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Morphological Differentiation of Closely Related Species of the Genus *Bucculatrix* Zeller, 1839 (Lepidoptera, Bucculatricidae) with an Analysis of Their Distribution and Mechanisms of Reproductive Isolation

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Abstract—Morphological studies of the representatives of the genus *Bucculatrix* Zeller, 1839 from the gnaphaliella, albedinella, frangutella, bechsteinella, cidarella, and ulmella species-groups revealed numerous pairs of closely related and almost indistinguishable species. Their delimitation is only possible by the tiny differences in the genitalia structures, which are more clearly expressed in females than in males. Within the cidarella and ulmella species-groups, some species are found to form more compact subgroups. The analysis of distribution shows overlapping of the ranges for the majority of the studied pairs. The reproductive isolation of the examined sibling species may be generated, similarly to several gelechioid families (Momphidae, Cosmopterigidae), not so much by inconspicuous differences in their morphology but rather by the peculiarities of their biology (different host-plants and different life cycles) and ethology (different mechanisms of finding a mating partner).

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The vast genus *Bucculatrix* Zeller, 1839, which comprises the overwhelming majority of species belonging to the family Bucculatricidae, is characterized by a rather uniform general appearance of moths. As early as half a century ago an unusual abundance of sibling species within this genus was recorded, which demonstrate very slight differences (Braun, 1963). Some species (*B. separabilis* Braun, 1963 and *B. variabilis* Braun, 1963) were found to be only slightly differentiated in the moth morphology, with the synchronized development of larvae on the same host plant. Others (*B. arnicella* Braun, 1963 and *B. tridenticola* Braun, 1963), showing virtually identical external characters of moths, reveal inconspicuous dissimilarity in the male genital structures and in the larval host plants, but inhabit different landscapes (the former species is confined to the forest zone, whereas the latter occurs in the sagebrush semidesert). Several American sibling species (e.g., *B. evanensis* Braun, 1963 and *B. benenotata* Braun, 1963), on the contrary, may be better discriminated by the pattern of the fore wing, which is accompanied by a pronounced similarity of genitalia (Braun, 1963). The study of the Palaearctic representatives of the genus *Bucculatrix* belonging to groups of gnaphaliella, albedinella, frangutella, and bechsteinella, established earlier by us, also revealed numerous pairs of morphologically close species (Baryshnikova, 2013; Baryshnikova, 2014). The groups of cidarella and ulmella were found to contain not only pairs but complexes of species nearly indistinguishable from each other, whose morphological characters have been earlier analyzed in the comparative aspect for generating the identification key (Baryshnikova, 2013).

At present, studies of cryptic species and search for new taxa within various groups of mining moths develop integrative approaches including (in addition to comparative morphological analysis) analyses of mitochondrial and nuclear DNA (e.g., Laštůvka et al., 2013; Kirichenko et al., 2015).

The focus of the present study lies in a more detailed discussion of characters for reliable diagnostics of species of the genus *Bucculatrix*, with regard to their distribution and host plants, as well as in hypothesizing factors determining their reproductive isolation and subsequent speciation.

MATERIALS AND METHODS

A total of 225 specimens belonging to 22 species of the genus *Bucculatrix* have been examined. This mate-

