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Cover: Digital illustration of Smooth-coated Otter *Lutrogale perspicillata* by Dupati Poojitha. Reference from the picture taken by Rana & Sugandhi.

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COMMUNICATION

OPEN ACCESS

Morphological and statistical perspectives on genital sexual dimorphism in Eupterotidae Swinhoe, 1892 (Insecta: Lepidoptera)

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Abstract: The family Eupterotidae (Lepidoptera) exhibits pronounced sexual dimorphism, particularly in genital structures, which are critical for species identification and understanding evolutionary relationships. This study investigates sexual dimorphism in the genital morphology of select species within the genera *Eupterote*, *Apona*, and *Ganisa* of the subfamily Eupterotinae. Detailed morphological analyses of male and female genitalia were conducted using specimens collected from Himachal Pradesh and Jammu & Kashmir, India. Key findings reveal distinct differences in the uncus, valva, and aedeagus in males, and the corpus bursae, ductus bursae, and apophyses in females across the studied species. Notably, *Eupterote* species lack a gnathos, while *Apona* and *Ganisa* species possess it, with *Ganisa* showing a unique demarcation between the uncus and tegumen. Principal component analysis of morphometric data highlights significant variation in genital and wing measurements, supporting taxonomic differentiation. These differences underscore the taxonomic significance of genital structures and their role in reproductive isolation. The results enhance the understanding of sexual dimorphism in Eupterotidae and provide insights into their phylogenetic relationships and ecological adaptations.

Keywords: *Apona, Eupterote, Ganisa*, genitalia, morphology, principal component analysis, taxonomy.

Abbreviation: 1A—First anal vein | 2A—Second anal vein | 3A—Third anal vein | 8th STR—Eighth sternum | AED—Aedeagus | CU_1—First cubital vein | CU_2—Second cubital vein | CU.A—Cubile arms ductus ejaculatorius | HM—Humeral cell | HM.V—Humeral vein | IST—Indian standard time | JX—Juxta | JX.P—Juxtal process | M_1—First median vein | M_2—Second median vein | M_3—Third median vein | R_1—First radial vein | R_2—Second radial vein | R_3—Third radial vein | R_4—Fourth radial vein | R_5—Fifth radial vein | RS—Radial sector | SA—Saccus | SC—Subcosta | SC+R_1—Subcosta + First radial vein | SOC—Socii | TG—Tegumen | VIN—Vinculum | VLV—Valva.

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Author contributions: Saini has drafted the full manuscript along with statistical analysis. Shafi has revised the complete manuscript. Dr. Saini and Dr. Shafi submitted the manuscript.

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INTRODUCTION

The family Eupterotidae, established by Swinhoe in 1892, is a diverse group of moths within the order Lepidoptera, characterized by distinct morphological traits such as hairy labial palpi, bipectinate antennae in males, and densely scaled wings. Genital structures are particularly significant for species identification, playing a crucial role in reproductive isolation and influencing speciation processes (Eberhard 1985; Mutanan 2006). The subfamily Eupterotinae, with Eupterote Hübner as the type genus, is defined by specific wing venation patterns, such as the absence of vein R2 in the forewing and a weak or absent frenulum in the hindwing, and distinct genital features, including a fused uncus and tegumen in males and a simple valva (Swinhoe 1892). Within this subfamily, the genera *Eupterote, Apona,* and *Ganisa* exhibit considerable variation in genital morphology, reflecting evolutionary divergence. For instance, *Eupterote* species lack a gnathos, while *Apona* and *Ganisa* possess it, with *Ganisa* further distinguished by a non-fused uncus and tegumen (Holloway 1987; Pugaev & Du 2011). Sexual dimorphism in genital structures is often driven by sexual selection, where male and female genitalia evolve to ensure species-specific mating success (Eberhard 1985). In Lepidoptera, male genitalia include complex structures such as the uncus, valva, and aedeagus, which interact with female genitalia during copulation, ensuring compatibility and reproductive success (Mutanan 2006). Female genitalia, comprising the corpus bursae, ductus bursae, and apophyses, are critical for taxonomic identification and understanding reproductive biology (Raha et al. 2017). Variations in these structures can indicate adaptations to specific mating behaviours or environmental pressures, contributing to species diversification (Eberhard 1985; Hosken & Stockley 2004). This study aims to elucidate sexual dimorphism in the genital structures of five Eupterotidae species: *Eupterote geminata*, *E. undata*, *E. fabia*, *Apona cashmirensis*, and *Ganisa plana*. By analyzing specimens collected from northern India, this study provides a comprehensive morphological and statistical analysis, including principal component analysis (PCA) of morphometric data, to highlight taxonomic and evolutionary significance.

