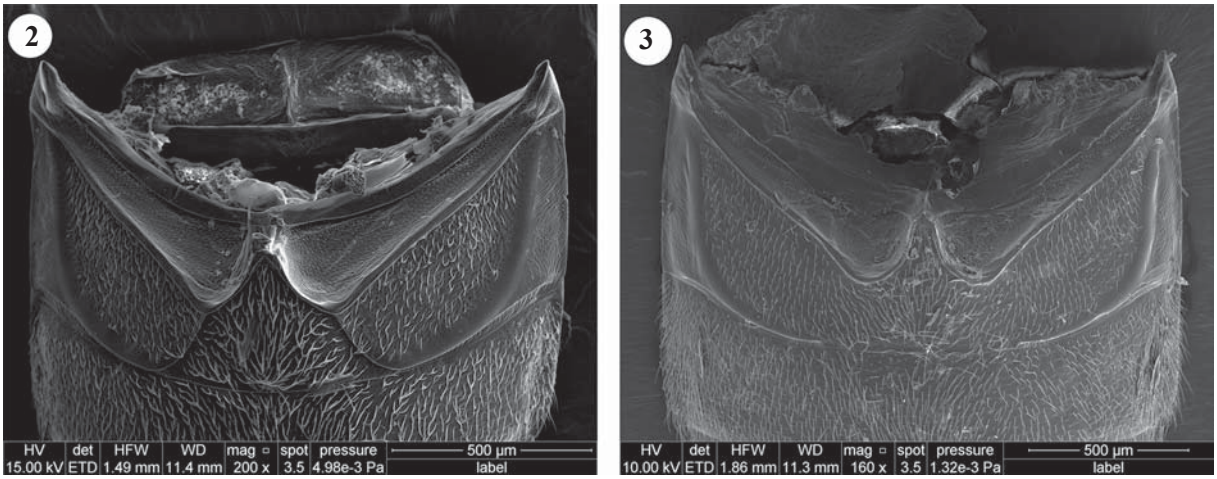
The present study is based on 18 specimens of 16 species. Only two species were represented by a male and a female specimen. For measurements of the stridulatory file of the abdominal segment and the posterior femur plectrum, we used Image Pro (Media Cybernetics, USA). We measured the following parameters of the stridulatory file: the length of the stridulatory file from the first ridge on the carina to the last ridge at the medial part (μm), the number of ridges at 50 μm, the length of ridges (average length of ridges ± SD, μm), the distance between ridges (average distance between ridges ± SD, μm), and the width of ridges (average width of ridges ± SD, μm). For the plectrum, we measured the maximum length (μm).

Result

A comparative description of the stridulatory organs was based on SEM images of 16 species of two genera. The stridulatory file comprises a series of thin parallel ribs. As can be seen in Figs 2–3, the abdominal cuticular ridge is complete in the genus *Augyles* and incomplete in the genus *Heterocerus*. The stridulatory file starts from the carina of the first abdominal segment and is completed at the medial part of the dorsal area of the first abdominal segment.

Table 1. The list of species and localities of the studied samples.
Таблица 1. Список видов и место сбора исследованных экземпляров.

Species	Locality	Coordinates	Date
Augyles dilutissimus (Reitter, 1887)	Altyn-Emel NP (Kazakhstan)	44°10’28”N 79°22’52”E	15.VI.2018
A. flavidus (Rossi, 1794)	Koktal (Kazakhstan)	43°57’49”N 79°36’19”E	1.VII.2015
A. hispidulus (Kiesenwetter, 1843)	Zamyany (Russia)	46°49′00”N 47°37′00”E	7.VII.2016
A. interspidulus (Charpentier, 1979)	Abakan (Russia)	53°42’49”N 91°30’20”E	27.VII.2019
A. obliteratus (Kiesenwetter, 1843)	Koktal (Kazakhstan)	43°57’49”N 79°36’19”E	1.VII.2015
A. sericans (Kiesenwetter, 1843)	Agoy (Russia)	44°09’33”N 39°03’24”E	20.VIII.2016
A. turanicus (Reitter, 1887)	Koktal (Kazakhstan)	43°57’49”N 79°36’19”E	3.VII.2010
Heterocerus fenestratus (Thunberg, 1784)	Krasnodar (Russia)	45°02’56”N 38°52’22”E	3.VII.2019
H. flexuosus Stephens, 1828	Zhagabulak (Kazakhstan)	48°33’51”N 57°36’19”E	26.IV.2012
H. fossor Kiesenwetter, 1843	Abakan (Russia)	53°42’49”N 91°30’20”E	27.VII.2019
H. fusculus Kiesenwetter, 1843	Krasnodar (Russia)	45°02’57”N 38°52’22”E	3.VII.2019
H. heydeni Kuwert, 1890	Shalkar (Kazakhstan)	47°49′30″N 59°46′495″E	1.V.2014
H. kaszabi Charpentier, 1979	Taishir (Mongolia)	46°39’01”N 96°52’45”E	8.VIII.2012
H. marginatus (Fabricius, 1787)	Nizhny Novgorod (Russia)	56°17’36”N 43°55’53”E	9.VII.2015
H. obsoletus Curtis, 1828	Krasnodar (Russia)	45°02’57”N 38°52’22”E	3.VII.2019
H. parallelus Gebler, 1830	Taishir (Mongolia)	46°39’01”N 96°52’45”E	8.VIII.2012

