Final report of the project 116694 –
Taxonomy, Phylogeny and Biogeography of the Subtribe Poliina (Lepidoptera, Noctuidae)

Aims of the project

Our surveys constituted the continuation of our earlier Noctuidae projects between 2004-2013 (**47005**, 2004-2007; **73597**, 2008-2010; **110122** book, 2013), focused, however, on taxonomic relations, biogeographic and phylogenetic connections of the surveyed group.
We hypothesised the following main regularities:

(i) The phylogenetically basal groups have the original bilaterally symmetrical form of the male genital capsula.
(ii) The core area of the species diversity overlaps with the range of the phylogenetically most ancient groups which are forming the basal splits morphologically and according to the sequences of the barcoding gene (CO1).
(iii) Originating from the bilaterally symmetrical structures there are two main divergent trends of change: *(α)* the enhanced complexity of external genitalia connected with secondary asymmetrisation of the functionally active parts (clasping, stimulation, sensual function), combined with less complexe structures of inner genitalia, i.e. there is a trade-off in the complexity of external vs. internal genitalia *(β)* the simplification of genital capsula is combined with sophistication of the internal genitalia in both sexes: i.e. there is an other trade-off in the complexity of external vs. internal genitalia.
(iv) The two most diverse and widely distributed genera (*Polia* vs. *Ctenoceratoda*) show this basic two types of trade-offs, the first in Holarctic arboreal zonobiomes, the latter in Central Palearctic orobiomes + eremic habitats.
(v) The speciation in these genera proceeded basically by allopatric segregation, after expansion crossing some barriers. Therefore the mostly derived forms of the diverse genera should occur at the peripheries of the genus' range of distribution.

As we explain in the **Results** and **Discussion,** we could fully confirm these main expectations.
Our working hypotheses required to answer the main questions as follows.

- Which are the main phylogenetic trends of morphological change in external genitalia? Which characters can be qualified as plesiomorphic vs. apomorphic in Poliina genera?
- Which are the core areas of species diversity *(i)* of the genera which share the most plesiomorphic characters and *(ii)* of the species of the genera which have conserved the most plesiomorphic characters?
- Can we uncover some connections between morphological character changes, geographical expansivity and species diversity?
- Which are the infrageneric, monophyletic species groups within the mostly diverse genera *Polia* and *Ctenoceratoda*?
- Which species groups of *Polia* have a Holarctic or wide Trans-Palaearctic distribution? Which patterns of geographical variation exist in such species?
- Which are the vicariant sister species within the Holarctic species groups of *Polia*? Which level of molecular differentiation exists in such species?

All these questions were answered in the **Results** and **Conclusions**.

Materials and Methods

The method of our surveys was basically classical taxonomic, using all modern facilities from electronic databases and large collection of digitalised microscopic genitalia slides to molecular surveys (CO1 gene of mtDNA), since we mostly worked with conserved museum material. We also used light and scanning electronic microscopy to uncover the finer structural details of antennae in closely related species. For morpho-taxonomic studies, we collected and evaluated the photographic documentation of comprehensive type materials and other important voucher specimens of Poliina species from several internationally important European collections (Berlin, Bonn, Budapest, London, Munich, Vienna and St. Petersburg).
We established the biogeographical character of all *Poliina* species based on the information harboured in an electronic database of the distribution data. From all species we prepared series of standard genital slides (more than two thousand) with special consideration on the type specimens. We designated several lectotypes, recognised and stated synonymies and composed a morphological matrix of genital characters (see: Varga et al. 2017, 2020).

Using the genital morphological data matrices, we produced trees based on these characters (Trees 1-2 and Supplement in Varga et al. 2020). Two independent runs were allowed on data for females and males, separately, iterating for 10,000,000 generations logging every 1000. The Mkv model (Lewis 2001) for morphological data available in BEAST v2.4.7. (Bouckaert et al. 2014) was applied in all sets. Maximum clade credibility trees were created from 10,000 trees after removing 10% (50% for combined) burn-in using TreeAnnotator v2.4.7.(Bouckaert et al. 2014) for males only (1), for females only (2), and for males and females combined (3).

For our molecular taxonomic investigations, we collected the sequence data of all accessible Poliina species from published barcoding results of the North American, Central andNorthern European species, and the known gene bank sequences (Tree4). Sequences for *cytochrome c oxidase subunit 1* (CO1) were gathered from GenBank (http://ncbi.nlm.nih.gov/). The average length of DNA sequences was 649.2 base pairs. MAFFT (Katoh et al. 2005) was applied for aligning gene sequences. Then the alignments were cleaned with Gblocks (Castresana 2000). The 'ape' (Paradis et al. 2004) and 'seqinr' (Charif & Lobry 2007) packages in R v3.4.1 (R Development Core Team 2017) were used to facilitate these steps (Varga et al. 2020).

A maximum likelihood approach (RAxML, Stamatakis 2006) was applied to find the starting tree for the Bayesian MCMC analyses conducted in BEAST v1.8.3 (Drummond & Rambaut 2007). The best fitted substitution model (GTR+G+I) according to the model test available in the 'phangorn' package (Schliep 2011) was set, and a birth-death model of speciation was applied in the analyses (Gernhard 2008). Two independent BEAST runs were allowed iterating for 50,000,000 generations logging every 5000. A maximum clade credibility tree was created from 20,000 trees after removing 10% burn-in using TreeAnnotator v1.8.3. (Rambaut & Drummond 2016). Effective samples sizes were checked in Tracer v1.7.6. (Rambaut et al. 2018), for the analyses based on morphological and also on genetic data, which allowed evaluating the initial settings for the MCMC runs.

Results (I)

Taxonomical results with phylogenetic considerations

We presented a revised checklist of the subtribe Poliina Hampson, 1902. In this publication (Varga et al. 2017) we described one new genus (*Multisigna* gen. n.), three new subgenera (*Atropolia*, *Leuconephropolia* and *Protopolia* subgen. n.) and a new species (*Polia* (*Atropolia*) *posterodiluta* sp. n.). In an other publication (Varga et al. 2018) we described one more new subgenus (*Metallopolia*) and its taxonomic position was discussed. The subtribe Pachetrina Beck, 1996 was synonymised with Poliina; two genera (*Kollariana* Hacker, 1996 and *Spiramater* McCabe, 1980) were transferred to the subtribe Mamestrina Hampson, 1902. A number of lectotype designations and new combinations were given; the newly designated lectotypes and the genitalia of the disputed taxa were illustrated.