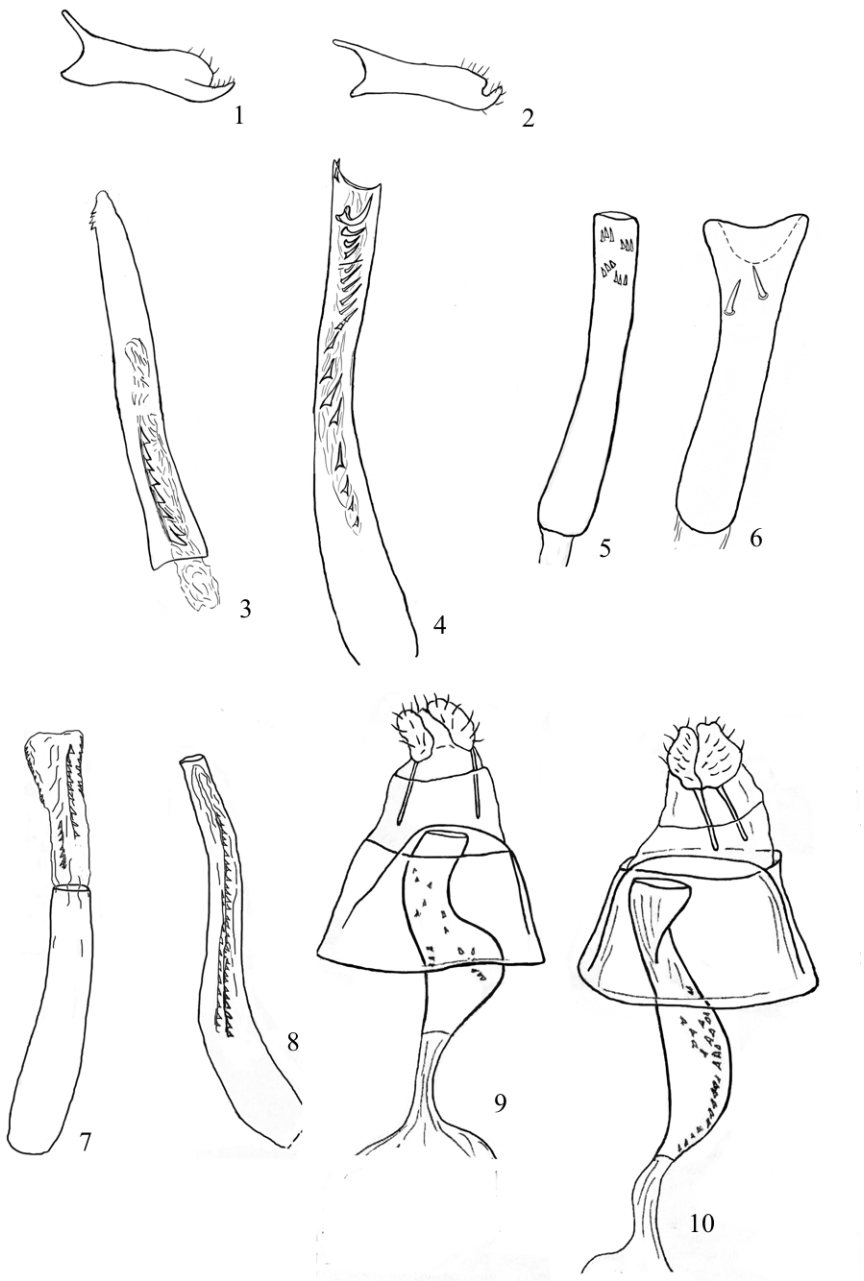
rial is kept in the collection of the Zoological Institute, Russian Academy of Sciences, in St. Petersburg (ZIN). The principal method of study was the comparative analysis of male and female genital structures in morphologically closely related species. The terminology of parts of the copulatory apparatus follows that in the recently published monograph on the genus *Bucculatrix* of the fauna of Russia (Baryshnikova, 2013). The data on distribution and trophic associations were mainly borrowed from the reviews of Nearctic and Palaearctic representatives of this family (Braun, 1963; Kobayashi et al., 2010; Baryshnikova, 2013).