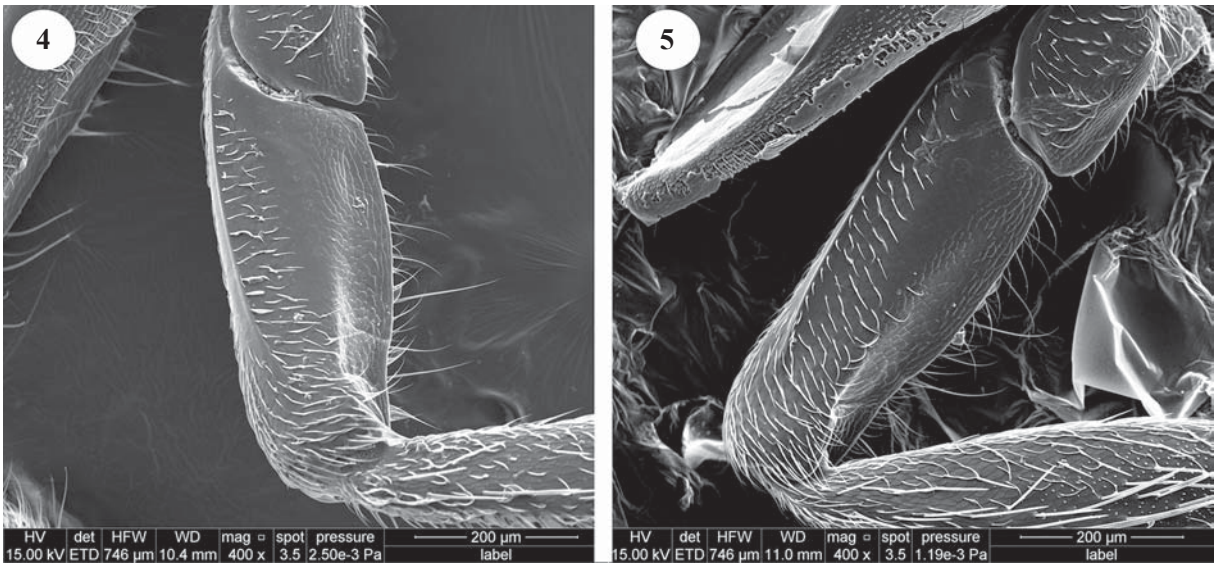


Figs 2–3. The abdominal cuticular ridge with the stridulatory file: 2 — complete abdominal ridge of female *Augyles interspidulus* (post-metacoxal lines of first abdominal segment present); 3 — incomplete abdominal ridge of female *Heterocerus heydeni* (post-metacoxal lines of first abdominal segment absent).

Рис. 2–3. Брюшной кутикулярный гребень со стридуляционным полем: 2 — полная бедренная линия самки *Augyles interspidulus*; 3 — неполная бедренная линия самки *Heterocerus heydeni*.

The length of the stridulatory file was measured from the first thin parallel rib to the top ridge of the dorsal area of the first abdominal segment. The number of ridges at 50 µm was measured on the area where they apparently produce the sound. The plectrum is the single ridge that starts from the anterior part of the femur and does not reach the tibia (Figs 4–5).

