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Phylogeny, character evolution and tribal classification in Crambinae and Scopariinae (Lepidoptera, Crambidae)

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Abstract. Crambinae (2047 spp.) and Scopariinae (577 spp.) are two major groups of pyraloid moths with a worldwide distribution. Their larvae feed predominantly on Poales and Bryophyta, with many cereal crop pests. We present the first molecular phylogeny of the two groups based on five nuclear genes and one mitochondrial gene (total = 4713 bp) sampled for 58 crambine species representing 56 genera and all tribes, 33 scopariine species representing 12 genera, and species in several other crambid lineages. Maximum likelihood and Bayesian analyses of the molecular data resolve suprageneric relationships in Crambinae and Scopariinae, whereas relationships between these and other subfamilies remain ambiguous. Crambinae and Scopariinae are each recovered as monophyletic groups, and Erupini, formerly regarded as an ingroup of Midilinae, is recovered as a possible sister group of Crambinae. The tree topology suggests the following two major changes within Crambinae: Prionapterygini Landry **syn.n.** of Ancylolomiini Ragonot **stat. rev.** and Myelobiini Minet **syn.n.** of Chiloini Heinemann. Argyriini Munroe is monophyletic after the transfer of *Pseudocatharylla* Bleszynski and *Vaxi* Bleszynski to Calamotrophini. Crambini, Diptychophorini and Haimbachiini are monophyletic after the exclusion of *Ancylolomia* Hübner, *Euchromius* Guenée, *Micrelephas* Dognin and *Miyakea* Marumo from Crambini, as well as *Microchilo* Okano from Diptychophorini. Euchromiini **tribe n.** is described for *Euchromius*. *Microcramboides* Bleszynski **syn.n.** and *Tortriculladia* Bleszynski **syn.n.** are synonymized with *Microcrambus* Bleszynski. In Scopariinae, *Caradjaina* Leraut **syn.n.** and *Cholius* Guenée **syn.n.** are synonymized with *Scoparia* Haworth, and, in addition, *Dasyscopa* Meyrick **syn.n.**, *Dipleurinodes* Leraut **syn.n.** and *Eudipleurina* Leraut **syn.n.** are synonymized with *Eudonia* Billberg. *Micraglossa melanoxantha* (Turner) (*Scoparia*) **comb.n.** is proposed as a new combination. We analysed 27 morphological characters of wing venation, tympanal organs, male and female genitalia, as well as host plant data and egg-laying behaviour. The ancestral character-state reconstructions confirmed previous apomorphies and highlighted new apomorphies for some of the newly recovered clades. The derived, nonadhesive egg-dropping behaviour is found to have evolved at least twice in Crambinae and is associated with the use of Pooideae as host plants.

This published work has been registered in ZooBank, http://zoobank.org/urn:lsid:zoobank.org:pub:1A84282D-930A-4C32-8340-D681BFF27A12.

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Introduction

With about 15 500 species, Pyraloidea is one of the most diverse superfamilies of Lepidoptera. The Crambinae (2047 species) and the Scopariinae (577 species) together account for about 18% of described pyraloid species (Nuss *et al.*, 2019). The Crambinae typically have elongated forewings that provide them with camouflage on grasses, while the Scopariinae generally exhibit a greyish wing pattern matching that of rocks and tree trunks where they rest during the day. The Crambinae and Scopariinae are encountered worldwide, with the Scopariinae being more diverse in tropical mountain areas and islands (Munroe & Solis, 1998). The majority of Crambinae larvae are known to feed upon grasses (Poaceae). Many species of *Chilo* Zincken, *Coniesta* Hampson, *Diatraea* Guilding and *Eoreuma* Ely are recognized as economically important pests of various crops, including maize, millet, rice, sorghum and sugarcane, and a few species of Crambini referred to as sod webworms are pests of lawns in North America (Hill, 1987, 2008; Khan *et al.,* 1991; Capinera, 2001). The majority of Scopariinae larvae, as well as some species of the crambine genera *Agriphila* Hübner, *Catoptria* Hübner, *Chrysoteuchia* Hübner, *Gadira* Walker, *Glaucocharis* Meyrick and *Platytes* Guenée feed on mosses (Munroe & Solis, 1998; Goater *et al.*, 2005; Slamka, 2008; Glime, 2017). Other larvae have been reported to feed on Cyperaceae, various dicotyledons (Robinson *et al.*, 2010), on dried leaves (Millière, 1868), and on lichens for two species of *Eudonia* Billberg (Nuss in Goater *et al.*, 2005). Crambinae larvae are either stem borers (Chiloini, Haimbachiini), root feeders or ground-living leaf feeders (many Crambini) (Munroe & Solis, 1998). Crambinae females are reported to glue their eggs onto their host plant in the Haimbachiini (*Coniesta*, *Eoreuma*, *Xubida* Schaus), *Argyria* Hübner, *Calamotropha* Zeller, *Chilo* and *Myelobia* Herrich-Schäffer (Peterson, 1963; Youm & Gilstrap, 1994; Legaspi *et al.*, 1997; Landry *et al.*, 2015), whereas most Crambini drop nonadhesive eggs while perched or during a slow, undulating flight (Ainslie, 1930; Peterson, 1963; Matheny & Heinrichs, 1972; Marshal, 1988; Ponomarev, 2016).

The Crambinae were historically split into two groups, the 'Ancylolomiidae' and the 'Crambidae' (designating here all Crambinae except Ancylolomiini) (Ragonot, 1888), a classification followed by Forbes (1920), who recorded the two groups as subfamilies of Pyralidae. The subsequent attempt towards a classification of the Crambinae was made by Bleszynski, who recognized a *Chilo* complex, including *Chilo*, *Diatraea* and *Myelobia*, and an *Acigona* Hübner (synonym of *Haimbachia* Dyar) complex (Bleszynski, 1966). The Crambinae were later divided into the following tribes: Chiloini (Klots, 1970), Diptychophorini and Crambini (Gaskin, 1972, 1975), Argyriini (Klots, 1983), Ancylolomiini and Myelobiini (Minet, 1982). Gaskin also used the name Calamotrophini (Gaskin, 1988) for the genus *Calamotropha* but he did not describe the tribe. The first phylogenetic analysis of the group was based on 45 morphological characters and highlighted two new tribes – the Haimbachiini and the Prionapterygini – and confirmed the monophyly of the Crambini and the Diptychophorini (Landry, 1995). Landry's work was mostly restricted to North American taxa and failed to recover the relationships among the tribes or to assign some genera to a given tribe. The Crambinae are currently classified into 176 genera (Nuss *et al.*, 2019). The Scopariinae genera were reviewed by Nuss (1999), and possible relationships within the group were presented by the same author (Nuss, 2003), but the subfamily has never been analysed phylogenetically. The current classification divides the Scopariinae into 24 genera, with *Eudonia* Billberg (263 species) and *Scoparia* Haworth (242 species) making the bulk of their diversity (Nuss *et al.*, 2019). *Hoploscopa* Meyrick was described in Scopariadae (now Scopariinae) and *Heliothela* Guenée in Hercynidae (now Odontiinae). The latter was placed by Amsel (1961) in its own subfamily, the Heliothelinae, which was later supported by Minet (1982) and Nuss (1998). The latter author considered the spine in the corpus bursae of the female genitalia to be a synapomorphy of the group, in which he also placed *Hoploscopa*. Other authors either treated the Heliothelinae as subgroup of the Scopariinae (Hannemann, 1964; Leraut, 1980; Robinson *et al.*, 1994), or synonymized it with the Scopariinae (Munroe & Solis, 1998). The Erupini constitute another tribe whose phylogenetic relationships to other Crambidae lineages are not clear. *Erupa* Walker was placed in the Schoenobiinae (Bleszynski, 1966), then moved to the Crambinae (Lewvanich, 1981). Munroe (1995) introduced the tribe Erupini to accommodate *Erupa*, *Lancia* Walker, *Neerupa* Hampson and *Schoenerupa* Hampson, and placed the tribe in the Crambinae. Most recently, Hayden (2012) transferred the Erupini to the Midilinae.

The wing venation provides characters that have been used widely (Ragonot, 1888; Forbes, 1920; Dyar & Heinrich, 1927; Shibuya, 1928; Okano, 1962; Bleszynski, 1964, 1966, 1970a; Klots, 1970; Landry, 1995) to define and segregate groups within the Crambinae: forewing vein R_{S4} is either 'free', i.e. connected to the end of the cell, or stalked with $R_{S2} + R_{S3}$; the hindwing cell is either 'open' or 'closed' by a cross-vein; the base of hindwing M_1 is either adjacent or fused to Sc + R, or it originates near the middle of the cell. The variation in male genitalia structures, such as the pseudosaccus, the gnathos tip, the coecum penis, and the papillae anales in female genitalia, were recognized as key to understanding the phylogeny of the Crambinae (Okano, 1962; Bleszynski, 1966, 1970a; Klots, 1970; Landry, 1995), but the lack of well-resolved phylogenies impeded investigations of the evolution of these structures.

Regier *et al.* (2012) found the Crambinae and the Scopariinae to be sister groups in the first molecular phylogeny of the Pyraloidea. However, their study did not include Erupini and Heliothelinae, while Crambinae and Scopariinae were only represented by three and two species respectively, raising the question of whether both groups would remain monophyletic when analysing a more diverse taxon sampling. We present here the first molecular phylogenetic analysis based on five nuclear markers and one mitochondrial marker, including a large sampling of Crambinae and Scopariinae, along with Erupini and Heliothelinae, in order to: (i) decipher the evolutionary relationships within these groups and, accordingly, to revise the classification; (ii) infer the evolution of morphological characters and identify potential apomorphies; (iii) understand

the evolution of nonadhesive egg-laying behaviour and host plant use.

Material and methods

Taxon sampling

Samples were collected by light-trapping or during the daytime with a net and preserved either in a dried condition or in alcohol (see Table S1 for all relevant information). A number of specimens were obtained from colleagues (see Acknowledgements). Specimens were identified based on wing pattern and genitalia, and identifications were cross-checked by blasting the COI barcode sequence against the 'All Barcode Records' of the 'Current Database' on the Identification Engine in BOLD (http://boldsystems.org/; Ratnasingham & Hebert, 2007). Fifty-eight species representing 56 of the 176 genera and all tribes of Crambinae, as well as 33 species representing 12 of the 24 genera of Scopariinae were selected. Additionally, *Erupa* sp. (Erupini), *Heliothela wulfeniana* (Scopoli) (Heliothelinae) and *Hoploscopa* sp. (Hoploscopini) were included. Published DNA sequences for 13 additional taxa were also included (Mutanen *et al.*, 2010; Kawahara & Breinholt, 2014), including all subfamilies of the Crambidae except for the Cybalomiinae, the Lathrotelinae and the Linostinae. Correct identifications of specimens was proofed on BOLD for all samples except for MM07046 [*Hellula undalis* (Fabricius)] for which no COI barcode sequence was available. *Patania ruralis* (Scopoli), *Syllepis* cf. *marialis* (Spilomelinae) and *Anania hortulata* (Linnaeus) (Pyraustinae) were chosen as outgroups because the clade including the Pyraustinae and the Spilomelinae was highlighted as a sister group to all other Crambidae subfamilies (Regier *et al.*, 2012).