We provided a re-description of the subtribe Poliina and confirmed that the typical leg spining described by McCabe (1980) represents a synapomorphy with the sister subtribus Mamestrina, and this trait separates this clade from other Hadenini. According to our morphological analysis Poliina moths proved to be monophyletic based on shared characteristic "ground plan" of genital structures (including some lock-and-key mechanisms, earlier hypothesised by Varga and Ronkay 2013) with several synapomorphies. We considered as such ones, (***α***) in males, (i) the identical structure of ampulla-harpe complex, (ii) the clavus fused to the dilated dorsal costa of sacculus, (iii) the elongate and regularly (*Haderonia, Polia*) asymmetrical saccular processes with tufts of specialised brushes (Fig. 1), (iv) the long, tubular endophallus (vesica) without subbasal diverticulum and cornutus but (v) with long medio-subterminal field of fasciculate cornuti, (***β***) in females, (vi) the globular corpus bursae and (vii) the tubular appendix bursae.

As plesiomorphic structure, the distal part of valvae is usually ended in spatulate cucullus with corona and anal spine. The paired trifine brush organ (*TBO*, Zilli & Di Giulio 1996) is regularly present on the second abdominal segment (Fig. 2), often showing species- or species group-specific differences. The last abdominal segments often show specific strongly sclerotised, shield-shaped structures. On the shield-shaped, strongly sclerotised male eight sternite, the posterior abdominal brush (*PAB*, Zilli 1997) is strongly reduced, substituted by easily removable hairs only, and the sack of *PAB* is substituted by a weakly sclerotized, dotted stripe at the proximal part of the abdominal segment. In female genitalia, ovipositor is usually soft, densely covered with fine hairs, ductus bursae is elongate, strongly or moderately sclerotised, bursa is saccate with various number and pattern of signa; the shape and sclerotisation of appendix bursae shows co-adapted traits to the shape of the male vesica.


Fig. 1. Asymmetrical male genital capsula of *Polia tiefi* Fig. 2. Paired ***trifine brush organ*** with pockets of *P. tiefi*

All Poliina genera share these basic morphological characters but they also have some external differential traits. The species of the moderately diverse genus *Haderonia* have relatively simple, usually bilaterally symmetrical genital capsula (Figs. 4-5.). It was subdivided into two species groups from which the species of the smaller ones are rather similar externally while the five larger ones can be easily separated from each other. All *Tricheurois* species only show a very slight external differentiation, sharing a nearly unicolourous blackish-brown colouration with typical bronze shine. *Ctenoceratoda* species have very long, in males typically pectinate antennae (name!) and elongate, acute shape of forewings, and colourful patterns somewhat like to *Cucullia* species. *Polia* is the most diverse and heterogenous genus of the subtribe. However, it is taxonomically strongly subdivided, and all of the oligotypic subgenera, such as *Atropolia* Varga, Ronkay & Ronkay, 2017, *Metallopolia* Varga, Ronkay & Ronkay, 2017 and *Protopolia* Varga, Ronkay & Ronkay, 2017 display a very special look. All genera but also the subgenera of *Polia* show very typical, recognisable genital characters. These have been described and characterised in detail in four publications (References) and in the monographic book (Noctuinae III. – Poliina. Taxonomic Atlas of the Eurasian and North African Noctuoidea. Volume 11.).

Based on the categorical absence of the T-shaped vesica in Poliina, typifying numerous Mamestrina genera (see also: Varga & Ronkay 1991), and also on the absence of the subbasal diverticulum with cornutus, certain genera and species formerly associated with Poliina have been excluded from this subtribe. The genus *Kollariana* includes three large, externally confusingly *Polia*-like species, the genitalia of which demonstrate, however, their close relationship with the *Sideridis* clade of the subtribe Mamestrina Hampson, 1902. This genus is transferred, therefore, into this latter supraspecific unit. It is worth to mention that there are some additional large-sized, *Polia*-like species occurring in the mountains of the SE frontier of the Tibetan plateau (e.g. the taxa of the genus *Irene*, the two members of the newly described genus *Multisigna* (“*Polia*” *costirufa* Draudt, 1950 and “*P.*” *hofer* Saldaitis, Benedek & Behounek, 2016), and the still less investigated “*Hyssia*” *hadulina* Draudt, 1950, etc.) which all belong to Mamestrina.

Results (II)

Differential characters and re-definitions of genera

Beside of these general characters, the species of some Poliina genera can be easily recognised by some external traits. According the original description (Hampson 1905), ***Tricheurois*** is characterised by upturned palpi, obliquely rounded frons, large and hairy eyes, head and thorax with simple hairing without any crests, and with spined middle and hind tibiae. The genus was also characterised by the veins of the fore and hind wings (3 and 5 near to the angle of the cell, 6 from the upper angle of the cell, 9 from 10 anastomosing with 8 to form an areole; and 3, 4 from angle of the cell, 6, 7 shortly stalked, respectively). Thus, this genus was diagnosed in the original desciption as “Agrotinae” based on the tibial spining, resulting in the junior synonymy *Acanthopolia* Boursin, 1943 who later recognised it as Hadeninae (Boursin 1965) based on several external and genital characters and placed between *Haderonia* and *Sideridis*. He correctly characterised the male genital capsule as a modification of the genital apparatus of *Haderonia*.

Fig. 3. Symmetrical genital capsula of *Tricheurois cuprina* with modified cuculli

Male genitalia are characterised by the very strong sclerotisation of the clasping apparatus, the characteristic modification of the spatulate cucullus with reduced corona but with strong anal spine and with long, arcuate “neck”, the tiny, weakly sclerotised ampulla, the short and symmetric saccular processes, the sclerotisation of the rugulose scaphium, the elongate and arcuate tube of aedeagus with only slightly stronger sclerotised carina and elongate, helicoidally tubular vesica with a long medio-terminal stripe of fasciculate cornuti.

Female genitalia show the general “Poliina-type” with weakly sclerotised anal papillae, and tubular, somewhat rugulose appendix bursae. Seventh abdominal sternite is strongly sclerotised, shield-shaped.

The original description of the genus ***Haderonia*** Staudinger, 1896 was completely merged with the description of the type species *H. subarschanica*. Some traits are similar to *Tricheurois*, such as the the hairy, upwards directed palpi, the hairy eyes and the fully developed proboscis. The long, pectinated antennae, the mesothoracic and abdominal hair crests were mentioned as differential characters. The genital capsule shows the configuration being typical for the subtribus Poliina characterised by a relatively simple, nearly symmetrical form of valvae with well-developed spatulate cucullus and developed corona with anal spine. Clavus is fused to the inflated dorsal costa of sacculus. Saccular processes are short or moderately elongate, symmetrical in shape but with differentiation or asymmetrical reduction of hair brushes. Harpe and ampulla are weakly sclerotised, short or reduced. Uncus is acute, strongly or moderately sclerotised; tegumen and saccus broad triangular. The trifine brush organ (TBO) is well developed. In female genitalia, papillae anales are weakly sclerotised, densely covered with long, fine hairs. Antrum broad U-shaped or infundibular, ductus bursae is elongate, dorso-ventrally flattened, moderately or strongly sclerotised. Corpus bursae is saccate, without signa; appendix bursae tubular, moderately elongate. We separated two species groups based on external morphology and the male genital capsule, mainly on the specific, partly secondarily asymmetrical modifications of the cucullus and sacculus (Figs 4-5).