The result of our measurements of different parameters of the morphology of structure showed variation between male and female structure for *Augyles dilutissimus* and *A. interspidulus*. The results are presented in Table 2. Figs 2–3 shows the general structure of the stridulatory file. Comparison of the structure of the stridulatory file on the abdomen for males and females of the species *Augyles dilutissimus* and *A. interspidulus* shows the general difference between these structure. The ridges of the female stridulatory file of *Augyles dilutissimus* (Figs 6–7) are more densely located and keep this structure up to the apex of the carina on the abdominal segment. In males, the position is less and the distance between the ridges is significantly increased to the top of the carina (Figs 8–9). It is interesting to note that the cuticular ridges look different in two parts of the first abdominal segment. Some of them are close, and the distance between ridges is almost equal to the width of the ridges on the dorsal area, which is the first-order. On the anterior part of the first abdominal segment, they are smoother, and the distance between the cuticular



Figs 4–5. The morphology of the plectrum: 4 — single ridge of female *Augyles hispidulus*; 5 — single ridge of female *Heterocerus marginatus*.

Рис. 4–5. Строение плектрума: 4 — простой гребень самки *Augyles hispidulus*; 5 — простой гребень самки *Heterocerus marginatus*.

Table 2. Features of *stridulatory file* and *plectrum* the researcher species of *Augyles* species.
Таблица 2. Характеристика стридуляторного поля и плектрума у исследованных видов рода *Augyles*.

Species	length of the stridulatory file	number of ridges at 50 μm	length of ridges (μm)			distance between ridges ± SD (μm)	width of ridges ± SD (μm)	length of plectrum (μm)
			mean ± SD	min	max			
Augyles dilutissimus ♀	408	19	30.49 ± 0.60	12.46	51.43	1.06 ± 0.02	1.85 ± 0.05	441.43
A. dilutissimus ♂	417	17	29.51 ± 0.60	11.84	44.53	1.27 ± 0.05	1.09 ± 0.04	366.99
A. flavidus	383	53	25.00–30.00			—	—	—
A. hispidulus ♀	413	19	37.04 ± 0.65	18.79	57.42	1.63 ± 0.09	1.84 ± 0.04	455.22
A. interspidulus ♀	390	17	39.65 ± 0.55	17.12	74.79	1.35 ± 0.04	2.33 ± 0.06	423.70
A. interspidulus ♂	434	16	49.59 ± 1.27	24.12	86.02	2.32 ± 0.21	0.86 ± 0.03	408.72
A. obliteratus	—	19	50.00–55.00			—	—	517.80
A. sericans	—	40	30.00–40.00			—	—	—
A. turanicus ♀	411	25	28.62 ± 0.27	17.47	37.35	0.81 ± 0.02	1.40 ± 0.05	385.34

Table 3. Features of stridulatory file and plectrum the researcher species of *Heterocerus* species.
Таблица 3. Характеристика стридуляторного поля и плектрума у исследованных видов рода *Heterocerus*.