RESULTS

Within the genus *Bucculatrix*, the gnaphaliella group whose species are mostly associated with asteraceous plants is characterized by a great number of species similar to each other. *B. maritima* Stainton, 1851 and *B. latviaella* Šulcs, 1990, which are nearly identical by their male genital structures may be taken as an example. Their morphological resemblance is emphasized by the peculiar, scarcely recorded in the genus *Bucculatrix*, pattern of moth fore wings displaying a basal streak as well as by narrow, acute tegminal lobes and deep subapical incisions of valvae in the male genitalia (Figs. 1, 2). The difference becomes observable only in the direct comparison of moths or genital preparations. The examination of additional material revealed that *B. latviaella* occurred not only in the western part of the Palaearctic Region (described from Latvia and recorded in several countries of Northern Europe); at least one specimen of this species, earlier attributed to *B. maritima*, has been collected in the southern part of Russian Far East (Baryshnikova, 2013) and one more individual (identification requires specification), in Transbaikalia. On the other hand, a specimen of *B. maritima* has been recently found by me among the material from Amur Province (Dubatolov et al., 2014). Thus, both species show extensive distribution ranges; their disjunctions in the Asian part of Russia are explained by the absence of mass collections there. At any rate, asters from the genus *Tripolium*, which are known as the host plants of *B. maritima*, have continuous ranges in the Palaearctic Region. A noticeable resemblance to *B. maritima* and *B. latviaella* is observed in several North-American representatives of the genus, especially in *B. angustata* Frey et Boll, 1876; this species whose larvae are trophically associated with the asteraceous genus *Aster* and, occasionally, with *Solidago* and *Erigeron* is widely distributed in the United States and Canada. In addition to pronounced similarity between *B. angustata* and *B. maritima* in the wing pattern and genital structures, as well as with closely related host plants, these species share a unique biological character: their larvae develop within mines throughout all its life and never feed openly (Braun, 1963); whereas larvae of most congeners mine only in early instars. Another example of sibling species within the gnaphaliella group is exhibited by the widely distributed Palaearctic species *B. artemisiella* Herrich-Schäffer, [1855] and the recently described from the Black Sea coast of Bulgaria *B. lerchianella* Tokár, 2015, which presumably develops on the wormwood. Despite the inconspicuous morphological differentiation of the new species from *B. artemisiella*, a reliable difference has been found in molecular genetic characters (Tokár, 2015). A marked difference by barcodes from West European individuals has been found in moths of the Armenian populations of *B. absinthii* Gartner, 1865, which may imply the species status (Tokár, 2015).

The representatives of the albedinella group, *Bucculatrix albedinella* (Zeller, 1839) and *B. altera* Seksjaeva, 1989, display only very tiny differences in the shape of the aedeagus as well as in the number and location of cornuti in the vesica: *B. altera* has a longer aedeagus with a markedly more stretched row of spiniform cornuti, one spine being always located at the apex of the aedeagus (Figs. 3, 4). Both species possess an eversible scale sac between the second and third abdominal segments representing, most probably, an androconial structure (Braun, 1963). The difference between the two species is better pronounced in the female genitalia; females may be reliably identified by the shape of the antevaginal plate (Baryshnikova, 2005, 2013, 2014). *Bucculatrix albedinella* is characterized by a widely disjunctive amphipalearctic range and is trophically associated with broad-leaved arboreals (elms and, presumably, limes), whereas *B. altera* is recorded only in the Russian Far East and in Japan (Baryshnikova, 2013); both species occurring in Primorskii Territory simultaneously in the same localities.

The frangutella group includes two closely related species: *B. frangutella* (Goeze, 1783), widely distributed in the Western Palaearctic and developing on *Rhamnus cathartica* and *Frangula alnus*, and *B. citima* Seksjaeva, 1989, described from Primorskii Territory



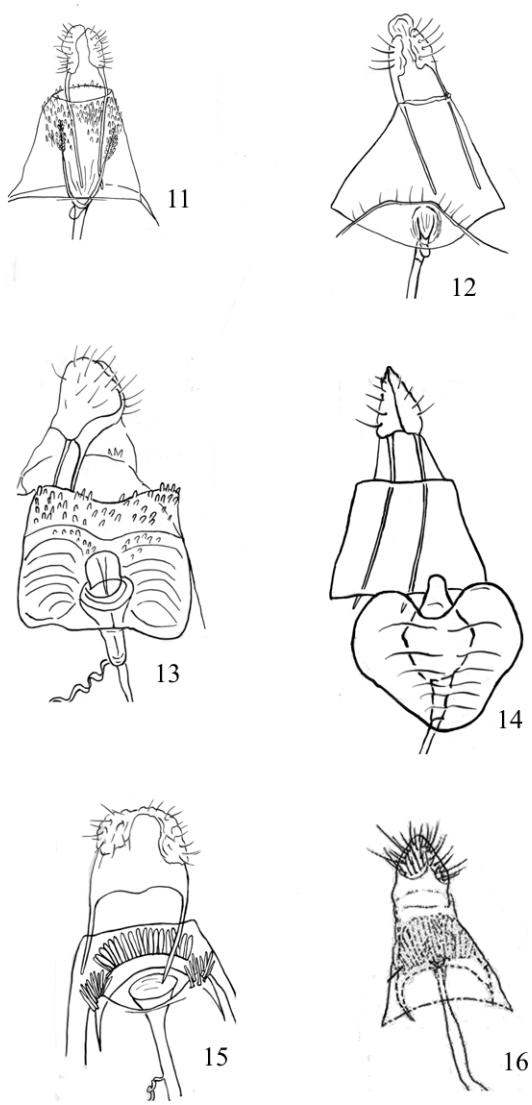
Figs. 1–10. *Bucculatrix* Z., male [(1, 2) right valva; (3–8) aedeagus] and female [(9, 10) bursa copulatrix omitted] genitalia: (1) *B. latviaella* Šulcs, (2) *B. maritima* (Stt.), (3) *B. albedinella* (Zeller), (4) *B. altera* Seksjaeva, (5) *B. citima* Seksjaeva, (6) *B. frangutella* (Goeze), (7, 9) *B. bechsteinella* (Bechstein et Scharfenberg), (8, 10) *B. malivorella* Baryshnikova; (1–6, 9, 10) after Baryshnikova, 2013; (7, 8) orig.