Fig. 4. Genital capsule of *Haderonia persimilis*

Fig. 5. Genital capsule of *Haderonia lasiofallax* sp.n.

The genus ***Ctenoceratoda*** Varga, 1992 was separated from *Haderonia* Staudinger, 1896 (type species: *Haderonia subarschanica* Staudinger, 1895 by monotypy) based on significant differences in genitalia of both sexes, different external look and very long, in male typically strongly bipectinate antennae (see the *derivatio nominis*). The very sophisticated lock-and-key apparatus of the vesica and the corpus/appendix bursae complex occurring in all species, in combination with certain simplifications of the completely symmetrical genital capsule were considered as main generic traits, which allow a clear differentiation both from the closest related genera, *Haderonia* and *Polia*. Based on these characters, eleven *Haderonia* species (sensu Boursin 1964) were transferred to the newly erected genus (Varga 1992) and additional four new species have been described. We (Varga et al. 2018) defined ten basic characters of the genus, partly shared with *Polia* and *Haderonia* as tentative synapomorphies, and considered these genera as forming a larger monophyletic unit together with *Ctenoceratoda*. The species content of the genus has been remarkably increased with the descriptions of seven new species.

Taxonomic subdivision of Polia, phylogenetic considerations

According to the genital morphology of both sexes, *Polia* (s.l.) was primarily subdivided (Varga et al. 2020) with full support into two main branches: *(i)* the *nebulosa* clade vs. *(ii)* all other species groups. Within the latter, more heterogenous group the monophyly of the *Pachetra-Metallopolia* twin clade is mostly supported which share (i) the symmetrical shape of clasping apparatus and (ii) the elongate, in *Pachetra* also spiralised vesica, without diverticula and covered by extremely numerous, nearly evenly dispersed tiny spinulose cornuti (Figs. 6-7.).

The subgenus *Metallopolia* (Varga et al. 2018) contains seven species representing three major lineages, the *culta*-, the *subviolacea*- and the *kalikotei*-lineages. From taxonomic point of view, the *culta*-line is the most diverse, displaying sympatric differentiation and allopatric speciation, as well, in the western and southern Himalaya (from Kashmir to eastern Nepal and Sikkim) vs. south-western and western China (Sichuan, Yunnan, Gansu). These taxa are characterised by the partly sympatric occurrence of a “small” and a “large” species. The western Himalayan *P. (M.) culta* and *P. (M.) kisculta* and the Chinese *P. (M.) dysgnorima* and *P. (M.) metagnorima* twin species show this remarkable duality, with clearly recognisable differences in the genitalia of both sexes, too. The two Chinese species remained undescribed until yet due to their external similarity to *P. (M.) ignorata* and *P. (M.) subviolacea*. The allopatric differentiation in the populations of the *kalikotei*- and the *subviolacea*-lineages resulted in a smaller morphological divergence which was considered here as subspeciation (Varga et al. 2018).

The closest related lineage of *Metallopolia* is the *praecipua-sublimis-minae* species-group, described as *Protopolia* subgen. n. (Varga et al. 2017). The differential characters are as follows: 1) male antenna of *Metallopolia* is broadly bipectinated, even the pectination of the *kalikotei*-line is remarkably longer than in *Protopolia*; 2) in *Metallopolia* the saccular processes are much longer and more asymmetrical, with differentiated setose areas while in *Protopolia* the saccular processes are shorter and symmetrical with simple, unspecialised setose areas; 3) the ampulla is absent, only the basal plate of the harpe (clasper) is present, while in *Protopolia* (and in other lineages of *Polia*) a variably shaped ampulla is present; 4) in *Metallopolia* the vesica is longer, tubular and basally curved, with large subbasal diverticulum and with long, more or less twisted cornuti field while the vesica of *Protopolia* is similar to *Polia s.str.*, shorter and broader tubular, shortly recurved, with short and weak spinulose field; 5) the ductus bursae and the antrum are fused into a sclerotised and flattened tube; 6) the appendix bursae is much longer than in the main groups of *Polia* (except the *subcontigua*- and the *mortua*-lineages), long, sausage-shaped or coiled and basally twisted, with long sclerotised basal-subbasal crests, while it is a subconical or semiglobular structure in the subgenus *Protopolia*.

The widely distributed Holarctic subgenus, *Polia* Ochsenheimer, 1816 s. str. is most strongly differentiated from all other subgenera of *Polia*, characterised by the asymmetrical male genital capsule, combined with the co-evolved simplification of vesica and appendix bursae, respectively, and by the presence of allopatric Nearctic-Palaearctic species pairs (Varga et al. 2019, 2020).

The coherence of the externally rather diverse *Atropolia mortua–Polia hepatica–P. griseifusa–P. subcontigua* complex is morphologically strongly supported (Figs. 6-7). These species are also characterised by the plesiomorphic, symmetrical character state of valvae without differentiation of saccular bristles, as in the widespread *P. hepatica* and *P. subcontigua*, or the reduction of saccular bristles in the West Chinese endemic *P. griseifusa*. Furthermore, *P. hepatica* also shows a helicoidal configuration of the long, tubular vesica, and, in female, the co-adapted sausage-shaped appendix bursae. These traits are present in somewhat similar form also in some other genera of Poliina as *Haderonia*, *Ctenoceratoda*, *Tricheurois* which share also the plesiomorphic symmetrical shape of the genital capsule. It is worth to note that an extreme form of the helicoidal configuration of vesica is present in the other widespread species, *P. subcontigua*, combined with the reduction of the subterminal fascia of cornuti as a unical derived character.

The saccular processes are relatively short and without well differentiated tufts of bristles in the externally distinct species *P. subcontigua*, *P. hepatica* and *P. griseifusa* (Fig. 8, left columne). In all other species, there are some trends in the asymmetrisation of the genital capsule and – as a trade-off – in the simplification of the vesica. The saccular processes are more elongate and are bearing a brush of stronger bristles only on the left side in the arctic-boreal species group: *P. lamuta*, *P. richardsoni* and *P. rogenhoferi* (Fig. 8, right columne). This "triplet" of species is also strongly supported on the genital morphological trees (Figs. 6-7).