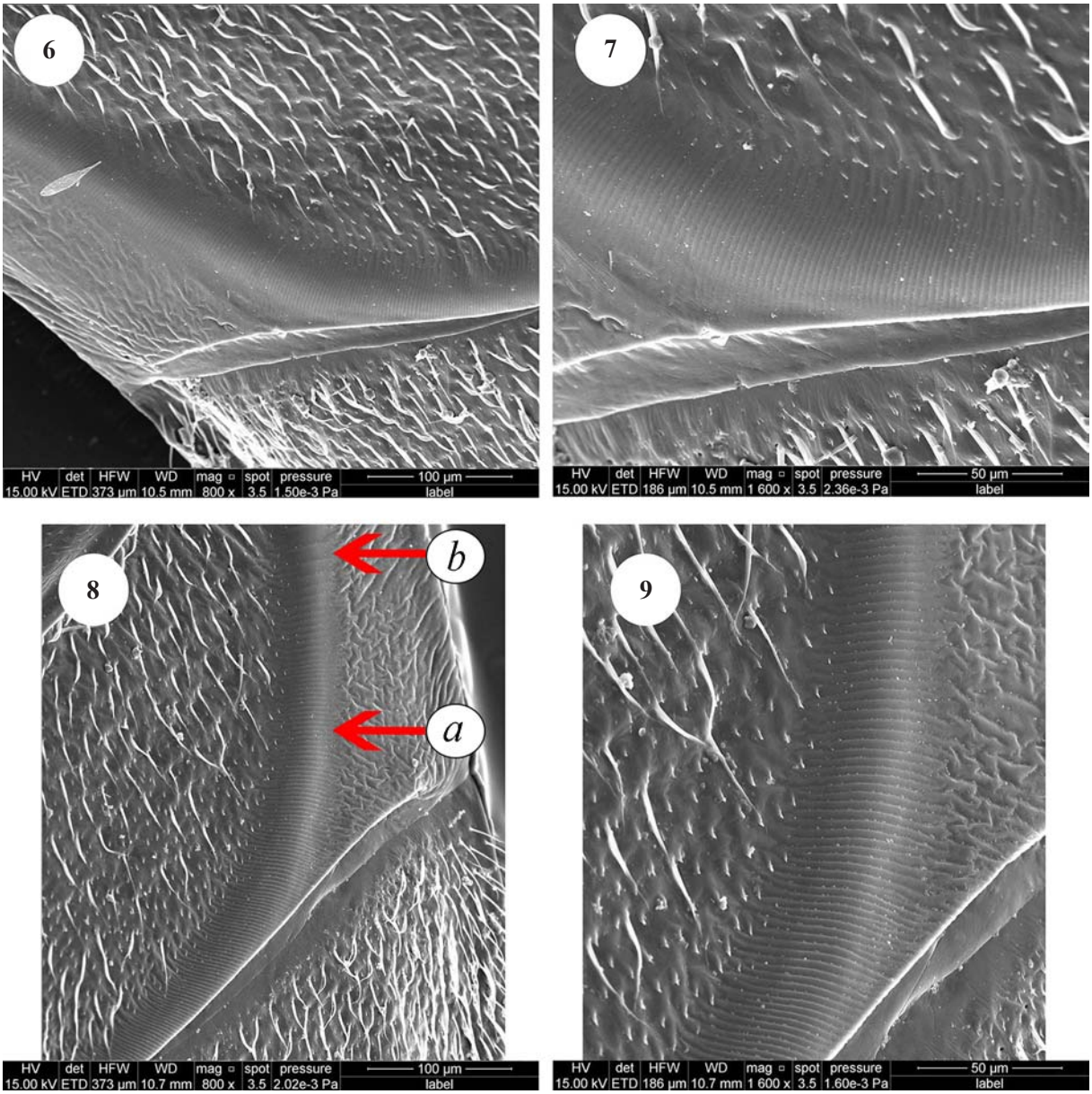
Species	length of the stridulatory file	number of ridges at 50 μm	length of ridges ± SD (μm)			distance between ridges ± SD (μm)	width of ridges ± SD (μm)	length of plectrum (μm)
			mean ± SD	min	max			
Heterocerus fenestratus ♂	431	21	33.83 ± 0.82	8.17	66.26	1.49 ± 0.04	1.26 ± 0.05	389.21
H. flexuosus ♂	426	25	41.08 ± 0.44	26.08	56.35	1.10 ± 0.02	1.06 ± 0.03	-
H. fossor ♀	475	17	44.04 ± 0.76	10.27	71.95	1.26 ± 0.02	1.96 ± 0.03	518.18
H. fusculus ♂	369	30	23.05 ± 0.69	1.54	51.53	0.94 ± 0.02	0.87 ± 0.01	322.27
H. heydeni ♀	468	20	39.73 ± 0.78	8.36	64.27	1.01 ± 0.02	1.07 ± 0.02	-
H. kaszabi ♂	475	22	35.20 ± 0.34	19.34	51.53	1.29 ± 0.02	1.13 ± 0.04	-
H. marginatus ♀	392	24	27.67 ± 0.43	3.39	40.66	0.75 ± 0.02	2.01 ± 0.04	416.42
H. obsoletus ♂	328	20	41.54 ± 0.92	11.72	65.03	1.08 ± 0.02	1.05 ± 0.05	574.00
H. parallelus ♀	399	15	66.00 ± 1.42	14.62	109.09	1.43 ± 0.03	1.78 ± 0.03	505.59

ridges unequally increases, which is the second-order (Fig. 8 *a*, *b*).