Molecular work

Dried specimens collected less than 2 years prior to DNA extraction were considered for PCR. DNA was extracted from abdomens following a nondestructive method (Knölke *et al.*, 2004) with the NucleoSpin Tissue kit (Macherey-Nagel, Germany) according to the manufacturer's protocol. Abdomens from alcohol-preserved specimens were dried 24 h before extraction to remove ethanol. Standard primers from Wahlberg & Wheat (2008) in combination with universal T3 and T7 tails were used to amplify six genes – carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase (CAD), cytochrome oxydase subunit I (COI), glyceraldehyde-3-phosphate dehydrogenase (GAPDH), isocitrate dehydrogenase (IDH), cytosolic malate dehydrogenase (MDH) and ribosomal protein S5 (rps5) – giving a 4710 bp dataset in total (see Table S1 for respective gene length). Nested primers were designed from gene alignments by eye and with GENEFISHER2 (Giegerich *et al.*, 1996; bibiserv.cebitec.uni-bielefeld.de/genefisher2/) and were subsequently checked for melting temperature, 3′ complementarity, self-annealing and potential hairpin formation on the online platform of OLIGOCALC (www.basic.northwestern.edu/biotools/OligoCalc.html) (Table S2). BIO-X-ACT Short DNA Polymerase (Bioline, U.K.) was used following the PCR protocol of Wahlberg & Wheat (2008) with minor optimizations (Table S3). Hi-Spec Additive (Bioline) was added to samples with lower yields. PCR programmes from Wahlberg & Wheat (2008) with the annealing temperature optimized for each primer set or the TouchDown PCR programme (Regier, 2007) were used (Table S4). Amplification success was checked by electrophoresis on 1% or 2% agarose gels subsequently stained with ethidium bromide and visualized under UV light. For PCR products with weak or multiple bands, bands of interest were excised from the gel and DNA was extracted using the PCR clean-up gel extraction kit from Macherey-Nagel. Alternatively, the 'gel-picking' method was used (Decker, 2003): the band of interest was briefly touched with a sterilized toothpick, which was used to inoculate a new PCR and reamplified. PCR products were cleaned by adding 0.3 μL ExoSAP-IT (Affymetrix, Cleveland, Ohio, U.S.A.) and 1 μL H_2O to 10 μL of PCR product, and we then followed the manufacturer's protocol. Cleaned PCR products were sequenced forward by Macrogen (South Korea) or alternatively at the SMTD on a 3730 DNA Analyzer (Applied Biosystems, U.S.A.) using the T7 sequencing primer or PCR primers. Reverse sequencing with the T3 sequencing primer or PCR primers was performed for poor-quality samples.

DNA sequences processing

Sequences were aligned by eye and trimmed to first codon position on PHYDE 0.9971 (Müller *et al.*, 2005). Single-gene datasets were concatenated with help of a bash script. Gene sequences of conspecific samples sharing identical COI barcode were concatenated for the following species: *Chilo quirimbellus* Bleszynski (LEP1576 and LEP1577), *Eudonia mercurella* (Linnaeus) (LEP109 and LEP2677), *Heliothela wulfeniana* (LEP550 and LEP837), *Pseudocatharylla argenticilia* (Hampson) (LEP1905 and LEP1906) and *Urola nivalis* (Drury) (LEP2671 and LEP2672). Illumina transcriptome reads of *Myelobia smerintha* (Hübner) from Kawahara & Breinholt (2014) were retrieved from the Dryad depository and searched for the genes of interest with reciprocal BLAST using gene sequences of *C. quirimbellus* and *Diatraea saccharalis* (Fabricius), with subsequent identification of orthologues done under RAXML. The six gene sequences were concatenated into a 4710 bp dataset with 17.8% of missing data. The alignment of the DNA sequences was straightforward with no insertion or deletion detected, with the exception of the CAD gene of *Thaumatopsis pexella* (Zeller) (Crambinae) being one codon longer. Sequencing success and GenBank reference numbers are reported in Table S1. Codon saturation for each third codon position was visualized by plotting the substitution rate between each pair of taxa on the branch length separating them (Klopfstein *et al.*, 2013). The best-scoring RAXML tree calculated from the standard concatenated dataset was used in order to compare

substitution rates for each gene and codon position with a reference branch length. Substitution rate plots revealed saturation of the COI third position at deep relationships of the phylogenetic tree (Fig. S1). Two datasets were thus analysed, the whole dataset (conc6genes) and one with the COI third codon position deleted (conc6genes_COI1 + 2). Unless otherwise mentioned, the conc6genes is implicitly considered.

Partitioning

The best partition scheme was searched for on the dataset prepartitioned per gene and codon position (total = 18 subsets) using PARTITIONFINDER (Lanfear *et al.*, 2017). Greedy algorithms (Lanfear *et al.*, 2012) for the models GTR, GTR+G and GTR+I+G with the corrected Akaike information criterion model selection approach were used. The best partition scheme that was found contained 16 character sets, with the first codon position of CAD and IDH merged into one character set and the second codon position of GAPDH and rps5 merged into another character set.

Phylogenetic analyses

Maximum likelihood (ML) analyses were performed using RAXML (Stamatakis, 2006) with the GTR + G evolution model. Branch support was assessed by performing 1000 bootstrap replicates using the GTRCAT approximation. The best-partitioning scheme found with PARTITIONFINDER was used. Bayesian inference (BI) analyses were performed with MRBAYES (Huelsenbeck & Ronquist, 2001), with the dataset partitioned following the best PARTITIONFINDER model and nst = 6, rates = gamma, revmatpr = dirichlet (1,2,1,1,2,1) (prior assumption of twice as many transitions as transversions), ratepr = variable, brlenspr = unconstrained: exponential (1.0) applied. While the parameters revmat, tratio, statefreq, shape, pinvar were set unlinked, other parameters were left unchanged. Two independent runs were done on 100 mio generations, each with three heated and one cold chain. Good mixing of the Markov chains Monte Carlo (MCMC) was estimated by visualizing the effective sample size (ESS) for all parameters on TRACER (Rambaut *et al.*, 2018), with ESS > 200 indicating sufficient sampling. Posterior trees produced before reaching the log-likelihood plateau were discarded from the sampling. The consensus tree was generated with TREEANNOTATOR from the BEAST package (Bouckaert *et al.*, 2014). The ML and BI analyses were run on the CIPRES portal (Miller *et al.*, 2010).

Identification of rogue taxa

Taxa flagged as 'rogues' appear at different and often contradictory positions in phylogenetic trees, thereby affecting the stability of a topology (Wilkinson, 1994; Aberer *et al.*, 2013). ROGUENAROK (Aberer *et al.*, 2013) was used to identify rogue taxa from the complete taxon sample with the following options: majority-rule search, support optimized, maximum dropset size = 2, algorithm = ROGUENAROK. The ROGUENAROK analysis returned *Hellula undalis* (Glaphyriinae) and *Microchilo* cf. *elgrecoi* (Crambinae) as the two most unstable taxa among the bootstrapped trees (respective scores, 1.15556 and 1.18611) and were removed from the final taxon sampling. Six further *Eudonia* species, three *Scoparia*, one *Caradjaina* Leraut and two species of *Microcrambus* Bleszynski, as well as *Microcramboides meretricellus* Bleszynski and *Tortriculladia* cf. *pentaspila* recovered within *Microcrambus*, were removed from the final taxon sampling to limit the sampling to one or few representatives per genus.

Character evolution

Dissection and slide-mounting methods follow Landry (1995). Ratios of structures were measured with an eyepiece micrometer. Male and female specimens used for morphological investigations were associated based on wing pattern and COI barcode sequence. Twenty-seven characters analysed by Landry (1995) and Nuss (2003) were investigated. These comprise the wing venation, tympanal organs, and male and female genitalia. Additional information on characters was retrieved from the following papers: Bleszynski (1963, 1965, 1966), Clarke (1965), Gaskin (1975), Landry (1995) and Song *et al.* (2009). Wing venation nomenclature follows Wootton (1979). The characters were coded in a morphomatrix using MESQUITE (Maddison & Maddison, 2017) (Table S5) and illustrated using Adobe ILLUSTRATOR CS6 (Fig. 1).

Ancestral character states were estimated with the *ace* function of the R package APE (Paradis *et al.*, 2004) using ML (Pagel, 1994) for discrete characters, and with the *fastAnc* function of the PHYTOOLS package in R (Revell, 2012) using ML and tree rerouting (Felsenstein, 1985) for continuous characters (scripts available as Files S1 and S2, respectively). These packages were used in previous publications for reconstructing character evolution in plants (Soltis *et al.*, 2013; Landis *et al.*, 2018; Spriggs *et al.*, 2018) and animals (Tingle *et al.*, 2017; Irisarri *et al.*, 2018). Minor modifications of the source code of the 'ace' function as suggested by Emmanuel Paradis (personal communication) were done to take uncertain character states (coded '?') into account (File S3). The best RAXML tree of the molecular data was used as input file. The 'ER' (equal rates) model was chosen for the 'ace' analyses. Ancestral continuous character states were mapped using the 'contMap' function of the PHYTOOLS package. The phylogenetic signal for continuous traits was estimated with the PHYTOOLS function 'phylosig' (Revell, 2012) using Pagel's λ (Pagel, 1999) and Blomberg's K (Blomberg *et al.*, 2003) methods.

Records of egg adhesiveness and larval life habits were retrieved from the literature and the Lepiforum website (www.lepiforum.de), and host plant records were retrieved from the literature and the HOST database (Robinson *et al.*, 2010) (Table S7). Information associated with congeneric species was used for species for which character traits were missing in order

to incorporate the maximum amount of information for these traits. Due to the scarcity of the information record, these traits were not formally analysed like morphological characters.

Results

Phylogenetic relationships

The ML and BI analyses of conc6genes and conc6genes_COI1+2 delivered similar topologies except for the placement of *Donacoscaptes* Zeller, *Eudonia truncicolella* (Stainton) and *Heliothela*, but none of these conflicting placements were supported by either of the analyses (BS < 70). The 'CAMMSS clade', including the Acentropinae, Crambinae, Midilinae, Musotiminae, Schoenobiinae and Scopariinae, was recovered as monophyletic, with Erupini, Heliothelini and Hoploscopini also included [bootstrap support (BS) = 92, posterior probability (PP) = 1; Fig. 2]. Heliothelinae was not supported as monophyletic. *Heliothela* was sister to the Crambinae and Scopariinae in the ML analysis of conc6genes, whereas it is recovered as the most basal lineage of the CAMMSS clade in the BI of conc6genes as well as in the ML and BI analyses of conc6genes_COI1+2. However, none of the analyses provided good support for these placements. *Hoploscopa* was sister to the Musotiminae in all analyses, but only the BI analyses provided good support for this topology (PP = 0.98 in analyses of both datasets). The sister-group relationship between Scopariinae and Crambinae + *Erupa* was not supported in the ML analyses, but was weakly and strongly supported in the BI of conc6genes (PP = 0.96) and conc6genes_COI1+2 (PP = 1.00; Fig. S3).