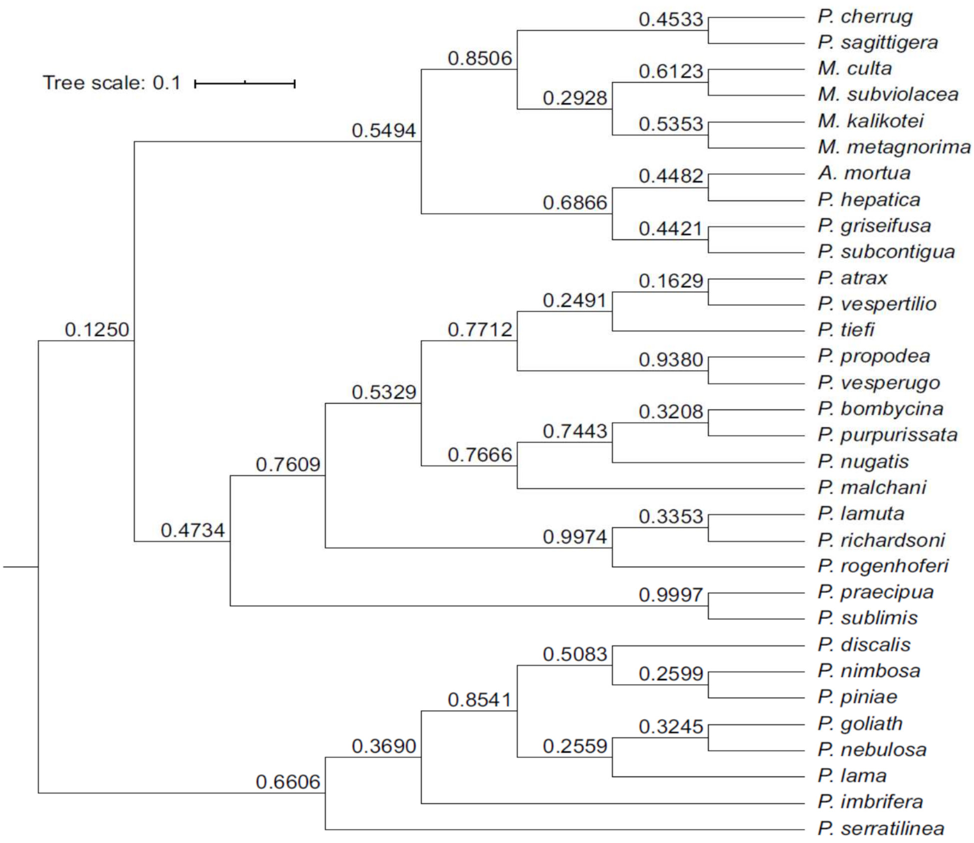


Fig. 6. Consensus tree of Poliina based on discrete characters of the male genitalia

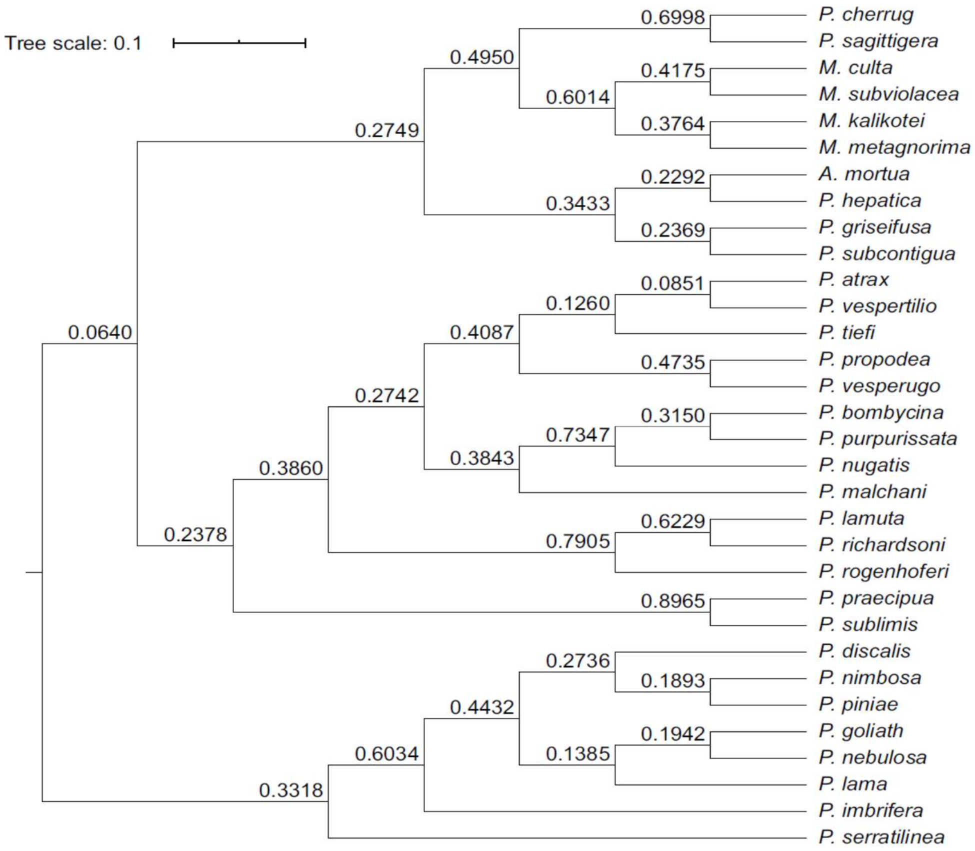


Fig. 7. Consensus tree of Poliina based on discrete characters of male and female genitalia

The diverse “Siberian” species group seems to be closely allied to the former species since they also have a bundle of strong bristles only on the left saccular process and the bristles on the left side are not differentiated into two tufts (Fig. 8, right column). The processes are, however, more differentiated in the species of this group, i.e. these are often more or less spatulate (*P. tiefi, P. vespertilio*) or obtuse (*P. malchani*) on the left side, and the terminal part of the right process shows a moderate trend of bifurcation (*P. vesperugo, P. tiefi*). This character is also present in the North American *P. propodea* which appears as a strongly supported sister species of *P. vesperugo* (Figs. 6-7). The bifurcation of the terminal part of the right process is manifested in extreme form in the externally dissimilar *P. serratilinea*, characterised also by a subdivision of bristles on the left process.