The ridges of the female *Augyles interspidulus* are smoother (Figs 10–11) than those of the males. The ridges are more densely distributed, and the distance increases gradually to the apex of the abdominal carina. In males, the increase in the distance between the ridges occurs sharply, approximately in the middle of the stridulatory file (Figs 12–13). On the Fig. 12 (*a*, *b*) shows the same structure of stridulatory organs as in Fig. 8 (*a*, *b*). There are the first-order ridges and second-order ridges.

The stridulatory file consists of a series of teeth with an average size of 383 μm (*A. flavidus*) to 434 μm (*A. interspidulus*). It is interesting here that *A. interspidulus* (16) has a minimum number of ridges on 50 μm and *A. flavidus* has a maximum number of ridges on 50 μm (53). The distance between ridges in males of *Augyles dilutissimus* (1.06 ± 0.02 μm) shorter than in females (1.27 ± 0.05 μm). The peak of the ridges is pronounced more strongly in males, as confirmed by the width of ridges: 1.09 ± 0.04 μm in males and 1.85 ± 0.05 μm in females. *Augyles interspidulus* has the same patterns in the structure of the stridulatory file. The plectrum is represented by a single ridge on the femur.

The genus *Heterocerus* comprises species with a larger stridulatory file. As seen in Figs 14–15, the stridulatory file consists of a series of very fine, parallel



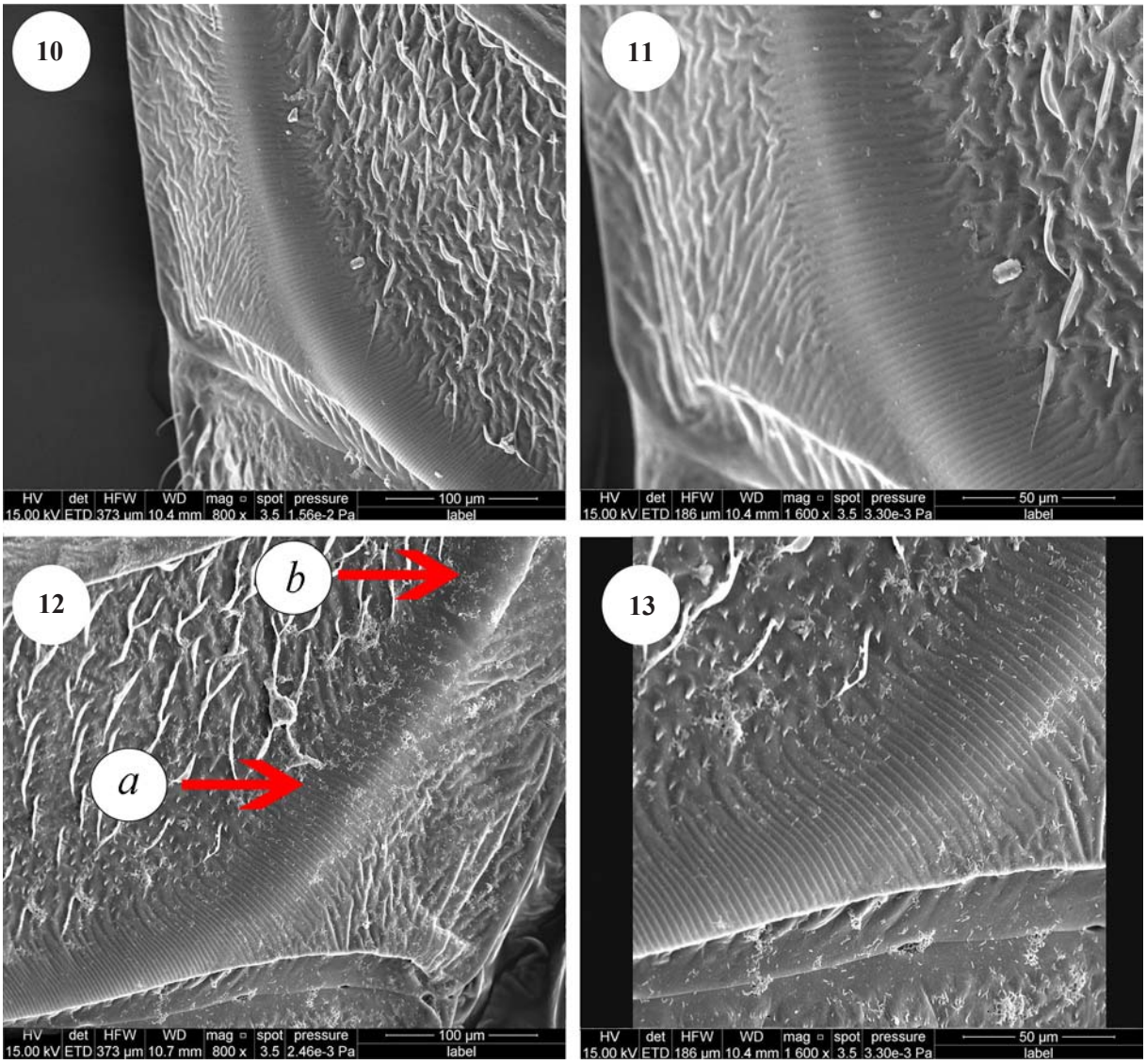
Figs 6–9. The stridulatory file of *Augyles dilutissimus*: 6–7 — female; 8–9 — male; *a* — the first-order ridges; *b* — the second-order ridges.