and later recorded in Japan, where it is trophically associated with the Far Eastern species of the buckthorn (Kobayashi et al., 2010). The basic difference between these species seems to be the absence of the scale sac in the male abdomen of *B. frangutella* as well as a different number and position of cornuti: *B. citima* is characterized by small cornuti forming 4 groups, whereas *B. frangutella* exhibits only 2 comparatively large spiniform cornuti (Figs. 5, 6). Females of these two species, similar to the preceding species pair, diverge more clearly (Baryshnikova, 2014). Earlier *B. frangutella* and *B. citima* were regarded as vicarial species; however, *B. citima* has been recently reported in the southern part of Ukraine (Baryshnikova, 2013) and *B. frangutella* was once recorded in the Russian Far East (Caradja, 1920). Therefore, in spite of the latter determination based on the external characters should be probably regarded as erroneous,

the overlapping of the distribution ranges in Eastern Europe may be considered as reliable.

The bechsteinella group comprises a pair of hardly distinguishable species, *B. bechsteinella* (Bechstein et Scharfenberg, 1805) and *B. malivorella* Baryshnikova, 2007. These species show a great resemblance by the male genitalia and by the presence of a scale sac in the male abdomen, but reveal clearer differences in the female genitalia (Figs. 7–10). *Bucculatrix bechsteinella* develops on various fruit rosaceans and demonstrates a wide distribution range from Western Europe and Northern Africa towards Transbaikalia, while *B. malivorella* has been still recorded on apple trees and occurs in Middle Asia. It is probable, nevertheless, that the published data on the presence of *B. bechsteinella* in Middle Asia region should be attributed to *Bucculatrix malivorella*; at least, the material from Tajikistan, Uzbekistan, Kirgizia, and Sothern Kazakhstan examined by me represents only *B. malivorella*.

The cidarella group comprises 6 extraordinarily similar species: *B. cidarella* (Zeller, 1839), *B. bifida* Seksjaeva, 1989, *B. locuples* Meyrick, 1919, *B. muraseae* Kobayashi, Hirowatari et Kuroko, 2010, *B. similis* Baryshnikova, 2005, and *B. parasimilis* Baryshnikova, 2005. The two latter species show inconspicuous difference in the width of the tegminal lobes (markedly wider in *B. parasimilis*) and in the shape of the scale sac, which is rather small and lined with narrow scales in *B. similis*, whereas in *B. parasimilis* it is divaricated and lined with wide scales (Baryshnikova, 2005). *Bucculatrix muraseae*, described from Japan and displaying trophic relations with *Alnus japonica*, has been later recorded in Primorskii Territory of Russia (Baryshnikova, 2013). Males of this species, similar to those of *B. cidarella* and *B. bifida*, have no scale sac; however, the copulatory apparatus of *B. muraseae* is markedly larger, its tegminal lobes are slightly curved, and valvae apically pointed. *B. bifida* described from Primorskii Territory has a close resemblance to *B. cidarella* by the male genitalia (this species is widely distributed in the Palaearctic Region including Japan: Kobayashi et al., 2010, but it was not found in the Russian Far East), showing, however, somewhat differently shaped female genitalia (Figs. 11, 12). Judging by the label of the holotype, *B. bifida* was reared from buckthorn, which distinguishes it from *B. cidarella*, whose larvae mine leaves of alder species. *B. locuples* is known from the USA and Canada (Braun, 1963) and is associated with the alder (*Alnus serrulata*); it noticeably resembles *B. muraseae* by the male genitalia and has dark coloration and fore-wing pattern like *B. cidarella*, but with golden luster (Braun, 1963). The trophic associations of *B. similis* and *B. parasimilis*, which are known only by their type specimens from Primorskii Territory, remain unknown. Therefore, 5 out of 6 species of the cidarella group are found to occur in the Far East; however, it is impossible now to analyze the peculiarities of the total distribution of this group as well as to characterize in full measure the distribution ranges of individual species.