The Scopariinae (Heliothelinae excluded, following Nuss, 1998) were found to be monophyletic (BS = 100, PP = 1.00). *Anarpia* Chapman was found sister to all remaining Scopariinae, which form four major well-supported clades: (i) *Helenoscoparia* Nuss, *Gesneria* Hübner, *Cosipara* Munroe and an unidentified Scopariinae; (ii) *Micraglossa* Warren; (iii) *Scoparia*, including *Caradjaina* Leraut **syn.n.**, *Cholius* Guenée **syn.n.**; and (iv) *Eudonia*, including *Dasyscopa* Meyrick **syn.n.** and *Dipleurinodes* Leraut **syn.n.** Relationships among *Antiscopa* Munroe, *Micraglossa* and *Eudonia* + *Scoparia* are ambiguous. Weak evidence was found for *Micraglossa* as sister to *Eudonia* + *Scoparia* in the analyses of the conc6genes dataset (BS < 70, PP = 0.93), but analyses of conc6genes_COI1+2 provided better support for this topology (BS = 75, PP = 0.98). The two species-rich genera *Eudonia* and *Scoparia* are recovered as sister groups.

The Erupini, represented here only by *Erupa*, are sister to the Crambinae, a position not supported in the ML analysis but well supported in the Bayesian analysis (PP = 0.99). The Crambinae were recovered as monophyletic with strong support in all analyses (Fig. 2). The Diptychophorini were recovered as monophyletic, with *Diptychophora* Zeller, *Glaucocharis* and *Microcausta* Hampson sister to *Gargela* Walker, a genus

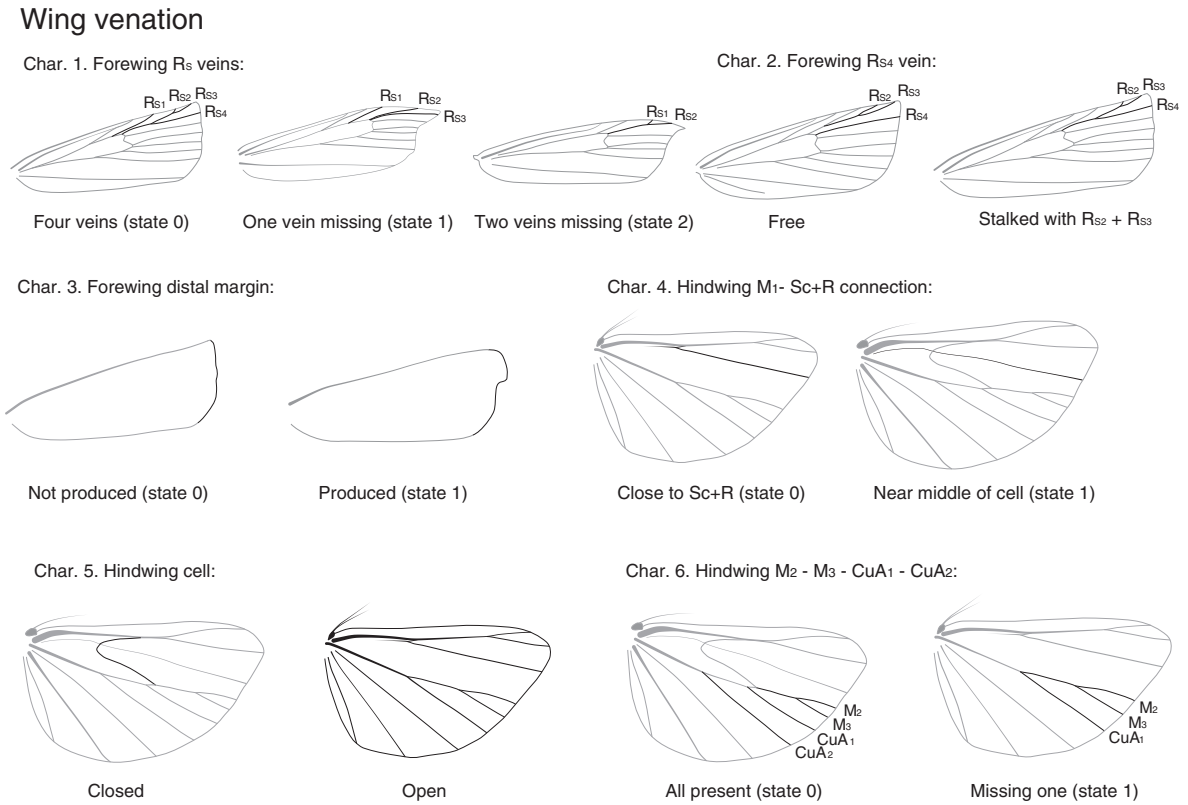

Fig. 1. List of the 27 morphological characters studied. State polarization reflects what is thought to be the derived state.

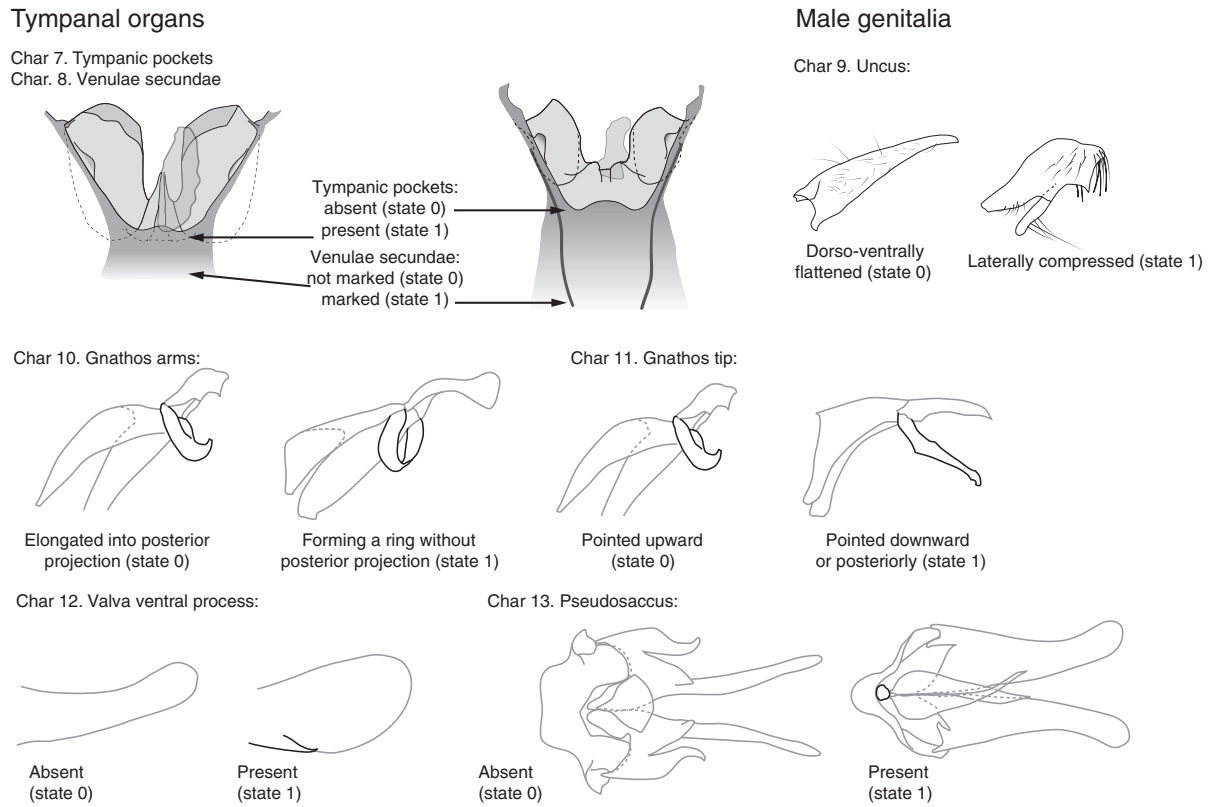

Fig. 1. Continued.

formerly unplaced to tribe (Song *et al.*, 2009). The Diptychophorini are sister to all remaining Crambinae, which form a well-supported monophyletic group, here referred to as the 'narrow winged clade'. *Microchilo* Okano, so far placed in Diptychophorini, has been recovered in our analyses within the 'narrow winged clade' (Fig. S2). There is a basal dichotomy between the Ancylolomiini + Chiloini and all remaining narrow-winged crambines, but the sister-group relationship between the Ancylolomiini and the Chiloini was only supported by the BI analysis (PP = 0.99). In the Ancylolomiini **stat. rev.**, *Ancylolomia* Hübner is nested within the Prionapterygini **syn.n.** (*Mesolia* Ragonot, *Prionapteryx* Stephens, *Pseudoschoenobius* Fernald), and *Prionotalis* Hampson is consistently found as sister group to the rest of the Ancylolomiini in all topologies, with moderate (PP = 0.97) and strong support (PP = 0.99) in the BI of conc6genes and conc6genes_COI1+2 respectively. The Chiloini were well supported in all analyses (BS = 99, PP = 1.00). The New Zealand *Gadira* and the Australian *Hednota* Meyrick form a monophyletic clade sister to *Chilo* + *Diatraea* + *Myelobia*. The East-Palearctic *Miyakea* Marumo, formerly placed in the Crambini (Nuss *et al.*, 2019), and the Hawaian *Orthomecyna* Butler form a well-supported monophyletic group (BS = 96, PP = 1.00) sister to the 'open cell clade'. The latter clade, including the Argyriini, Calamotrophini **stat. rev.**, Crambini, Euchromiini **tribe n.**, Haimbachiini, as well as *Catharylla* Zeller and *Micrelephas* Dognin was consistently recovered in all analyses (BS = 98, PP = 1.00). *Argyria*, *Catharylla*, *Urola* Wakler (part of the Argyriini) and *Micrelephas* – formerly placed in the Crambini (Landry, 2003) – form with the Haimbachiini a monophyletic clade (BS = 81, PP = 1.00), sister to the 'R_{S4} stalked clade'. The Argyriini sensu Munroe (1995), defined on the basis of the snow-white colour of the wings, are polyphyletic with *Argyria* and *Urola*, as well as *Catharylla*, belonging to one clade along with *Micrelephas* and the Haimbachiini, whereas *Pseudocatharylla* Bleszynski and *Vaxi* Bleszynski belong to the Calamotrophini. The Haimbachiini were recovered as monophyletic with strong support in all analyses (BS = 100, PP = 1.00). The 'R_{S4} stalked clade' includes the Calamotrophini, Crambini and Euchromiini (BS = 98, PP = 1.00), with the clade including *Euchromius* and the Calamotrophini found sister to the Crambini. *Euchromius*, formerly placed in the Crambini (Landry, 1995), is sister to the Calamotrophini, which includes *Calamotropha*, *Pseudocatharylla* and *Vaxi* (BS = 98, PP = 1.00). The Crambini (*Ancylolomia*, *Euchromius, Micrelephas* and *Miyakea* excluded) are monophyletic (BS = 100, PP = 1.00), with *Platytes* recovered as the most early diverging lineage. *Agriphila* and *Catoptria* form a sister clade to the remaining Crambini. Two major groups are observed in the remaining Crambini, the *Crambus* group (BS = 100,