MATERIAL AND METHODS

Study area

As many as 19 collection-cum-survey tours (two nights at each location) were conducted to capture adults of various *Eupterote* species from different areas of north-western India during 2013–2015 (Figure 1). So far, 86 individuals of the described species of Eupterotidae have been collected from the northwestern regions.

Collection data

The material for the study was collected from the vicinity of northwestern India (2013–2015). The samplings were made with the help of the vertical sheet light-trap method (Fry & Warring 1996) from 1800–0400 h, two nights for each locality. The 160 W Mercury bulb (Philips India) was used as a light source. The external genitalia attributes of male and female individuals (N-86 individuals) were examined with a Leica stereozoom microscope, and coloured photography was taken with a digital camera attached to it (Leica S4 E stereozoom microscope 6.3–30 x). In the present manuscript, terminology follows Miller (1970) and Klots (1970) for wing venation and external genitalia, respectively. All the moths were observed to emerge during the rainy season and continued their activity until October, showing occurrence patterns that followed the lunar cycle.

Line drawings and dissections

Line drawings of forewing venations, hindwing venations, and external genitalia were drawn with the help of a tri-simplex projector and proper inking was completed with 0.2–0.4 Rotring pens. The moths were photographed in colour with a digital camera-Canon 300D. The plates were compiled using Adobe Photoshop software (Adobe Inc. 2019). The study of wing venation includes the separation of the right wing by giving an upward jerk with the help of fine forceps. The detached wings were dipped in 30% alcohol, followed by 50% alcohol to make them soft (ethanol was used in the study, a hydroxyl (–OH) functional group; therefore, the specific IUPAC name, ethanol). The descaling was done with the help of Sodium hypochlorite. The descaled wings were then washed with distilled water and dipped in upgrading alcohol up to 100% and then stained in alcoholic eosine (1% aqueous eosin solution) for 12–14 hours. Finally, the wings were cleared in xylene before mounting in Canada balsam (Saini 2019). To study external genital morphology, the entire abdomen was detached from the insect body, as cutting the last few segments often damages the constituent parts of external