Figs. 11–16. *Bucculatrix* Z., female genitalia: (11) *B. bifida* Seksjaeva; (12) *B. muraseae* Kobayashi, Hirowatari et Kuroko; (13) *B. abrepta* Seksjaeva; (14) *B. eclecta* Braun; (15) *B. comporabile* Seksjaeva; (16) *B. tsurubamella* Kobayashi, Hirowatari et Kuroko; (11–15) after Baryshnikova, 2013; (16) after Kobayashi et al., 2010.

The ulmella group is subdivided into several subgroups based on morphological characters. One includes species associated with elms; these are *B. ulmifoliae* Hering, 1931 distributed in the western part of the Palaearctic Region, the Far Eastern *B. abrepta* Seksjaeva, 1989, and *B. eclecta* Braun, 1963 known up to the recent time only from North America. However more thorough examination of the available material provided the possibility to attribute a fraction of the Far Eastern specimens earlier determined as *B. abrepta* to *B. eclecta* (Baryshnikova, 2013). The males of *B. abrepta* are characterized by a small abdominal scale sac, nearly parallel-sided vinculum, very volumetric valva with beaked apex (on preparations with a cover glass, it looks almost rectangular), and aedeagus devoid of a dent on a wall); meantime, males of *B. eclecta* exhibit a comparatively large abdominal sac and triangular vinculum, valva with straight apex (slightly convex on the preparations with a glass), and aedeagus with a small dent on the wall (Baryshnikova, 2013). Female genitalia also reveal a difference between *B. eclecta* and *B. abrepta* (Figs. 13, 14). Interestingly, both species occur in the southern parts of Primorskii Territory in the same places and approximately at the same time. In North America, larvae of *B. eclecta* mine leaves of *Ulmus pumila*, an elm species which is not native but is widely distributed there, whose native range is located in the eastern regions of the Palaearctic Region.

Bucculatrix ulmifoliae, judging by the images of its lectotype, is similar to *B. abrepta* in the shape of the aedeagus, which displays, however, 2 dents on the wall (Mey, 1999; Langmaid et al., 2007). The vinculum of *B. ulmifoliae* caudally forms a marked rounded lobe, nearly indistinct in *B. abrepta*, whereas its valval apices are less pronouncedly stretched dorsolaterally on preparations. The female genitalia of *B. ulmifoliae* are well distinguishable from those of *B. abrepta*, but virtually identical to those of *B. eclecta*. It is quite possible that re-examination of the type material of *B. eclecta* will lead to future synonymization of this spices with *B. ulmifoliae*. The three aforementioned species are closely related to *B. ulmicola* Kuznetzov, 1962, which is known from the south-western part of the Palaearctic Region; however, this species is differs strongly from them in the shape of the male genitalia exhibiting small accessory growths of the tegminal lobes.