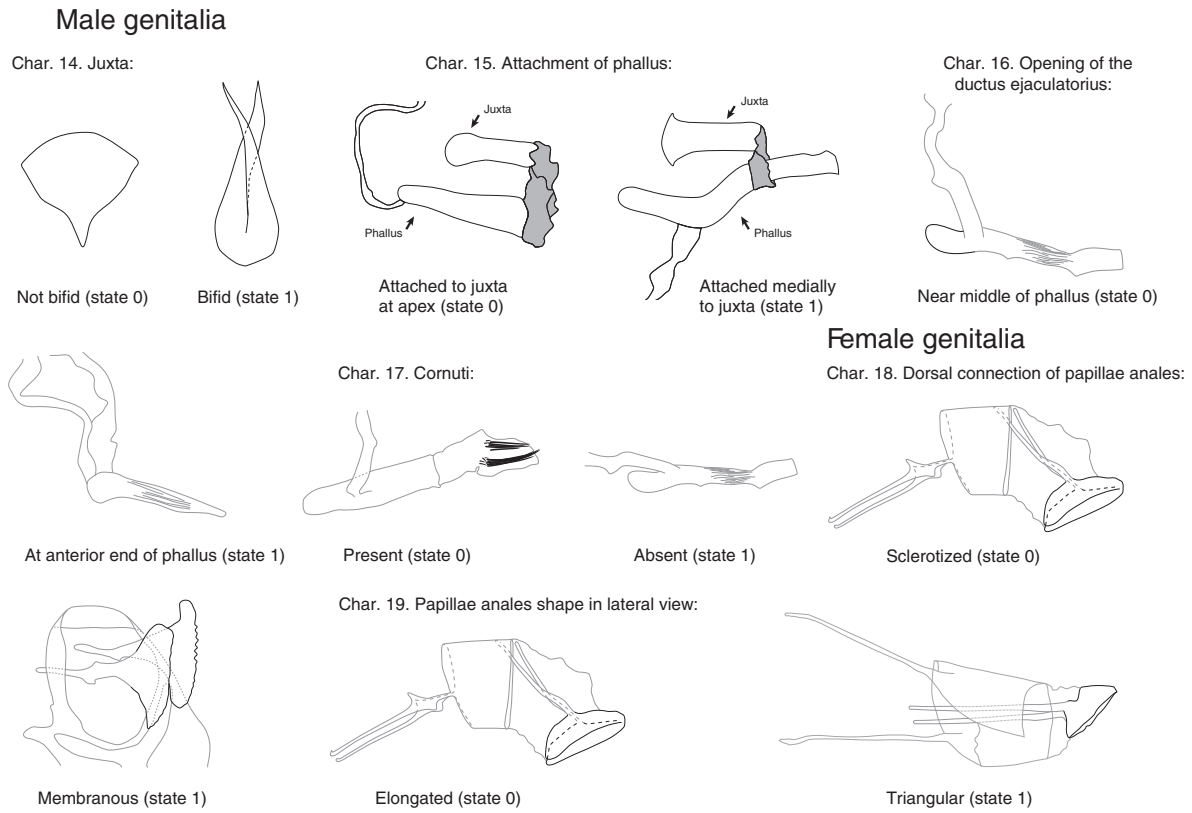

Fig. 1. Continued.

PP = 1.00) and the *Pediasia* group (BS = 75, PP = 1.00). Relationships within the *Crambus* group are well resolved, while basal relationships among the genera of the *Pediasia* group remain ambiguous. In the *Crambus* group, *Microcramboides* **syn.n.** and *Tortriculladia* **syn.n.** fall within *Microcrambus* (Fig. S2).

Evolution of morphological traits

Wing venation. Wing venation provides several characters varying in Crambinae, whereas no variation is observed in Scopariinae. Reduction in the number of veins in the forewing, with loss of the R_{S4} vein, and in the hindwing, with loss of one of the M_2–M_3 vein, is observed in 12 genera, five of them showing reductions in both the forewing and hindwing. Six of these genera belong to Crambini alone: *Culladia* Moore (one forewing R vein missing), *Fernandocrambus* Aurivillius, *Neoculladia* Bleszynski, *Novocrambus* Amsel, *Platytes* (one forewing R vein, one hindwing M vein missing), and *Raphiptera* Hampson (forewing CuA_2, two forewing R veins, and one hindwing M vein missing (Landry, 1995)). Species of *Euchromius* and *Microcrambus* are also reported missing one vein (Landry, 1995). Vein loss is found in several very small moths (< 7 mm in *Microcausta*, *Neoculladia*, *Novocrambus* and *Raphiptera*) and is associated with the narrowing of the wings or the reduction in size of the moths (Landry, 1995; Polilov, 2015).

The forewing R_{S4} vein is connected to the cell in all *Euchromius* species (R. Schouten, personal communication), *Platytes ornatellus* Leech, and *P. vobisne* Dyar, but missing in *P. alpinella* Hübner (Landry, 1995; Kirpichnikova, 1999), and stalked with $R_{S2} + R_{S3}$ in the Calamotrophini and in all Crambini sampled. Forewing vein R_{S4} stalked to $R_{S2} + R_{S3}$ (Fig. 1, character 2) is reconstructed as the most probable ancestral state in the Calamotrophini ($P = 0.9$; Table S6) and for the node including all Crambini sister to *Platytes* ($P = 0.992$), whereas no support for the R_{S4} vein stalked as the ancestral state of the R_{S4} clade was found ($P = 0.526$). The hindwing M_1 stem well separated from the Sc + R vein (character 4; Fig. 1) is reconstructed as the most probable ancestral state of the Crambinae ($P = 0.87$; Fig. 3) and constitutes a new apomorphy for the subfamily. The M_1 stem is closer to the Sc + R vein in the clade including *Chilo*, *Diatraea* and *Myelobia* and in the 'open cell clade'. In the latter, the hindwing cell is of the 'open' type, i.e. without a vein connecting the base of M_1 and M_2 (Fig. 1, character 5). The ancestral character reconstruction supports one origin of this trait in the open cell clade (Fig. 3), with the closed cell in *Calamotropha*, and the open cell in *Glaucocharis* (Diptychophorini) representing homoplasies.

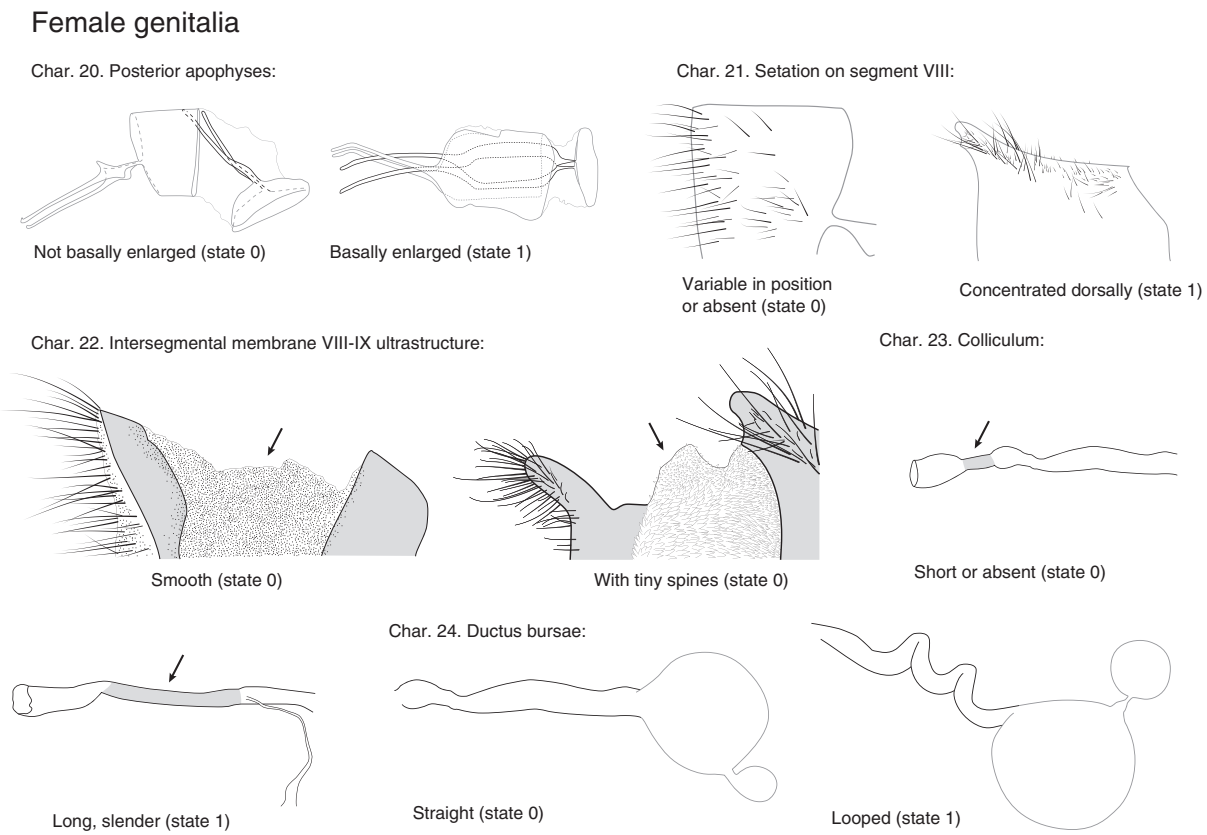

Fig. 1. Contined.

Tympanal organs. Two characters of the tympanal organs were investigated. Presence and absence of tympanic pockets (Fig. 1, character 7) are homoplastic. In the Crambidae, they are observed in the two Spilomelinae included here and in the Crambinae. Within the Crambinae, they are absent in Diptychophorini (but present in *Microcausta*), *Myelobia* + *Diatraea*, *Orthomecyna*, Haimbachiini and *Neoculladia* + *Novocrambus*. The ancestral character-state reconstruction revealed the presence of tympanic pockets as the ancestral state for the 'narrow winged clade' ($P = 0.99$). Strongly sclerotized venulae secundae (Fig. 1, character 8) are observed in *Helenoscoparia* (Scopariinae), and in Chiloini, Haimbachiini, *Calamotropha* and some Crambini (Crambinae). They are also found in *Acentria* Stephens (Acentropinae), *Erupa*, *Evergestis* Hübner (Glaphyriinae), *Patania* Moore (Spilomelinae) and *Schoenobius* Duponchel (Schoenobiinae), and are thus strongly homoplastic.