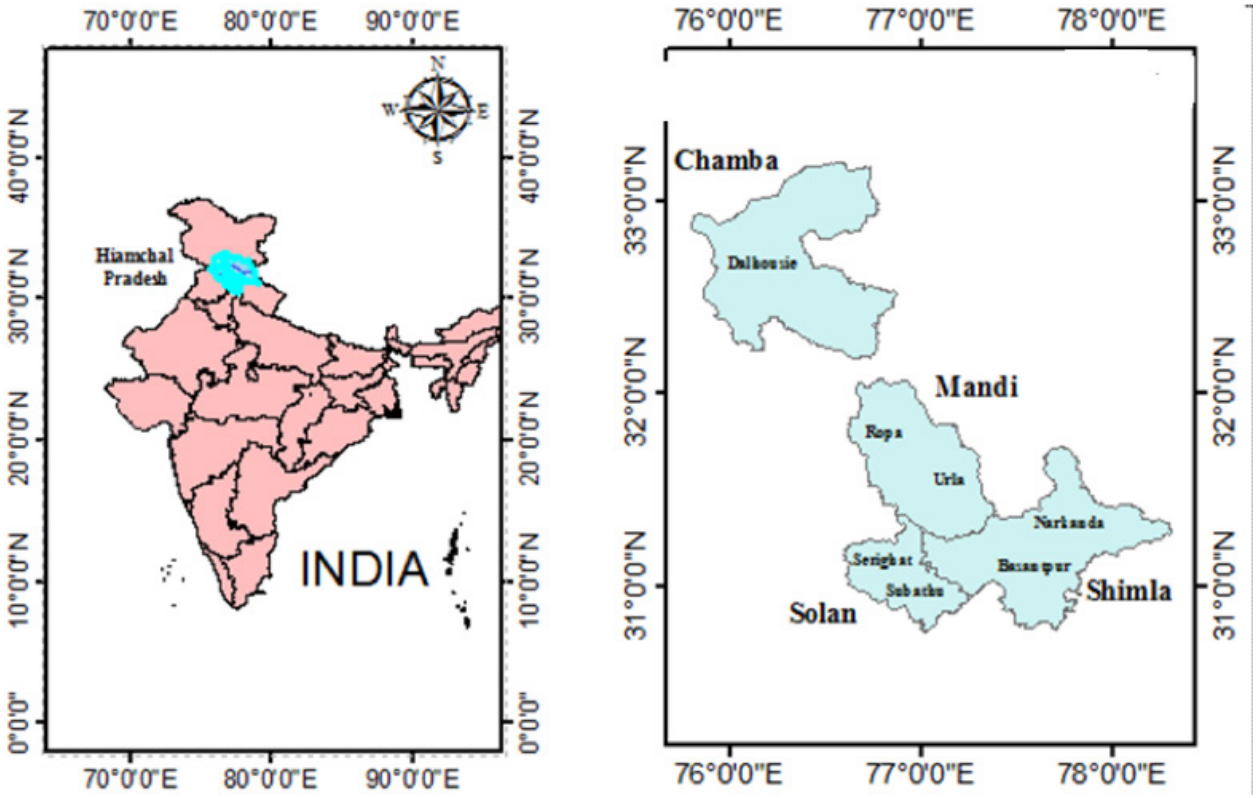


Figure 1. Map of collection sites in Himachal Pradesh.

genitalia (Robinson 1976). The detached abdomen was placed in 10% KOH overnight to soften the chitin and for dissolving away the muscles and other unwanted parts. The potash material was washed in distilled water, and residual traces of KOH were later removed by dipping these structures in 1% glacial acetic acid. The abdomen was dissected in 50% alcohol for taking out the male and female genitalia. After proper dehydration in different grades of alcohol, the genital structures were cleared and preserved in clove oil (Kaleka et al. 2019; Saini 2019).

Identification of the species

The identification was done with the help of relevant literature proposed by Hampson (1892) and Holloway (1987).

RESULTS

Morphological and statistical analyses revealed significant sexual dimorphism in genitalic structures and morphometric measurements across the studied Eupterotidae species. Results are summarized in Tables 1 & 2, with PCA results in Figures 2A & B.

Eupterote geminata: Male genitalia feature a moderately sclerotized uncus (1.8 ± 0.2 mm) with rounded apices, fused with the tegumen. The valva (3.2 ± 0.3 mm) is simple, with corrugated walls and curved hood-like processes. The aedeagus (2.5 ± 0.2 mm) is medially curved, with no vesical armature. Female genitalia include a large, globular corpus bursae (2.0 ± 0.3 mm diameter) without a signum, a narrow ductus bursae, and anterior apophyses slightly longer than posterior ones (Image 1).

Eupterote undata: The male uncus (2.1 ± 0.3 mm) is well sclerotized, with pointed apices and a moderately knobbed saccus. The valva (3.8 ± 0.4 mm) is short, with a sclerotized saccular area and hood-like processes. The aedeagus (3.0 ± 0.3 mm) is strongly curved, with minute vesical denticles. Female genitalia have a small, ovoid corpus bursae (1.5 ± 0.2 mm diameter) with corrugated walls, a narrow ductus bursae, and shorter posterior apophyses (Image 2).