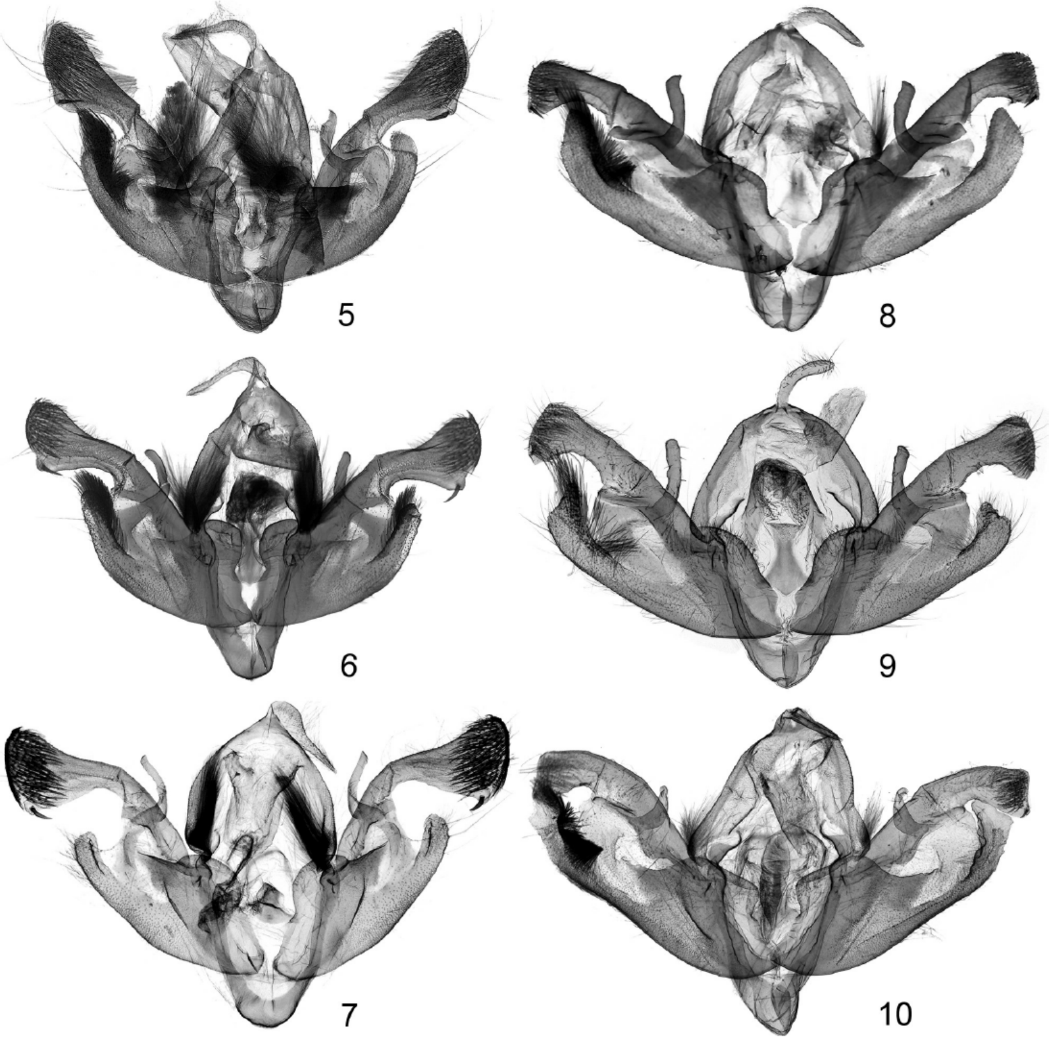
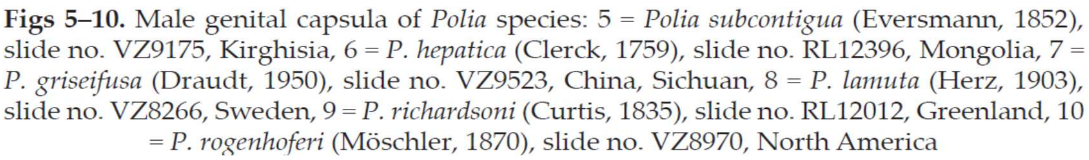


Fig. 8. Symmetrical (left columne) vs. secondarily asymmetrical (right columne) genital capsula of *Polia* species (from Varga et al. 2020)


Figs 5–10. Male genital capsula of *Polia* species: 5 = *Polia subcontigua* (Eversmann, 1852), slide no. VZ9175, Kirghisia, 6 = *P. hepatica* (Clerck, 1759), slide no. RL12396, Mongolia, 7 = *P. griseifusa* (Draudt, 1950), slide no. VZ9523, China, Sichuan, 8 = *P. lamuta* (Herz, 1903), slide no. VZ8266, Sweden, 9 = *P. richardsoni* (Curtis, 1835), slide no. RL12012, Greenland, 10 = *P. rogenhoferi* (Möschler, 1870), slide no. VZ8970, North America

The former boreo-montane group is closely allied with the Holarctic *P. bombycina* “triplet” of species. The monophyly of the Trans-Palaearctic, polytypic *P. bombycina* and its Nearctic sister species-pair, *P. purpurissata* and *P. nugatis* is also strongly supported (Figs. 6-7). These species show certain trends of subdivision of bristles into two tufts on the left saccular process and also the appearance of some stronger bristles on the right side, mostly in *P. purpurissata*, less in *P. nugatis*, and rather variably in different subspecies of *P. bombycina*.

The trifid brush organ (TBO) is generally present (Fig. 2.), both in the morphologically “basal” groups as in all *Metallopolia* species but also in certain *Polia* s. str. taxa as *P. hepatica*, *P. subcontigua*. They are also present in the species of the other species groups, with some exceptions, e.g. *P. malchani* in the Siberian group, in the Eurasiatic mountain steppic *P. serratilinea*, and also in three not closely related species of the *nebulosa*-group, i.e in the Central Asiatic *P. lama* and in the North American *P. nimbosa* and *P. imbrifera*.

The configuration of the female genitalia is rather uniform. Papillae anales are usually weakly sclerotised, rounded or obtuse, never acute. The whole antrum–ductus bursae–corpus bursae+appendix bursae apparatus is co-adapted to the elongated aedeagus and elongated tubular vesica. Ductus bursae is the shortest in the arctic-boreal species *P. richardsoni*, *P. lamuta* and *P. rogenhoferi*, and the longest, with mostly extended, funnel shaped antrum is present in the supposedly mostly derived *P. nebulosa* group. Unfortunately, in presence vs. absence of signa we could find no phylogenetic trend since the presence vs. reduction of this trait was rather eclectic in the different species groups and in closely related species, as well. Certain degree of variations was also observed even within one single species, in accordance with the previous findings of McCabe (1980).

Concerning the genital structures of both sexes, the the *P. nebulosa*-group proved to be the most homogenous group (Figs. 6-7) in which all species show a perfect separation of bristles on the left process into a subbasal and terminal group, and the appearance of a subbasal group of bristles on the right side. The vesica is characterised in this group by the presence of a subbasal and a medial diverticula. The distinctness of this group was already indicated by CO1 data (Zahiri et al. 2014; Varga et al. 2020; Fig. 9.).