Male genitalia. The tip of the gnathos in lateral view is either directed upwards, or posterad to downwards. In Scopariinae, it is directed downwards (Fig. 1, character 11), which represents the most probable ancestral state for the group ($P = 0.85$), but it is directed upwards in *Antiscopa*, *Helenoscoparia*, and upwards or posterad in *Eudonia*. A gnathos tip directed downwards or posterad is observed in *Calamotropha*, *Euchromius*, *Micrelephas*, *Ancylolomia*, *Prionotalis*, and in all Crambini where it is reconstructed as the most probable ancestral state ($P = 0.89$). The gnathos of *Mesolia*, *Prionapteryx* and *Pseudoschoenobius* is reduced to a ring without posterior extension. The pseudosaccus, a small sclerite nested between the antero-ventral angle of valvae and vinculum (Fig. 1, character 13), is observed in the Ancylolomiini + Chiloini clade and 'R_{S4} stalked clade'. The pseudosaccus was either gained once in the ancestor of the 'narrow-winged clade' with secondary losses in the clade including the Argyriini, *Catharylla*, *Micrelephas* and the Haimbachiini, as well as in *Miyakea* + *Orthomecyna*, or it is homoplasic, with two independent origins in the ancestors of the Ancylolomiini + Chiloini clade and the 'R_{S4} stalked clade'. The phallus is attached medially via the diaphragma to the juxta in all Crambinae (*Ancylolomia* and *Microchilo* excepted) and in *Musotima* Meyrick (Musotiminae) (Fig. 1, character 15), while it is attached apically in all Scopariinae and in other Crambidae subfamilies investigated here. The medial attachment of the phallus represents an apomorphy for the Crambinae, corroborating the findings of Landry (1995). The opening of the ductus ejaculatorius (Fig. 1, character 16) is situated in the anterior part of the phallus in all Ancylolomiini as well as in *Myelobia* + *Diatraea*, suggesting that this configuration evolved twice independently in Crambinae. A bifid juxta (Fig. 1, character 14) was observed in all Chiloini and was recovered as the most

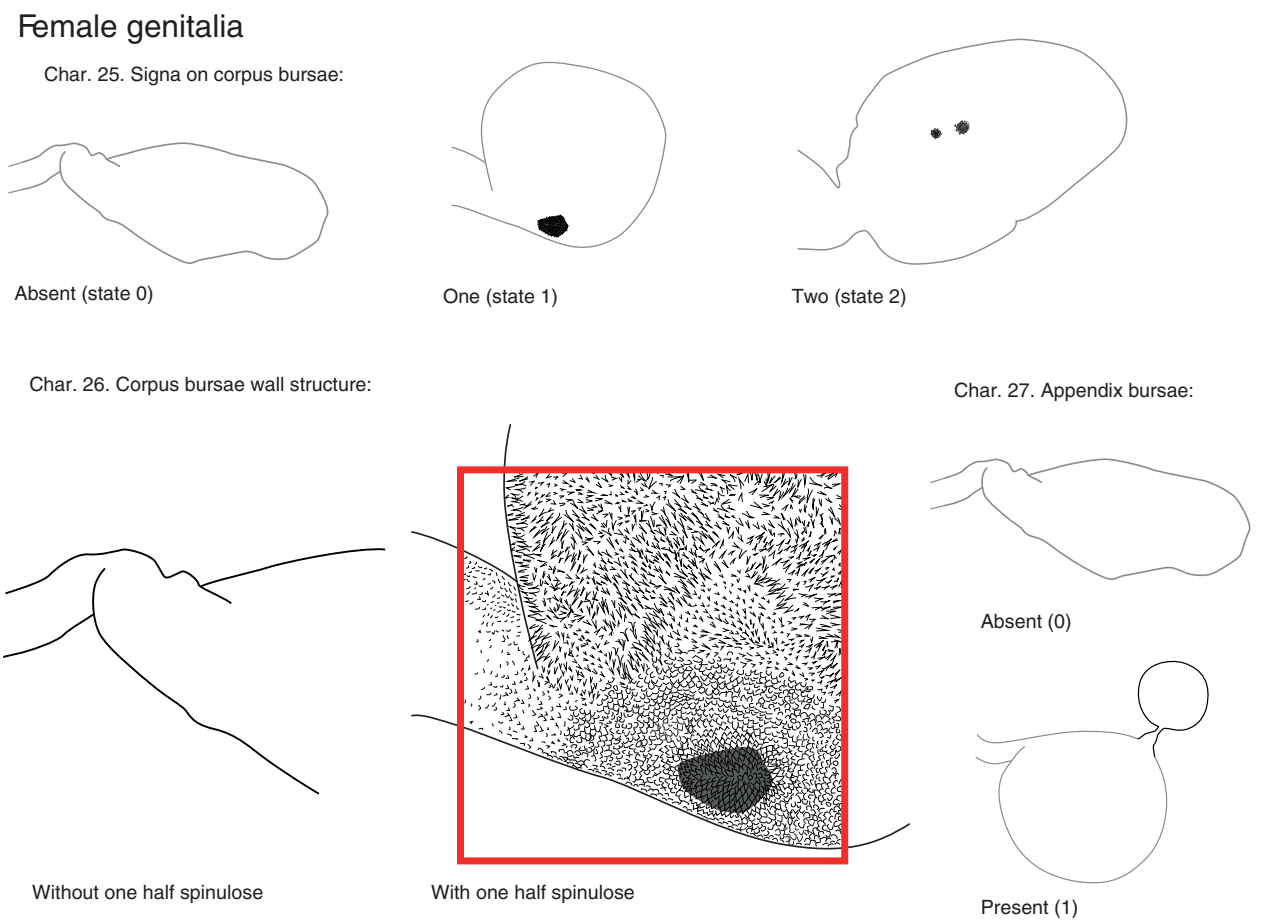

Fig. 1. Contined.

probable ancestral state of the clade, but convergent, nonhomologous structures were observed in *Diptychophora*, *Gargela*, *Micrelephas* and *Vaxi*.

Female genitalia. An appendix bursae (Fig. 1, character 27) is present in *Eudonia* + *Scoparia*, with one secondary loss in *Scoparia luteolaris* **comb.n.** An appendix bursae was also observed in outgroups *Anania* Hübner (Pyraustinae) and *Syllepis* Poey (Spilomelinae). A gain of this feature in the ancestor of *Eudonia* + *Scoparia* was supported and represents a synapomorphy for this clade.

The presence of a signum (or signa) in the corpus bursae (Fig. 1, character 25) was reconstructed as the ancestral state in the Crambinae, with the absence of signum recovered as the most probable ancestral state of the 'narrow-winged clade' ($P = 0.99$). In the Crambini, *Platytes* species bear one or two signa, while only one signum is present in all *Agriphila* and *Catoptria* species. The presence of two signa was recovered as the most probable ancestral state of the *Crambus* group (Fig. 3) with moderate support ($P = 0.83$), with secondary losses of one signum in *Conocramboides* Bleszynski and both signa in *Microcrambus*. Few species of *Chrysoteuchia* and *Orocrambus* Purdie only have one signum. No signum is observed in the *Pediasia* group, with the exception of few species of *Chrysocrambus* Bleszynski and *Parapediasia* Bleszynski with one or two signa.

The female oviscapt is made of evertible segments VIII and IX + X and intersegmental membranes VII–VIII and VIII–IX. Segments IX + X are modified into papillae anales that sometimes help to fix the eggs to the substrate (Kristensen, 2004). A membranous dorsal connection of the papillae anales (Fig. 1, character 18) was observed in six different taxa (*Ancylolomia*, *Diptychophora*, *Euchromius*, *Gadira*, *Miyakea* and all Crambini), and represents the most probable ancestral state of Crambini ($P = 0.94$). The anterior and posterior apophyses are attachments for muscles responsible for the retraction of the eighth and ninth segments after oviposition (Kuznetsov & Stekolnikov, 2001; Kristensen, 2004). While the pattern of distribution of the posterior apophyses lengths did not show any phylogenetic signal [null hypothesis stating no correlation not significantly rejected under the λ method ($P = 0.063$) or the K method ($P = 0.238$)], phylogenetic signal was found for the lengths of the anterior apophyses [null hypothesis significantly rejected with the λ method ($P = 5.18 \times 10^{-9}$) and the K method ($P = 0.049$)], indicating a tendency to anterior apophyses reduction in the 'open cell clade' (Fig. 4).

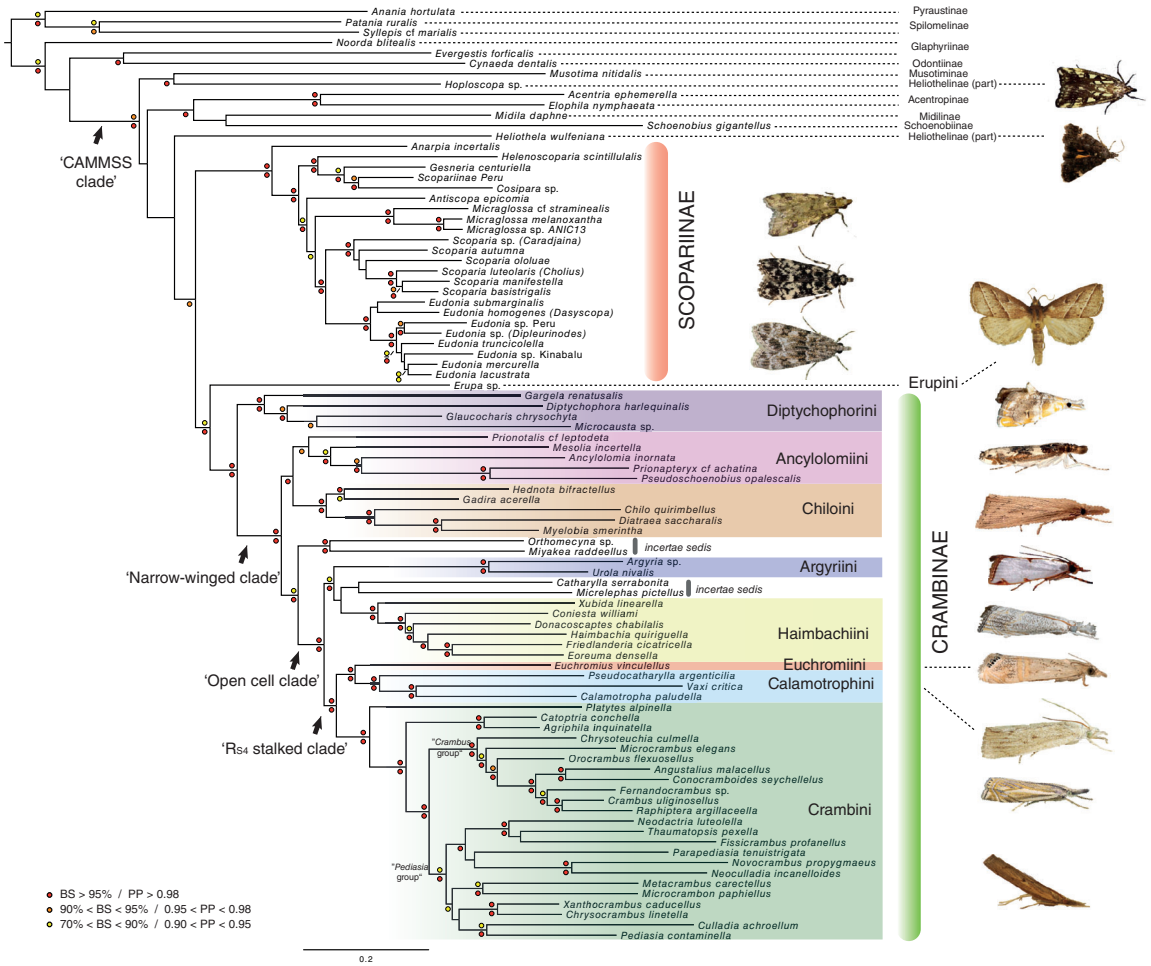

Fig. 2. Best-scoring maximum likelihood (ML) tree of the conc6genes dataset with best PARTITIONFINDER model applied. Filled circles above the nodes represent bootstrap support (BS) values from 1000 replicates, and filled circles below the nodes represent the posterior probability (PP) of the Bayesian analysis of the same dataset. Highlighted groups reflect the new classification adopted here. Species pictures (with photographers in brackets) from top to bottom are as follows: *Hoploscopa* sp. (T. Léger), *Heliothela ophideresana* (D. Hobern), *Micraglossa aureata* (jpmoth.org), *Scoparia exhibitalis* (D. Hobern), *Eudonia mercurella* (R. Coleman), *Erupa* sp. (T. Léger), *Glaucocharis* sp. (L. Shih), *Prionapteryx nebulifera* (L. Prosperi), *Diatraea saccharalis* (R. Gomes da Costa), *Argyria centrifugens* (Ian Morton), *Haimbachia squamulella* (P. Coin), *Euchromius ocelleus* (L. Hoy), *Calamotropha paludella* (D. Hobern), *Crambus lathoniellus* (F. Bauer), *Pediasia contaminella* (D. Hobern).