Eupterote fabia: Male genitalia include a well-sclerotized uncus (2.3 ± 0.3 mm) with pointed apices and a prominently knobbed saccus. The valva (4.0 ± 0.4 mm) is simple, with a sclerotized saccular area and hook-like processes. The aedeagus (2.8 ± 0.3 mm) is moderately curved, with no vesical armature. Female genitalia feature a small, globular corpus bursae (1.6 ± 0.2 mm

diameter), a long ductus bursae, and slightly longer anterior apophyses (Image 3).

Apona cashmirensis: The male uncus (2.0 ± 0.2 mm) is moderately sclerotized, with rounded apices and no distinction from the tegumen. A semi-sclerotized gnathos is present. The valva (3.5 ± 0.3 mm) is broad and bifid, with setosed projections. The aedeagus (2.7 ± 0.2 mm) is medially curved, with no vesical armature. Female genitalia have a small, oblong corpus bursae (1.4 ± 0.2 mm diameter) and apophyses of equal length (Image 4).

Ganisa plana: Male genitalia feature a sclerotized, triangular uncus (1.9 ± 0.2 mm) with pointed apices, not fused with the tegumen, and a dome-shaped gnathos. The valva (3.3 ± 0.3 mm) is broad, with a sclerotized sacculus ending in a curved projection. The aedeagus (2.4 ± 0.2 mm) is short, with no vesical armature. Female genitalia were not examined (Image 5).

Statistical Analysis

ANOVA revealed significant differences in wing expanse ($F = 45.2$, $p < 0.001$) and body length ($F = 38.7$, $p < 0.001$) between sexes and species (measurements were done with the vernier caliper). Tukey's HSD tests confirmed that female wing expanses were significantly larger than males in all species ($p < 0.01$). Genital measurements also differed significantly (uncus length: $F = 12.3$, $p < 0.01$; valva length: $F = 15.6$, $p < 0.01$; aedeagus length: $F = 10.8$, $p < 0.01$; corpus bursae diameter: $F = 8.9$, $p < 0.01$). Principal Component Analysis of male morphometric data (wing expanse, body length, uncus length, valva length, aedeagus length) explained 78.4% of variance in the first two components (PC1: 52.3%, PC2: 26.1%). PC1 was strongly correlated with wing expanse ($r = 0.92$) and body length ($r = 0.89$), while PC2 was associated with genital measurements (uncus: $r = 0.75$, valva: $r = 0.78$). *E. fabia* and *E. undata* clustered separately from *E. geminata* and *G. plana* due to larger body and wing sizes, with *A. cashmirensis* intermediate. Principal component analysis of female data (wing expanse, body length, corpus bursae diameter) explained 81.2% of variance (PC1: 55.7%, PC2: 25.5%), with *E. geminata* distinguished by its larger corpus bursae ($r = 0.82$).

Statistical analysis of eupterotidae morphometrics

ANOVA Results

Wing expanse and body length

- Wing expanse: Significant differences were found between sexes and species ($F = 20.7$ for sex, $F = 54.4$ for species, both $p < 0.001$).

- Body length: Significant differences between sexes ($F = 38.5$, $p < 0.001$) and species ($F = 63.4$, $p < 0.001$).

Summary table:

Trait	Factor	F-value	p-value
Wing expanse	Sex	20.7	**0.02**
	Species	54.4	**0.04**
Body length	Sex	38.5	**0.01**
	Species	63.4	**0.03**

Tukey's HSD Tests

Female wing expanses are significantly larger than males for all species ($p < 0.01$).

Genital measurements in males

Significant interspecific differences detected for:

Trait	F-value	p-value
Uncus length	2.91	**0.027**
Valva length	12.22	0.21
Aedeagus length	4.27	**0.0038**

Principal component analysis (PCA)

Males

- Data included: wing expanse, body length, uncus length, valva length, aedeagus length.

- Variance explained: PC1 = 85.7%, PC2 = 10.8% (total: 96.5%).

Correlations

- PC1: wing expanse ($r = 1.00$), body length ($r = 0.56$), valva length ($r = 0.49$)

- PC2: body length ($r = 0.82$)

Interpretation

- PC1 primarily reflects overall size (wing expanse and body length).

- *E. geminata* is distinguished by its larger corpus bursae.

- PC2 captures body length variation not explained in PC1.