Within the ulmella group, one more complex of species nearly identical in morphological characters is formed by *B. ulmella* Zeller, 1848, *B. comporabile* Seksajeva, 1989, and *B. tsurubamella* Kobayashi, Hirowatari et Kuroko, 2010. *B. ulmella* is peculiar in the absence of the scale sac in the male abdomen, whereas this structure is well developed in *B. comporabile* and *B. tsurubamella*. The two latter species may be discriminated by the valvae not fusing basally and forming no plate in *B. comporabile*, unlike those in *B. tsurubamella*. Females in this complex exhibit variously shaped and easily detachable scales forming somewhat differently located groups on the abdominal segment 8, their functional destination being vague (Figs. 15, 16). *B. ulmella* is widely distributed in Europe, being found outside Europe in eastern Georgia (Lagodekhi Nature Reserve). It has trophic associations with the West Palaearctic species of the oak, though it has been also found on elms and chestnuts. Two other species occur in Primorskii Territory of Russia and in Japan, where larvae of both species were found to develop on oaks. It is noteworthy that *B. comporabile* is recorded on several East Palaearctic oak species, while *B. tsurubamella* is described from *Quercus acutissima* which does not occur in Russia; maybe in Primorskii Territory it is associated with other *Quercus* species. Apparently, the two Far Eastern species can be considered as vicariants of a more widely distributed European-Caucasian species.

One more cluster within the ulmella group includes *B. applicita* Seksjaeva, 1989 described from the southern part of Primorskii Territory and presumably associated with the oak as well as the North American *B. zophopasta* Braun, 1963, *B. packardella* Chambers, 1873, and *B. polytita* Braun, 1963, whose larvae mine leaves of oak and birch. All these species are characterized by the marked morphological similarity of the genitalia in both sexes, which also provides a possibility to hypothesize vicariation.

DISCUSSION

The Palaearctic fauna of the genus *Bucculatrix*, as well as the Nearctic fauna, are found to contain numerous pairs and complexes of virtually indistinguishable species, most of them demonstrating overlapping of distribution ranges. The diagnostic characters are predominantly associated with the copulatory apparatus features not infrequently more developed in females than in males which hardly yields to a formal definition. In several cases, identification of such

a species may be based on the presence or absence of the eversible scale sac in the male abdomen (*B. frangutella* and *B. citima*), as well as on the basis of its size and the shape of the covering scales (*B. abrepta* and *B. eclecta*). Lack of serial material for the majority of the species under discussion provides no possibility to apply now the methods of statistical analysis for their study.

The analysis of distribution in most examined species pairs and complexes revealed a partial overlapping of their ranges; closely related species could be found simultaneously in the same localities (*B. albedinella* and *B. altera*). At the same time, there are cases of a great geographic disjunction of ranges in allied species (*B. ulmella*, on the one hand, and *B. comporabile* and *B. tsurubamella*, on the other). The host plants of such species, if established, are identical or belong to a single genus, though a more pronounced difference in the food specialization may be detected, as in pairs of *B. bechsteinella–B. malivorella* or *B. bifida–B. cidarella*.

The reproductive isolation of sympatric and morphologically similar species is presumably associated with the presence of an eversible scale sac in the male abdomen, which represents a unique structure of Bucculatricidae developed in the majority of the known species and being, most probably, of androconial nature (Braun, 1963). Dissimilarities in its size and shape as well as in the structure of its scales suggest different behavioral responses as in the representatives of cidarella and ulmella groups. Several species probably have androconial structures on the female genital segments (*B. comporabile* and *B. tsurubamella*).

Involvement of visual signals in the maintenance of reproductive isolation in sympatric closely related species, such as *B. cidarella* and *B. muraseae*, which differ by coloration and wing pattern, cannot be ruled out. Probably, these signals operate in cases of similar wing pattern in species differentiated by the presence/absence of characteristic luster of the wing plate, e.g. in *B. cidarella* and *B. locuples*; however, overlapping of distribution ranges in not yet recorded.

Unfortunately, the value of phenological and ecological difference in the divergence of closely related sympatric species is hard to ascertain now owing to scarcity of collected material and insufficient knowledge of their trophic associations.

Therefore, the factors affecting speciation in the genus *Bucculatrix* can be ascertained only judged about only approximately, but even the rather scant available data provide a possibility to assume that the speciation mechanisms in this genus resemble those hypothesized for several other phytophagous lepidopterans (Sinev, 1989), which are characterized by a small body size, poor flight activity, and comparatively intricate precopulatory behavior.

On the basis of the cidarella group, consisting of a complex of closely related and morphologically poorly distinguishable species, it may be supposed that their differentiation occurred not long ago and is associated with a rise of dissimilarities in the mechanisms of attraction of individuals of the opposite sex and/or using different host plants. However, it is hard to say what evolutionary changes appeared first and triggered a the process of speciation.

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