Discussion

Phylogenetic relationships

Our six-gene dataset recovered the monophyly of the Crambinae and the Scopariinae with strong support, with 60% of their inner relationships recovered with strong support (BS > 90%) and 19% with moderate support (70% < BS < 90%), while support for relationships among different subfamilies remained weak. *Erupa*, *Heliothela* and *Hoploscopa* were included for the first time in a molecular phylogeny. Our results do not support a close relationship between *Heliothela*, *Hoploscopa* and the Scopariinae, but low support in the backbone of the phylogeny precludes any definite statement concerning their positions. *Erupa* was consistently found as sister group to the Crambinae, though without support (BS < 70) in the ML analysis. Although the Crambinae and the Scopariinae were recovered as sister groups in Regier *et al.* (2012), the topology (Crambinae + *Erupa*) + Scopariinae was recovered here, but only with good support in the Bayesian analysis. The sister-group relationship Crambinae + Scopariinae shows an increase in bootstrap support (58% vs 88%) when *Erupa*, *Heliothela* and *Hoploscopa* are removed from our dataset (not shown), therefore resulting in a topology similar to that of Regier *et al.* (2012). The placement of *Erupa*, *Heliothela* and *Hoploscopa* with respect to Crambinae and Scopariinae remains uncertain and must be adressed in further studies with a greater number of genes and broader sampling of Crambidae.

Our phylogeny confirmed some of the tribes treated by Landry (1995) (e.g. the Diptychophorini, Haimbachiini), while others were found to be polyphyletic (Argyriini, Crambini). The snow-white colour of the forewing in *Argyria*, *Catharylla*,

Fig. 3. Ancestral character-state reconstruction mapped on best-scoring maximum likelihood (ML) tree where change is most likely to have occurred. The ML probability of ancestral character state is represented as pie charts. Pies are colored as follows: white (state 0), red (state 1), green (state 2). States for characters 1 (forewing Rs veins) and 6 (hindwing M_2 – M_3 – CuA_1 – CuA_2) are displayed on the first and second columns on the right, respectively.

Urola and *Vaxi*, the gnathos pointing downwards or posterad, or the noncoalesced papillae anales in *Ancylolomia*, *Euchromius, Miyakea* and the Crambini were shown to be homoplastic. In other cases, genera erected for species displaying 'odd' features, e.g. yellow colour of forewing in *Cholius* **syn.n.** or row of hairs on hindwing A_1 vein in males of *Dasyscopa* **syn.n.**, were found to represent highly apomorphic characters within *Scoparia* or *Eudonia*, respectively (Fig. 2). In Scopariinae, the clade *Micraglossa* + (*Scoparia* + *Eudonia*) was recovered in all analyses, although with weak support in ML analyses. The corpus bursae covered half with spines, half with scobinate patches (Fig.1, character 26) was suggested as a synapomorphy for the group including these genera (*Caradjaina* **syn.n.** excepted) by Nuss (2003), which argues in favour of this topology. In the

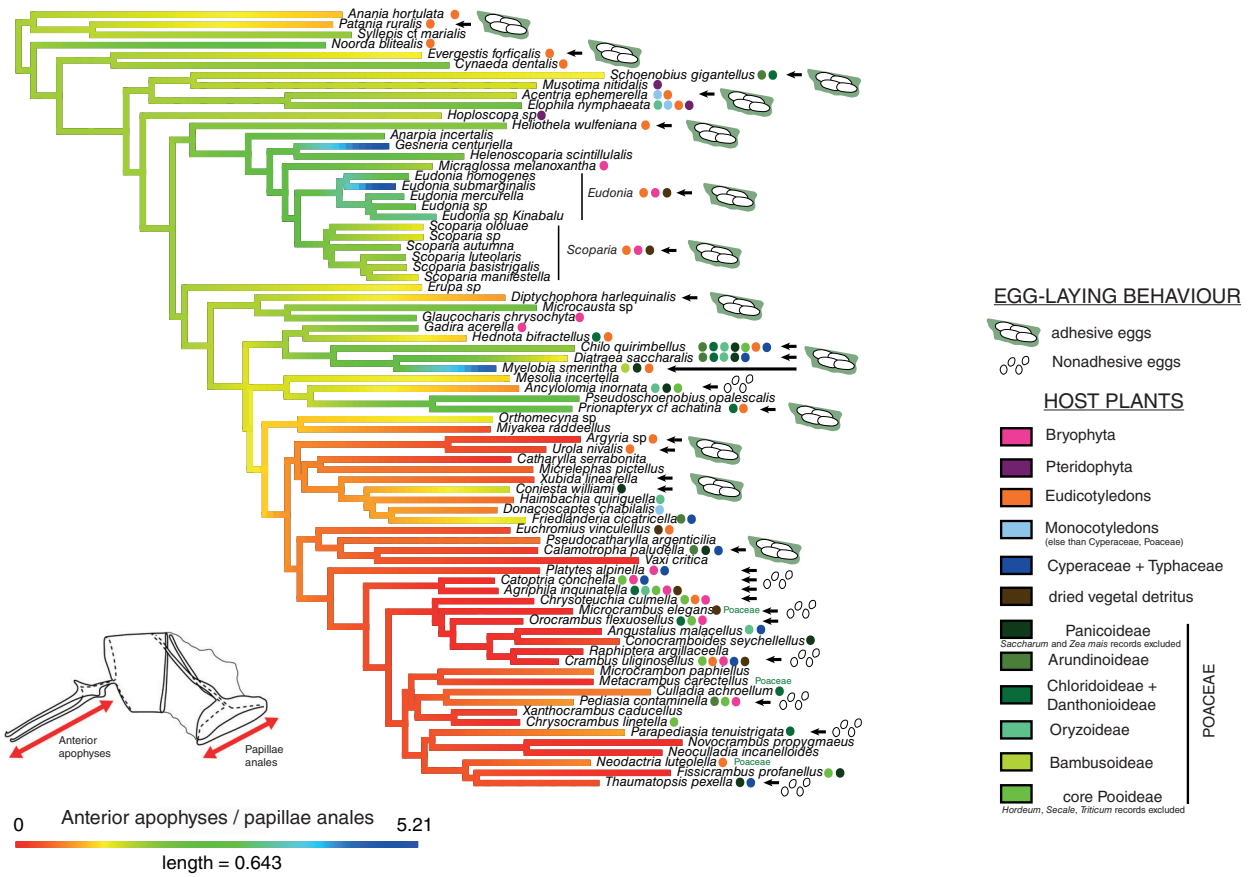

Fig. 4. Ancestral character reconstruction of anterior apophyses lengths compared with papillae anales lengths on the best-scoring maximum likelihood tree. Host plant and egg adhesiveness records for each species or congeneric species are displayed on the right.

Crambinae, the Ancylolomiini **stat. rev.** showed no support in the ML analysis (BS < 70%). *Ancylolomia* was recovered in the Crambini in the cladistic analysis of Landry (1995) on the basis of the gnathos pointing downward, the papillae anales dorsal connection membranous, and the hindwing cell open. The hindwing cell was coded as 'open' for *Ancylolomia japonica* Zeller in Landry (1995), although it is closed in *A. tentaculella* (Hübner) and was reported to be closed by other authors (de Joannis, 1932; Okano, 1962; Bleszynski, 1965; Kirpichnikova, 1999). Similarities in the morphology of *Prionotalis* and *Ancylolomia* (male genitalia with relatively short valva, strong vinculum, phallus as long as valva, with ductus ejaculatorius branching at anterior apex, female genitalia with papillae anales not coalesced dorsally and ductus seminalis originating on corpus bursae) (Bassi, 2016) support the Ancylolomiini hypothesis recovered here. However, the forewing distal margin produced, the ring-like gnathos without posterior extension and the valva with ventral process shared by *Mesolia*, *Prionapteryx* and *Pseudoschoenobius* are not observed in *Ancylolomia*, suggesting a possible misplacement of this genus as sister to *Prionapteryx* + *Pseudoschoenobius*.

Systematic changes

Our investigations provide the first comprehensive molecular phylogeny for the Crambinae and Scopariinae and the rationale for a number of changes in the classification. The enigmatic Erupini, weakly supported as sister to the Crambinae, do not share the Crambinae synapomorphies – hindwing M_1 near middle of cell and phallus connected medially to juxta – and are thus not considered as such. Their ambiguous position in our analyses restrained us from erecting this tribe as a subfamily for now. The taxa for which the composition is changed here are provided with a new diagnosis in the following, and their composition is summarized in Table S8.

SCOPARIIINAE
Scoparia Haworth, 1811

Diagnosis. Our ancestral character analysis recovered the ventral process of the valva in male genitalia as an apomorphy for the genus. *Scoparia* cannot be separated from *Eudonia*

based on external morphology, but has the following combination of characters in genitalia: male genitalia with uncus tip pointed, gnathos tip pointed and directed downwards, sacculus of valva sclerotized, with free distal extension and phallus (most species) with cornuti (Nuss, 1999); female genitalia with anterior and posterior apophyses as long as to twice as long as papillae anales, colliculum short, ductus bursae straight or slightly curved, corpus bursae spinulose on one lateral half, spatulate on other half, with an appendix bursae (absent in *Cholius* **syn.n.**).

Remarks. Molecular data support the monophyly of the clade, including *Caradjaina* and *Scoparia*, but do not support *Scoparia* as a distinct clade from *Caradjaina* (Figs 2, S2). Nuss (1999) highlighted the proximity between *Caradjaina* and *Scoparia* male genitalia, with *Caradjaina* displaying narrower shapes of the uncus, tegumen and base of valva. He suggested that the latter belonged to *Scoparia*, but no *Caradjaina* females were known then. Examination of female genitalia for the first time did not reveal any notable differences between *Caradjaina* and *Scoparia*. Thus, *Caradjaina* **syn.n.** is synonymized here with *Scoparia* and its only described species, *Scoparia kwangtungialis* (Caradja) **comb. rev.**, is transferred back to *Scoparia*. *Cholius* **syn.n.** is recovered in the molecular analysis as an ingroup of *Scoparia* and is thus synonymized here. *Scoparia luteolaris* (Guenée) **comb. rev.** and *S. leucopeplalis* (Hampson) **comb.n.** are transferred to *Scoparia*.