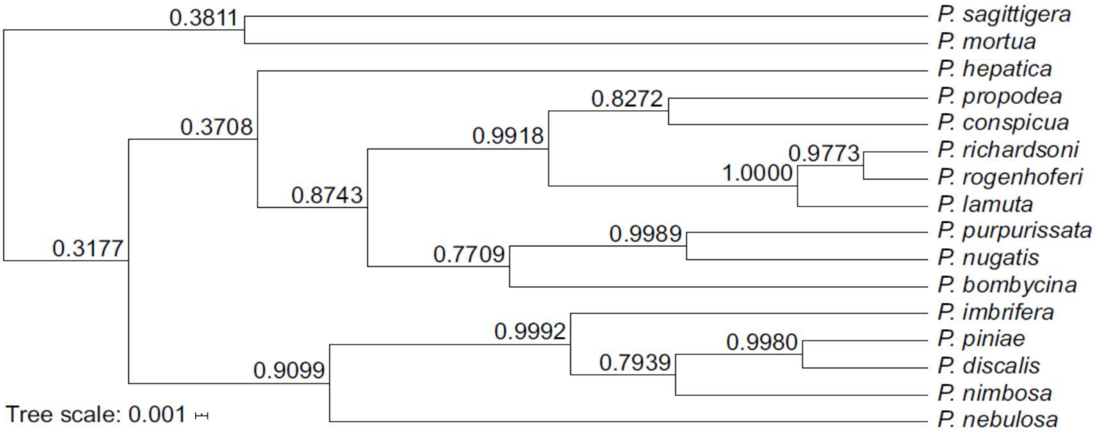


Fig. 9. Consensus tree constructed from the published North American, Central and North European CO1 data and the known gene bank sequences (GenBank, http://ncbi.nlm.nih.gov/)

According to the survey of the CO1 gene our results can be summarised as follows. Since *Pachetra sagittigera* and *Polia mortua* appear as outgroup of *Polia* s.str., the subgenus *Polia* can be considered as a monophyletic taxonomic unit (Fig. 9.). Within *Polia* the basal split was observed between the *nebulosa* species group and the rest of *Polia s.str*., in full agreement with our morphological analysis and with the earlier traditional taxonomic and molecular works (Heydemann 1944; McCabe 1980; Zahiri et al. 2014). In the *nebulosa* group the Eurasiatic *P. nebulosa* seems to be the sister group of all Nearctic species in which *P. imbrifera* is separated from the other three species with a high support.

In the other group of species, *P. hepatica* has shown the basal split which is plausible also according the plesiomorphic characters of the genital capsule and the helicoidal configuration of the long, tubular vesica (see also Figs. 6-7. and 9.). The other species are subdivided into two main groups: the mostly boreal (Siberian) and Arctic species as opposed to the species triplet of the temperate Holarctic *P. bombycina* group which is also well founded both on the morphological characters and geographical distribution of species. In this group *P. purpurissata* and *P. nugatis* are well differentiated sister species as opposed to the widely distributed Eurasiatic *P. bombycina*. In the Boreo-Arctic group the species triplet *P. lamuta* + (*P. richardsoni* + *P. rogenhoferi*) is clearly separated from the *P. propodea*-*P. vesperugo* species pair. However, the Siberian species which are close to the latter group (*P. tiefi, P. vespertilio, P. malchani*) remained unconsidered due to lack of the available barcode sequences.

Discussion and Answers

Based on the genital morphological characters, our first statement was that *Polia* s.str., other subgenera as *Metallopolia*, *Atropolia* and *Protopolia* but also *Pachetra* together are forming a higher monophyletic unit (Figs. 6-7). This result was also supported by the CO1 tree in some extent. According to CO1 data, however, *Pachetra* and *Atropolia* appear as outgroup of *Polia* s. str. which latter proved to be monophyletic. Thus, our first question whether *Polia* s.str. should be considered as a monophyletic taxonomic unit was answered.

Within this group we have found a basal split between the *P. nebulosa* species group as opposed to all other species groups of *Polia* s.str. Although the monophyly of the *P. nebulosa* group was fully supported, the subdivision of the other species groups appears to be more complicated. While the basal split of *P. hepatica* was shown with a rather low support due to its some plesiomorphic traits (symmetry of saccular processes, long tubular and helicoidal vesica), some other species groups seem to be strictly monophyletic. The most closely relationship was found in the – possibly most recently differentiated – Holarctic Arcto-alpine species "triplet" *P. richardsoni – P. rogenhoferi – P. lamuta*, in concordance with the published traditional morpho-taxonomic (Heydemann 1944, McCabe 1980) and CO1 (Zahiri et al. 2014) results. We also could confirm the monophyly of the Holarctic-temperate *P. bombycina*-group with high support, in which the polytypic Trans-Palearctic *P. bombycina* can be opposed to the Nearctic sibling pair *P. purpurissata – P. nugatis*.

The genital morphological trees have shown the same trends with the basic difference, however, that the strictly monophyletic *P. nebulosa*-group appears to be the counterpart of all other *Polia* subgenera and *Polia* s.str. species groups. Although we did not weight the characters, some seemingly autapomorphic characters of the *nebulosa*-group as *(i)* the into two groups differentiated tufts of bristles on the left saccular process, *(ii)* the reduction of the right saccular process and *(iii)* the elongate ductus bursae with funnel-shaped antrum could cause some imbalance in the trees. In addition, some former taxonomic statements were strongly confirmed as *(i)* the taxonomic position of "*Polia*" *cherrug* as *Pachetra*, as member of the "western" phylogenetic group, sensu Heydemann (1944) and *(ii)* the monophyly of the Sino-Himalayan *Metallopolia*. The morphological analysis also strongly supported the close relationship of the West Chinese relict species *P. atrax* with the southern Siberian mountain species *P. tiefi* and *P. vespertilio* but also with the Holarctic boreal sibling pair *P. vesperugo – P. propodea*. This diverse group of species is closely related both to the *P. bombycina* group, but also to the Arctic-Boreal "triplet" of species (*P. rogenhoferi, P. richardsoni, P. lamuta*) which already was predicted by Heydemann (1944). All these species groups show the same, less extreme type of asymmetry of saccular processes with a single brush of bristles on the left side.

The bilateral symmetry of the male genital capsule was considered as a plesiomorphic but also rather complex trait. It includes the symmetry vs. asymmetry of sclerotised parts, e.g. of the dilated clavus but mostly the shape of the saccular processes, on the one side, and the symmetry vs. asymmetry of bundles of bristles on the saccular processes, on the other side. While the differentiation of the brushes of fixed bristles emerges typically on the left side, the dilatation or bifurcation (and also the reduction) of the distal part of saccular processes usually appears on the right side (see Varga & Ronkay 2013). Both trends of asymmetry were observed in different groups of species, however, mostly expressed in the *P. nebulosa*-group. This group proved to be monophyletic based on the genital morphological characters of both sexes (Figs. 6-7.) but strongly supported also according to the CO1 sequence (Fig. 9.). This group was already predicted (McCabe 1980) and recently shown (Zahiri et al. 2014) as the sister group of all other North American *Polia*.