Рис. 6–9. Стридуляционное поле *Augyles dilutissimus*: 6–7 — самки; 8–9 — самца; *a* — гребни первого порядка; *b* — гребни второго порядка.

lines (ridges, or ribs), which consist of the first-order ridges and the second-order ridges (Fig. 14 *a*, *b*). These can be identified as a series of ridges where the length is from 23.05 ± 0.69 μm to 66.00 ± 1.42 μm. Figs 4–5 shows the general appearance of the plectrum. The end of the plectrum does not reach the tibia and looks similar in all species. Table 3 shows a considerable differences between the structures of the stridulatory organs of different *Heterocerus* species.

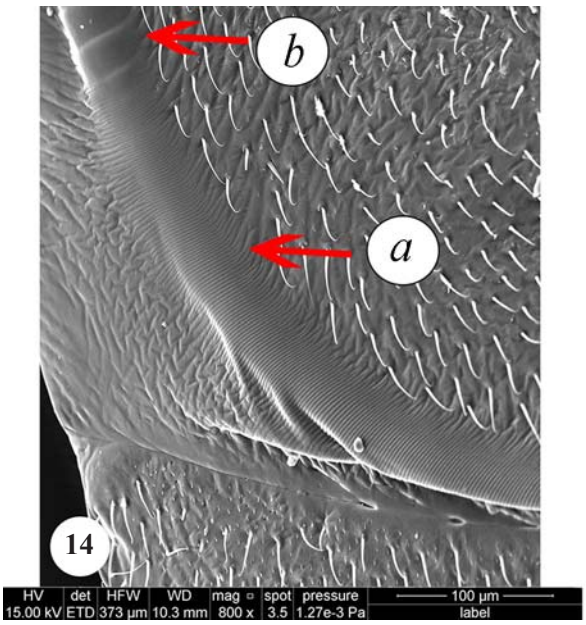
The stridulatory file is similar to those of species of the genus *Augyles* and is shown in Figs 4–5. The size of the ridges ranges from 328 μm (*Heterocerus obsoletus*) to 475 μm (*Heterocerus kaszabi*). The teeth are located more freely on the keel of the abdomen and rather tightly on the 2nd segment of the abdomen. The maximum length of ridges is 66.00 ± 1.42 μm for male *H. parallelus* and the smallest value is 23.05 ± 0.69 μm for *H. fusculus*. The number of ridges on 500 μm is from 15 (*Heterocerus parallelus*) to 30 (*H. fusculus*) and depends on the sex of the species. The structure of the plectrum is the same as in the previously described genus (Figs 4–5).