Eudonia Billberg, 1820

Diagnosis. Ancestral character analyses recovered the upward-directed (posterad in several cases) gnathos and the absence of cornuti in male genitalia as well as the long and slender colliculum and the looped ductus bursae in female genitalia as apomorphies for the genus. Further characters allow the segregation of *Eudonia* from other Scopariinae: in male genitalia, uncus tip blunt or notched, valvae generally without sclerotized sacculus – sclerotized in a few species, but without free distal extension; in female genitalia, anterior and posterior apophyses two to six times as long as papillae anales, corpus bursae spinulose on one lateral half, spatulate on other half, with a distinct signum and an appendix bursae.

Remarks. According to our molecular analyses, *Dasyscopa* **syn.n.** and *Dipleurinodes* **syn.n.** are nested within *Eudonia* and are thus synonymized here. *Eudipleurina* Leraut **syn.n.**, not included in our taxon sampling, resembles *Dipleurinodes* by the shared distinct sacculus without free distal extension (Nuss, 1999). It displays all apomorphies of *Eudonia* and is thus synonymized here with this genus. The following species are transferred to *Eudonia*: *Eudonia ambrensis* (Leraut) **comb.n.**, *Eudonia ankaratrella* (Marion) **comb.n.**, *Eudonia axeli* (Nuss) **comb.n.**, *Eudonia barbipennis* (Hampson) **comb.n.**, *Eudonia bueaensis* (Maes) **comb.n.**, *Eudonia comorensis* (Leraut) **comb.n.**, *Eudonia homogenes* (Meyrick) **comb.n.**, *Eudonia mineti* (Leraut) **comb.n.**, *Eudonia nigra* (Leraut) **comb.n.**, *Eudonia phaeopalpia* (Hampson) **comb.n.**, *Eudonia tavetae* (Maes) **comb.n.** and *Eudonia viettei* (Leraut) **comb.n.**

Micraglossa Warren, 1891

Diagnosis. No apomorphies are known for *Micraglossa* (Nuss, 1999). The inwardly directed spine in male genitalia observed in some *Micraglossa* species is a possible synapomorphy. *Micraglossa* can be separated from most other scopariine genera by the presence of golden shiny scales on the head, thorax and abdomen (Nuss, 1999; Li *et al.*, 2010). The genus is thought to be closely related to the Neotropical *Gibeauxia* Leraut, not sampled in this study, with which it shares the golden shiny scales and the presence of an elongated signum in corpus bursae (Nuss, 1999; Li *et al.*, 2010).

Remarks. The Australian *Micraglossa melanoxantha* (Turner) **comb.n.** is here transferred from *Scoparia* to *Micraglossa*.

CRAMBINAE

In light of the phylogenetic results, changes in the tribal classification are needed for all tribes except the Haimbachiini.

Diptychophorini Gaskin, 1972

Type genus. Diptychophora Zeller, 1866

Diagnosis. The moths of this tribe are small, with a forewing length not exceeding *c*. 8 mm and reaching only 3.5 mm in some specimens of *Microcausta*. Apomorphies for the tribe mentioned by Landry (1995), i.e. ratio of the forewing length over its width < 2.0 and the erect terminal scales on the flagellomeres, are not shared by *Gargela*. The forewing outer margin of many Diptychophorini is also often notched near M_1 and M_2, but this is not the case for *Gargela*. Also, the gelechioid-like recurved labial palpi of *Gargela* are a unique feature for a crambine group.

Remarks. The Diptychophorini include 10 genera (Nuss *et al.*, 2019). *Microchilo*, placed by Gaskin (1971) in the Diptychophorini, is recovered in the 'narrow-winged clade' (Fig. S2) and thus excluded from this tribe. *Gargela*, which was thought to be related to *Glaucocharis*, *Roxita* Bleszynski (Diptychophorini) and *Calamotropha* (Calamotrophini) based on wing pattern (Song *et al.*, 2009), is placed here confidently in the Diptychophorini.

Ancylolomiini Ragonot, 1889 **stat. rev.**

Type genus. Ancylolomia Hübner, 1825.

Diagnosis. The closed hindwing cell, its M_1 base near the middle of the cell, as well as the large valva, the short and broad tegumen arms with a wide dorsal connection and the ductus ejaculatorius branching at the anterior end of the phallus in male genitalia allow the Ancylolomiini to be segregated from other Crambinae.

Remarks. Ancylolomiini is a senior synonym of Prionapterygini Landry, 1995 **syn.n.** The Nearctic *Eufernaldia* Hulst, and Old World *Surattha* Walker (placed in the Prionapterygini by Landry, 1995), the Palearctic *Prionapteron* Bleszynski and *Talis* Guenée, as well as the Afrotropical *Aurotalis* Bleszynski and *Zovax* Bleszynski are placed here in the Ancylolomiini (Landry, 1995; Bassi, 2013, 2016). *Prionapteron* and *Talis* also share a ringed gnathos and a valval ventral sclerotization in male genitalia with *Mesolia*, *Prionapteryx* and *Pseudoschoenobius*, as well as triangularly shaped papillae anales in female genitalia with *Prionapteryx* and *Pseudoschoenobius*.

Chiloini Heinemann, 1865

Type genus. Chilo Zincken, 1817

Diagnosis. The ancestral character reconstruction recovered the two-armed juxta as the most probable ancestral state of the Chiloini and it thus represents an apomorphy for this tribe. Further characters allow segregation of this tribe from other Crambinae: hindwing cell closed, tympanal organs with marked venulae secundae, pseudosaccus present in male genitalia.

Remarks. In all, 11 genera are assigned here to this tribe: *Chilandrus* Bleszynski, *Chilo*, *Chiqua* Bleszynski, *Diatraea*, *Eschata* Walker, *Gadira*, *Hednota* Meyrick, *Leonardo* Bleszynski, *Malgasochilo* Bleszynski, *Myelobia* and *Tauroscopa* Meyrick. The Myelobiini Minet **syn.n.** including *Eschata* and *Myelobia*, share the thick venulae secundae and the bifid juxta in male genitalia with other members of the Chiloini. We could not examine specimens or the descriptions of *Hemiptocha* Dognin and *Pseudometachilo* Bleszynski and thus cannot assign them to this tribe with confidence. Klots (1970) provided a brief description of the tribe, although in a more restricted sense, while Gaskin's concept of the tribe is here revised substantially.

Argyriini Klots, 1983

Type genus. Argyria Hübner, 1818

Diagnosis. The following combination of characters allows the segregation of Argyriini from other Crambinae: labial palpi less than twice the eye width, forewing ground colour snow white, forewing R_{S4} starting from cell, hindwing cell open, in male genitalia conspicuous lateral coremata, pseudosaccus absent, in female genitalia anterior apophyses short (Landry, 1995).

Remarks. The Argyriini are presently limited to the two genera *Argyria* Hübner and *Urola* Walker. Bleszynski synonymized *Urola* with *Argyria* (Bleszynski, 1967), and a close relationship between these two genera was suggested by Landry (1995). *Catharylla* and *Micrelephas* could not be reliably assigned to either the Argyriini or the Haimbachiini (Fig. 2), but share all diagnostic features of Argyriini, except for the forewing R_{S4} stalked to $R_{S2} + R_{S3}$ and the absence of lateral coremata in *Catharylla*, and the brown and orange scaling on the forewing in some *Micrelephas* species (Landry, 2003; Landry *et al.*, 2013; Léger *et al.*, 2014). The Australian *Australargyria* Bleszynski and *Neargyrioides* Bleszynski, and the Oriental *Pseudargyria* Okano all share the snow-white colour of the wings, the forewing R_{S4} connected to the cell (connected to $R_{S2} + R_{S3}$ in *Australargyria*), the hindwing cell open and the absence of a pseudosaccus (present in *Pseudargyria*) with the Argyriini, and might belong to this tribe (Okano, 1962; Bleszynski, 1965, 1970b).

Calamotrophini Gaskin, 1988 **stat. rev.**

Type genus. Calamotropha Zeller, 1863

Diagnosis. The combination of the following characters will serve to single out the members of this tribe from other Crambinae: labial palpi more than twice the eye width, forewing with R_{S4} stalked to $R_{S2} + R_{S3}$; in female genitalia, papillae anales with dorsal connection sclerotized and apophyses anterior and posterior short.

Remarks. Calamotropha Zeller, *Pseudocatharylla* Bleszynski and *Vaxi* Bleszynski are included in this tribe. Other Afrotropical genera not included in our sampling, *Chrysocatharylla* Bassi, *Classeya* Bleszynski and *Pseudoclasseya* Bleszynski, have male genitalia similar to those of *Pseudocatharylla* (Bassi, 1999), the latter two sharing asymmetrical male valvae with *Pseudocatharylla*. All three share the hindwing cell open and the forewing R_{S4} vein connected at base with $R_{S2} + R_{S3}$ with the Calamotrophini and are thus placed in this tribe.

Euchromiini Léger, Landry & Nuss **tribe n.**
http://zoobank.org/urn:lsid:zoobank.org:act:1A84282D-930A-4C32-8340-D681BFF27A12.

Type genus. Euchromius Guenée, 1845

Diagnosis. The combination of the following morphological characters are regarded as diagnostic: forewing with single or double yellowish medial fascia, subterminal line starting from tornus and ending at middle of termen above terminal black dots, with a row of large black terminal dots on dorsal half; forewing R_{S4} vein originating from cell, hindwing M_1 vein close to $Sc + R$, hindwing cell open; in male genitalia, gnathos apex pointed downwards, pseudosaccus present; in female genitalia, papillae anales dorsally connected by a membrane (Schouten, 1988, 1992).

Remarks. The Euchromiini encompass the single genus *Euchromius*. *Metaeuchromius* Bleszynski and *Miyakea* Marumo show a similar wing pattern to that of *Euchromius* but the closed hindwing cell, the hindwing M_1 vein placed near the middle of the cell, the gnathos tip pointed upwards and the missing pseudosaccus discriminate them from *Euchromius*.

Crambini Latreille, 1810

Type genus. Crambus Fabricius, 1798

Diagnosis. The membranous connection of the papillae anales in female genitalia is recovered as an apomorphy for the tribe. The gnathos tip pointed downwards or posterad in male genitalia is another possible apomorphy, but this state is also observed in the closely related *Euchromius* and *Calamotropha* and might also be the ancestral state of the R_{S4} stalked clade. Further characters allow this tribe to be segregated from other Crambinae: forewing R_{S4} stalked (except some *Platytes* species) with $R_{S2} + R_{S3}$, hindwing cell open; in male genitalia pseudosaccus present; in female genitalia tergite VIII narrow, anterior and posterior apophyses short.

Remarks. Ancylolomia, *Euchromius*, *Micrelephas* and *Miyakea* are excluded from the Crambini. *Microcramboides* Bleszynski **syn.n.** and *Tortriculladia* Bleszynski **syn.n.** are nested within *Microcrambus* and are thus regarded as synonyms. Their original descriptions mentioned that *Microcramboides* and *Tortriculladia* are close to *Microcrambus* based on the genitalia and the sclerites of the male eighth abdominal segment (Bleszynski, 1967). The absence of signa in the female corpus bursae differentiating *Microcrambus* from *Crambus* (Bleszynski, 1963) is shared by *Microcramboides* and *Tortriculladia* and not observed in other members of the '*Crambus* group'. Crambini is the most speciose tribe of Crambinae, containing 61 genera with 783 species.