We could confirm the hypothesized trade-off between the genital capsule and the vesica. In genera with relatively simple, symmetrical genital capsula (*Haderonia, Tricheurois, Ctenoceratoda*) we regularly have observed more complicated, helicoidal structures of vesica, combined with co-evolved "lock" structures of female genitalia (sclerotised subgenital plate, sclerotised long ductus burse and tubular appendix bursae). Oppositely, the sophisticated, asymmetrical capsule is regularly combined with some simplifications of the vesica, e.g. in the arctic-boreal species group of *Polia*, or the loss of the fasciculate cornuti in *P. subcontigua* (combined with the autapomorphic helicoid shape of vesica), or the reduction of the medio-lateral diverticula in *P. serratilinea.* A widespread plesiomorphic character is the presence of the abdominal brush organ of males which can be reduced in several independent cases in not closely related species and species groups as homoplasy. Completely similar situation was already described in the closely related genus *Ctenoceratoda* (Varga et al. 2017) but also in the phylogenetically distant *Apamea zeta*-group (Noctuinae, Apameini; see: Zilli et al. 2009).

Phylogenetic biogeographical considerations

The genera of Poliina are either Holarctic (*Polia* Ochsenheimer, 1816) or only Eurasiatic with centre of diversity in the monsoonic mountainous areas of South-Eastern Asia (Himalayan – Sino-Tibetan faunal type). The genus *Polia* (and the subgenus *Polia*) obviously had an Eurasiatic origin since all closely related genera sharing the plesiomorphic, nearly symmetrical genital capsule (Varga et al. 2017) are South-East Palearctic (*Haderonia* Staudinger, 1896, *Tricheurois* Hampson, 1905) or Central Asiatic (*Ctenoceratoda* Varga, 1992). The great majority of *Polia* taxa are also East Palearctic, including the members of the recently separated and described subgenera *Atropolia, Metallopolia* and *Protopolia* (Varga et al. 2017). Twenty-six species of *Polia* are present in Eurasia and thirteen species in North America; from them only one is a Holarctic, circumpolar species (*P. richardsoni*). Further genera of the subtribe are exclusively Palaearctic, the most diverse genus is *Ctenoceratoda* with more than thirty, mostly Central Asiatic species.

The next step is the analysis of the biogeographical connections of the outlined phylogenetic subdivision. One of the main questions was, which are the exclusively Palaearctic species and species groups, and which are those species groups in which Palaearctic-Nearctic connections can be observed. We concluded that the highest species diversity of *Polia* is typical for the boreo-temperate zone of eastern Eurasia, and most species are typical for the montane taiga belt of southern Siberia (mostly Altai and Sayan mountain systems). Some of these species, however, as *P. subcontigua, P. malchani, P. vespertilio,* occur also in the Southern Urals.

There are three species connected with arctic and/or alpine habitats. Only one polytypic species, *P. richardsoni* has a more or less disrupted Holarctic range. Its sister species *P. lamuta* also shows a long-distance disjunction, it has an arctic-subarctic range in Northern Europe (*P. lamuta rangnowi*) and mountain tundral ("goltsy") occurrences from Buryatia to Yakutia (*P. lamuta lamuta*). The Nearctic *P. rogenhoferi* occurs from the Rocky Mountains to Labrador with a seemingly huge hiatus in the boreal coniferous forest zone (see: McCabe 1980). The disjunctions of these ranges are obviously results of a late-glacial, postglacial regression and fragmentation due to the expansion of the Holarctic taiga belt.

A Trans-Beringian disjunction was observed also in the possibly sister species pair *Polia propodea* and *P. vesperugo*. While the former species is relatively rare and recognised relatively late (described by McCabe, 1980), its Palaearctic counterpart has a huge but probably scattered range from Northern Fennoscandia to the Russian Far East and is subdivided to at least three larger population groups described as subspecies (see: Appendix 1). However, the bulk of the mountain taiga faunal complex (*P. tiefi, P. vespertilio, P. malchani*) and also the mountain steppic *P. serratilinea* did not reach Beringia. It means that they could not be allied to the so-called mammoth steppe faunal assemblage. Comparing the distribution of the boreal forest and steppic, vs. the arctic-subarctic tundral species of some highly diverse genera, as *Xestia* s.l. (including *Pachnobia, Anomogyna, Schoeyenia, Estimata, Raddea*) or *Anarta* s.l. (including *Coranarta, Hadula, Calocestra, Cardiestra, Aglossestra*), we can conclude that the faunal complexes associated with taiga, boreo-montane forests and steppe (prairie) habitats show only scarce data of occurrence in Beringia, which refer – concerning this biogeographical group – only on a limited exchange during the Upper Pleistocene between the Nearctic and Palaearctic. In tundral habitats, in contrast, there are abundant data on Trans-Beringian faunal exchanges (Lafontaine & Wood 1988; Mikkola et al. 1991).

Two other species groups of *Polia* have Holarctic distribution and show more ancient splits. The three species of the *P. bombycina* group are clearly differentiated with high support both from the former species groups and from each other, as well, and also have sizeable intraspecific variation within their fairly widely extended ranges. All these fact suggest that the ancestor of the Nearctic sister species had crossed Beringia in a relatively early temperate phase of the Upper Tertiary and consequently should have had enough time for divergence and geographical differentiation.

The CO1 dendrogram shows that the oldest Trans-Beringian wave was most probably connected with the ancestor of the Nearctic species of the *P. nebulosa* group, however. We have multiple evidence for this postulate as follows:

First: there is a considerable differentiation among and within the Eurasiatic species. *Polia goliath,* described originally as "*Dichonia*", and its sister species *P. gigantea* sp.n. strikingly differ externally from all other known *Polia* species. The major geographical population groups (*P. goliath*: Russian Far East + Japan and Korea, *P. gigantea*: mountains of West China: Sichuan, Shaanxi, and Taiwan) are clearly differentiated, especially the nominotypic *P. gigantea gigantea* occurring in Taiwan. Although *P. nebulosa* and *P. lama* are externally rather similar, both species have autapomorphies, like the dentate crest on the left saccular process in *P. nebulosa* or the reduction of the abdominal brush organ in *P. lama*. Furthermore, both species show mentionable intraspecific geographical differentiation (Varga et al. 2019).

Second: there is a highly supported deep split between *P. nebulosa* and all North American species despite the external similarity with some Nearctic species as *P. discalis* and *P. piniae.* These species represent an obviously monophyletic lineage shown already by Zahiri et al. (2014).

Third: there is a considerable differentiation among the four Nearctic species, with highest support between *P. imbrifera* and the other three species. The most recent speciation step was shown between the allopatric sister species *P. discalis* and *P. piniae.* However, all these specific splits have very probably pre-glacial origin due to the strong differentiation of the CO1gene.