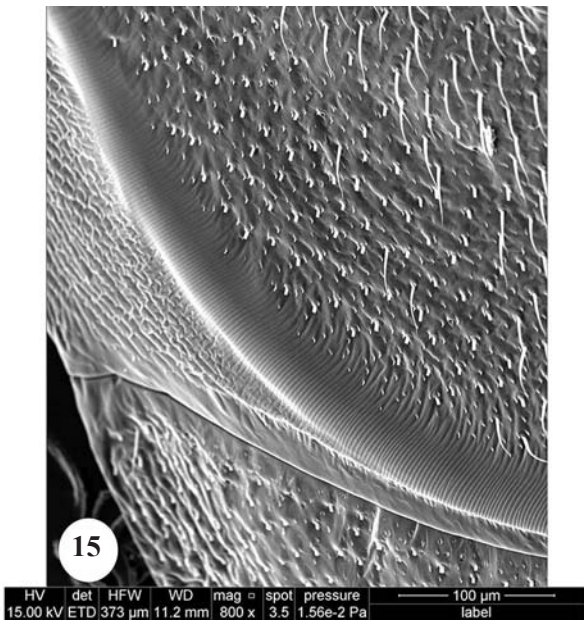
Comparing *Augyles* and *Heterocerus*, we concluded that the size of the stridulation file is more variable in *Heterocerus*. The lowest value of the length of the stridulatory file is 330 μm in *H. obsoletus* and 383 μm in *Augyles flavidus*. The size of the stridulatory file for species of *Augyles* varies from 383 μm (*Augyles flavidus*) to 434 μm (*Augyles interspidulus*). The stridulatory file of the genus *Heterocerus genus* varies from 329 μm (*Heterocerus obsoletus*) to 476 μm (*Heterocerus fossor*). But several ridges have the inverse proportion-



Figs 10–13. The stridulatory file of *Augyles interspidulus*: 10–11 — female; 12–13 — male; *a* — the first-order ridges; *b* — the second-order ridges.

Рис. 10–13. Стридуляционнное поле *Augyles interspidulus*: 10–11 — самки; 12–13 — самца; *a* — гребни первого порядка; *b* — гребни второго порядка.





Figs 14–15. The stridulatory file of *Heterocerus*: 14 — male *Heterocerus flexuosus*; 15 — male *Heterocerus kaszabi*; *a* — the first-order ridges; *b* — the second-order ridges.

Рис. 14–15. Стридуляционное поле *Heterocerus*: 14 — самец *Heterocerus flexuosus*; 15 — самец *Heterocerus kaszabi*; *a* — гребни первого порядка; *b* — гребни второго порядка.

ality of the size of the stridulatory file. The longest length of the stridulatory file is 476 µm for *H. fossor* and 435 µm for *A. interspidulus*. *Augyles dilutissimus* and *A. interspidulus* have a larger size of the stridulation file in males than in females. At the same time, the number of teeth is smaller: 19 (*Augyles dilutissimus*) and 17 (*Augyles interspidulus*) in males and 17 (*Augyles dilutissimus*) and 16 (*Augyles interspidulus*) in females. The number of ridges in the genus *Heterocerus* is much lower than in *Augyles*. The maximum number is 53 for *Augyles flavidus* and 16 for *A. interspidulus*. *Heterocerus fusculus* has the largest number of ridges (30), while *Heterocerus parallelus* has 15 ridges which is the smallest number of ridges in this genus.

As can be seen in Tables 1 and 2, the greatest recorded length of the ridges is 49.59 ± 1.27 µm in *Augyles* (male *A. interspidulus*) and 66.00 ± 1.42 µm in *Heterocerus* (female *H. parallelus*). Comparing species of the genus *Heterocerus*, we conclude that females have the greater length of the ridges except for *Heterocerus marginatu*s. This could be related to the body size of the species. Table 2 reveals a relationship of the distance between ridges and the width of the ridges. Males have sharper apices of the ridges, which is confirmed by the values obtained for *Heterocerus fenestratus*, *H. flexuosus*, *H. fusculus*, *H. kaszabi*, and *H. obsoletus*. The females *Heterocerus fossor*, *H. heydeni*, *H. marginatus*, and *H. parallelus* have smaller or almost the same distances between the ridge. The apices of the ridges are smoother. for the same is true of the males of *Augyles dilutissimus*, and *A. interspidulus* and of the females of females of *A. dilutissimus*, *A. hispidulus*, *A. interspidulus*, and *A. turanicus*.

The length of the plectrum ranges from 367 µm to 518 µm for the genus *Augyles*. At the same time, the size of the plectrum for females of *Augyles dilutissimus* and *A. interspidulus* is 441 µm and 424 µm against 367 µm and 409 µm for males of these species. *Heterocerus obsoletus* 574 µm of the length of the plectrum. This value is higher than those of the genus *Augyles*. *Heterocerus fusculus* has a plectrum 322.27 µm long.

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