Evolution of morphological features

Ancestral character-state analyses allow for the discovery of apomorphies for clades and for inferences about the placement of non-assigned taxa and fossils (Smith & Turner, 2005; Beutel & Kristensen, 2012). Among the 27 characters investigated, we recovered 21 synapomorphies supporting clades grouping more than one genus, thereby confirming 10 apomorphies in Landry (1995) and two apomorphies hypothesized in Nuss (1999, 2003). Nine characters provided apomorphies for newly recovered clades among the Crambinae. However, ancestral character reconstruction showed two major limitations: (i) character examination is limited to one or a few species, thereby overlooking trait variation within a taxon (Wiens, 1995); and (ii) ancestral character estimation analyses are based on the assumption that the true phylogenetic tree is used, which can lead to false reconstruction of ancestral character state, even within commonly accepted confident support values (> 90%) (Duchene & Lanfear, 2015).

The uncus and gnathos of the males are used in pairs in contact with the female's segment VIII during copulation in Crambidae (Cordero & Baixeras, 2015). Motion muscles activating the gnathos are mostly absent in the groundplan of Crambidae (Kuznetsov & Stekolnikov, 1979), suggesting a loss of the use of the uncus-gnathos claw function (Kuznetsov & Stekolnikov, 2001). By contrast, muscle M10, which lowers the gnathos by contraction, is present in the following Crambinae and Scopariinae species: *Agriphila straminella* (Denis & Schiffermüller), *Chrysoteuchia culmella* (Linnaeus), *Crambus pratellus* (Linnaeus), *Pediasia matricella* Treitschke, *Euchromius ocelleus* (Haworth) and *Scoparia ancipitella* (La Harpe) (Kuznetsov & Stekolnikov, 1979). All these taxa share a gnathos tip pointed downwards, which suggests a correlation between this trait and the presence of a gnathos-lowering muscle. In the light of our results and those of Regier *et al.* (2012), the presence of this muscle is a possible synapomorphy for the clade, including the Crambinae and Scopariinae. Male genitalia musculature reveals another interesting distribution pattern: the pair of valve extensors (muscles M3) connects the juxta to the anterior or medial part of the vinculum in Crambidae, whereas in *Euchromius ocelleus* and the four Crambini investigated by Kuznetsov & Stekolnikov (1979), M3 muscles attach to the pseudosaccus instead of the vinculum. The crucial role of the pseudosaccus in male genitalic musculature thus explains its conservation in the two groups of Crambinae where it is found.

Our ancestral character reconstruction recovered a loss of signum in the ancestor of the narrow-winged clade and the gain of two signa in the *Crambus* group. Signa are hypothesized to evolve as counter-adaptations against thick spermatophores (Cordero, 2005; Sánchez *et al.*, 2011). The presence of a signum is associated with polyandry, with both regarded as the plesiomorphic conditions in Lepidoptera, whereas the evolution of monandry is associated with the loss of signa (Sánchez *et al.*, 2011). Females of *Parapediasia teterrella* (Zincken) bear no signum and were shown to mate only once, whereas females of *Agriphila plumbifimbriella* (Dyar), which bear one signum, were reported to mate more than once (Marshal, 1988). No extensive data on the reproductive behaviour of the Crambinae are presently available, but the different number of signa observed among the Crambinae could reflect different reproduction behaviours.

Egg-laying behaviour and host plant use

Ecological traits such as nonadhesive eggs and larval life habits were not formerly investigated by ancestral trait reconstruction because of the paucity of available information, but the distribution of these traits on the recovered phylogeny provides insights into the evolution of the Crambinae and Scopariinae. The behaviour of dropping nonadhesive eggs is observed in Crambini and *Ancylolomia*, suggesting at least two independent origins in Crambinae (Fig. 4). Nonadhesive eggs are associated with two modifications of the female oviscapt. First, the dorsal connection of the papillae anales is not sclerotized (Fig. 1, character 18, state 1), which probably facilitates the nontargeted dissemination of eggs. Second, strongly reduced posterior and anterior apophyses as well as short membranes between segments VIII and IX suggest a reduced evertion of the oviscapt during oviposition. The membranous dorsal connection of papillae anales observed in *Euchromius*, *Gadira*, *Miyakea*, *Prionotalis*, *Aurotalis* and *Metaeuchromius* (the latter three were

not included in this study) indicate that the females of these species may also lay nonadhesive eggs. Furthermore, the series of steps followed during oviposition seem to differ in species laying nonadhesive eggs. In Lepidoptera species fixing their eggs to a substrate, the oviposition process implies the search for a suitable host plant, with subsequent evaluation of its surface with the antennae, tarsi, proboscis and oviscapt (Chadha & Roome, 1980; Marion-Poll *et al.*, 1992; Renwick & Chew, 1994). Females of *Agriphila plumbifimbriella* and *Parapediasia teterrella* were reported to drop their eggs in flight (Ainslie, 1922; Marshal, 1988), which means that host-searching is either skipped or oviposition is stimulated by habitat characters.

Host plant and larval life habits remain poorly investigated, with the exception of a few species of economic importance (Hill, 1987; Khan *et al.*, 1991; Capinera, 2001; Glime, 2017). Scopariinae are largely moss feeders, with a few *Scoparia* species feeding on dicotyledons (Nuss in Goater *et al.*, 2005). In the Crambinae, the early lineage Diptychophorini are most probably moss feeders, as suggested by the New Zealand *Glaucocharis* reported to feed on leaves of mosses (Gaskin, 1972). Despite the lack of host plant records for the Erupini (Hayden, 2012), moss-feeding habits are observed in the early-diverging lineage of the Crambinae, the Diptychophorini, as well as in *Gadira* and in all early-diverging lineages of the Crambini (*Platytes*, *Agriphila*, *Catoptria*, *Chrysoteuchia*), and suggest moss as the ancestral host plant of the Crambinae and Scopariinae. Crambinae belonging to the narrow-winged clade feed predominantly on Poaceae, with a few species feeding on sedges (*Calamotropha* spp., several Crambini), various dicotyledonous plants (*Argyria*, *Chrysoteuchia*, *Crambus*, *Prionapteryx*) or dry vegetal detritus (*Euchromius*) (Slamka, 2008; Robinson *et al.*, 2010). The wide range of host plants recorded for species of *Chilo* and *Diatraea* are thought to be the result of intense cultivation of cereal crops followed by an increase of their population size, which trigger host plant range expansion in herbivores (Castagneyrol *et al.*, 2016). Crambinae laying nonadhesive eggs (*Ancylolomia* and the Crambini) are reported to feed predominantly on grasses belonging to the 'core Pooideae' (Poaceae), i.e. the species-rich clade sister to the Brachypodieae (Saarela *et al.*, 2015). Their larvae live in silken tunnels near the ground, feeding at the base of grasses, unlike other larvae of Crambinae (*Calamotropha*, *Chilo*, *Diatraea*, *Myelobia*, Haimbachiini) which are stem borers in grasses.

Pooideae are thought to have experienced a major radiation in the early Oligocene (34–23 Ma), most likely in the northern hemisphere where they dominate the grass flora (Hartley, 1973; Bouchenak-Khelladi *et al.*, 2010). A host plant shift to Pooideae could explain the high diversity observed in Crambini (916 sp.), as host plant shifts are known to trigger speciation (Forbes *et al.*, 2017), leading to subsequent evolutionary radiations (Fordyce, 2010). Crambini account for 40% of the described crambine species and represent the bulk of crambine diversity in temperate areas (e.g. 80% of the Crambinae fauna in Canada) (Pohl *et al.*, 2018). Our analysis, including part of the diversity of the Crambini (25 of the 61 described genera), recovered the Holarctic genera *Platytes*, *Agriphila* and *Catoptria* as the earliest diverging lineages of Crambini, suggesting that diversification of the tribe took place there. A similar diversification scenario is found in the Satyrinae (Nymphalidae), which diversified on grasses during the drying and cooling period that triggered their expansion in the Oligocene (Peña & Wahlberg, 2008). Interestingly, similar cases of nonadhesive egg-laying behaviours are observed conjointly with the use of Pooideae as host plants in several Satyriinae genera (*Aphantopus*, *Arethusana*, *Brintesia*, *Erebia*, *Hipparchia, Lopinga*, *Maniola*, *Melanargia*, *Minois*, *Pyronia*) (Frohawk, 1924; Forster & Wohlfahrt, 1955; Wiklund, 1984; E. García-Barros, personal communication), suggesting that the evolution of nonadhesive eggs is an adaptation to the switch to Pooideae. Although the lack of fossils prevents us from any speculation about the time of their diversification, Crambini possibly followed a similar evolutionary path to that of the Satyrinae. Future studies with a broader sampling of the tribe covering all genera and biogeographical zones will shed more light on its evolution.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Fig. S1. Uncorrected p-distances of the third codon position for each gene plotted against the distance on the best-scoring ML tree of conc6genes.

Fig. S2. Best-scoring ML tree of the whole dataset with best PARTITIONFINDER model applied and bootstrap support values from 1000 replicates displayed on nodes.

Fig. S3. Best-scoring ML tree of the conc6genes_COI1+2 dataset with the best PARTITIONFINDER model applied. Bootstrap support (1000 replicates) displayed above the nodes, posterior probabilities displayed below.

Fig. S4. Node numbers used for 'ace' analyses (Table S6) plotted on best-scoring ML tree of the conc6genes dataset with best PARTITIONFINDER model applied.

File S1. R script of the 'ace' analysis (APE package; Paradis *et al.*, 2004).

File S2. R script of the 'fastAnc' analysis (PHYTOOLS package; Revell, 2012)

File S3. R script of the 'ace' function modified by E. Paradis to take uncertain states into account.

Table S1. List of the 111 taxa sampled along with their access numbers at the European Nucleotide Archive (https://www.ebi.ac.uk/ena) for each gene. Subfamilial and tribal assignments follow the current classification (prior to this study) according to GLOBIZ (Nuss *et al.*, 2019). The institute acronyms stand as follows: Muséum d'histoire naturelle de Genève (MHNG), Museum für Naturkunde der Humboldt-Universität Berlin (ZMHB), Senckenberg

Museum für Tierkunde Dresden (SMTD), Tiroler Landesmuseum Ferdinandeum, Innsbruck (TLMF), Zoologische Staatsammlung München (ZSM).

Table S2. PCR primers used. PCR product length for newly designed nested primers refer to the PCR product obtained with the corresponding standard primer from Wahlberg & Wheat (2008).

Table S3. PCR mix adapted from Wahlberg & Wheat (2008).

Table S4. PCR programmes adapted from Wahlberg & Wheat (2008) and Regier (2007).

Table S5. Character states matrix.

Table S6. Scores from the 'ace' analysis (ape package) for each node (see Fig. S4).

Table S7. Egg-laying behavior, host plant data and larval life habits in Crambinae, Scopariinae and other Crambidae subfamilies.

Table S8. Revised systematic classification of genera of Crambinae and Scopariinae investigated in this study. Support for systematic positions are provided by the analysis of the morphology only (*), or by the analyses of the morphology and molecular (**).

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