All these evidence support our hypothesis that Trans-Beringian migrations should have occurred at least three times in the zoogeographical history of *Polia*.

Conclusions

At first we had to answer some questions on the **phylogenetic evaluation of morphological traits**.

- It is a common sense that the original form of the paired male external genital appendages (modified gonopodia) is a bilaterally symmetrical one. This has the reference to Poliina noctuid moths, as well. We have found that the supposedly ancestral bilaterally symmetrical form of genital capsule is preserved *(i)* in oligotypic genera/subgenera and *(ii)* in taxonomically isolated species of highly diverse genera. Both are geographically restricted to the Sino-Himalayan area which is known as one of the main core areas of biodiversity not only in Eurasia but also globally (e.g. Myers et al. 2000; Boufford 2014).
- Therefore, we consider the secondary asymmetry of genital capsule represents an evolutionary trend, expressed in different traits, as the dyssymmetrisation of the shape of the clavus and of the saccular processes, and as the asymmetrical modifications (subdivision vs. reduction) of the specialised brushes on the saccular processes. Since these parts do actively function – mechanically or sensory – in the copulation, thus we have accepted that the trend of asymmetrisation was subjected to sexual selection.
- We also observed that some, mostly reductive changes can independently occur in species or species groups of different genera. Since the paired trifine brush organ (*TBO*, Zilli & Di Giulio 1996) is known as plesiomorphy in noctuid moths, we surveyed its occurrence and phylogenetic significance in Poliina genera. We have found that this trait is nearly without exception (*one pair of species) present in genera/subgenera which are restricted to the Sino-Himalayan core area (*Tricheurois, Haderonia**, *Polia* subg. *Metallopolia, Atropolia*) but occur also in the most diverse genera *Ctenoceratoda* and *Polia*. In *Ctenoceratoda* we characterised the species groups (details: Taxonomic Atlas Noctuinae III.) by presence vs reduction of this trait; while in *Polia* the picture is more fuzzy, from presence-absence in Palaearctic and Nearctic species pairs of the *P. nebulosa* group (details: Taxonomic Atlas Noctuinae III.) to the trend of reduction in the western subspecies of the Trans-Palaearctic species *P. bombycina*. Some other morphological reductions do not show any clear phylogenetic pattern, as the reduction of diverticula of vesica in males (it occurs independently in different species groups of *Polia*), or the reduction or the change in shape and number of signa in female genitalia (nearly in all genera).

We also answered some questions on the phylogeny and geographical history of Poliina and *Polia* s.str. using the combination of traditional morphological and molecular phylogenetic methods.

- The analysis of genital morphological traits and the CO1 sequence data have shown that there are *(i)* well defined species groups and *(ii)* sister species relations in the most widely distributed and diverse genus *Polia* and subg. *Polia.* The CO1 sequences have confirmed that subg. *Polia* is monophyletic, although it was already shown by Zahiri et al. (2014) for the Nearctic species only.
- Moreover, a basic split was shown between the *P. nebulosa*-group and all other species groups of *Polia* s.str. The latter diverse complex of species consist of three isolated species without any close relationship; while the others were subdivided *(i)* into a morphologically isolated (extreme asymmetry in male genitalia) polytypic steppic species (*P. serratilinea*), *(ii)* into a Holarctic species triplet (*P. bombycina*-group) in which the Nearctic sibling species pair is forming the sister group of the trans-Palaearctic polytypic species, and (iii) a Boreo-Arctic species complex, subdivided (*α*) into a trans-Palaearctic boreo-temperate, mostly East Palaearctic ("Siberian") group of species, (*β*) into a boreo-montane Palaearctic (*P. vesperugo*) vs Nearctic (*P. propodea*) species pair, and (*γ*) into a circum-Arctic Holarctic triplet of species.
- Our last conclusion concern the geographical history of *Polia*. Multiple evidence, but mostly the CO1 tree, supports that the Trans-Beringian migrations should have occurred at least three times in the zoogeographical history of *Polia*, at first in the *P. nebulosa* group (according to CO1 the common ancestor of *P. nebulosa* and the North American species), later the common ancestor of *P. bombycina* and the North American species pair, and most recently, during the allopatric speciation process of *P. vesperugo*/*P. propodea.*

According to our results **we could confirm all these regularities which were originally hypothesised**.

- ❖ The biogeographically most restricted, moderately diverse Sino-Himalayan genera (*Haderonia* and *Tricheurois*) are proved to represent the phylogenetically most ancient groups which have the original bilaterally symmetrical form of the male genital capsule but also with autapomorphic traits at generic and species group levels (in terminal parts of valvae and in variations of the tubular vesica with fasciculate cornuti).

- We could show that the core area of the generic and species (i.e. phylogenetic) diversity overlaps with the range of the phylogenetically relict-like Sino-Himalyan groups (*Haderonia, Tricheurois* and the Polia subgenera: *Leuconephropolia, Metallopolia, Atropolia* and *Protopolia*) which are forming the basal splits morphologically and according to the sequences of the barcode gene (CO1).
- In the two most diverse genera *Polia* vs *Ctenoceratoda* we could demonstrate that there are two divergent trends of change originating from the bilaterally symmetrical structures: *(α)* the enhanced complexity of external genitalia connected with secondary asymmetrisation of the functionally active parts (clasping, stimulation, sensual function), combined with less complexe structures of inner genitalia, i.e. there is a trade-off in the complexity of external vs. internal genitalia *(β)* the simplification of genital capsula is combined with sophistication of the internal genitalia in both sexes: i.e. there is an other trade-off in the complexity of external vs. internal genitalia.
- Additionally it was shown that these widely distributed genera *Polia* vs. *Ctenoceratoda* not only have this basic two types of trade-offs, but they also have antagonistic biogeographic trends. The first is mostly distributed in Holarctic arboreal zonobiomes, while the latter in Central and Inner Asiatic arid orobiomes and eremic habitats.
- The most diverse genera (*Ctenoceratoda*) and subgenera (*Polia* s.str.) were subdivided into strictly monophyletic species groups (see the congruences of the genital morphological and CO1 trees, Figs. 6-7. and 9.). Within these species groups or only pairs, the speciation proceeded basically by allopatric segregation, after the expansion crossing some barriers (see e.g. the *P. bombycina* and the *P. nebulosa* Holarctic species groups). Therefore the mostly derived forms of these diverse genera/subgenera occur at the peripheries of the genus' range of distribution.

As we explain in the **Results** and **Discussion,** we could fully confirm these main expectations.

Last not least: we produced a monographic book on the subtribe Polia in frames of the book series: **Taxonomic Atlas of Eurasiatic and North African Noctuoidea, Vol. 11., Noctuinae III. ISBN 978-615-5279-09-6**

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