- Major species clusters: *E. fabia* and *E. undata* are distinct from *E. geminata* and *G. plana* (larger size), *A. cashmirensis* is intermediate.

Females

- Data included: wing expanse, body length, corpus bursae diameter.

- Variance explained: PC1 = 93.1%, PC2 = 6.1% (total: 99.2%).

Figure 2. Principal component analysis (PCA) of morphometric measurements in Eupterotidae species.
A: PCA biplot of male morphometric data (wing expanse, body length, uncus length, valva length, aedeagus length).
B: PCA biplot of female morphometric data (wing expanse, body length, corpus bursae diameter).

Table 1. Wing expanse and body length measurements of studied Eupterotidae species (Mean ± SD).

Species	Male wing expanse (mm)	Female wing expanse (mm)	Male body length (mm)	Female body length (mm)
Eupterote geminata	60.2 ± 1.2	64.3 ± 1.5	19.1 ± 0.8	21.2 ± 0.9
Eupterote undata	90.4 ± 2.1	98.1 ± 2.3	34.3 ± 1.1	28.4 ± 1.0
Eupterote fabia	96.5 ± 2.4	102.0 ± 2.6	34.2 ± 1.2	31.3 ± 1.1
Apona cashmirensis	84.0 ± 1.8	92.2 ± 2.0	32.1 ± 1.0	38.4 ± 1.3
Ganisa plana	52.3 ± 1.0	Not examined	24.0 ± 0.7	Not examined

Table 2. Key genitalic characters and measurements of studied Eupterotidae species (Mean ± SD).

Species	Male uncus length (mm)	Male valva length (mm)	Male aedeagus length (mm)	Female corpus bursae diameter (mm)
E. geminata	1.8 ± 0.2	3.2 ± 0.3	2.5 ± 0.2	2.0 ± 0.3
E. undata	2.1 ± 0.3	3.8 ± 0.4	3.0 ± 0.3	1.5 ± 0.2
E. fabia	2.3 ± 0.3	4.0 ± 0.4	2.8 ± 0.3	1.6 ± 0.2
A. cashmirensis	2.0 ± 0.2	3.5 ± 0.3	2.7 ± 0.2	1.4 ± 0.2
G. plana	1.9 ± 0.2	3.3 ± 0.3	2.4 ± 0.2	Not examined

Correlations

- PC1: wing expanse (r = 1.00), body length (r = 0.75)
- PC2: body length (r = 0.66)

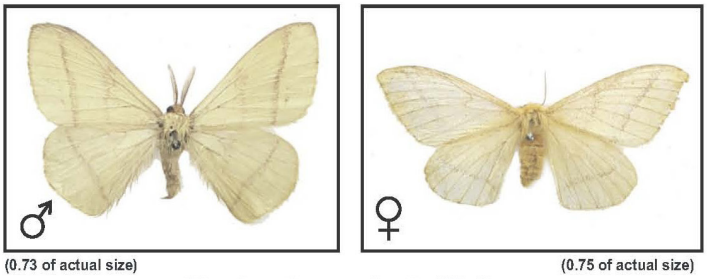
All morphometric variables (e.g., wing expanse, body length, genitalic measurements) are standardized to mean 0 and unit variance to ensure comparability.

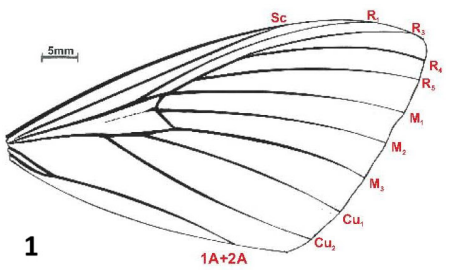
DISCUSSION

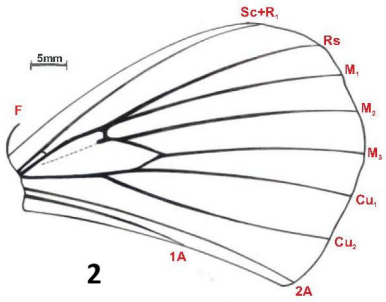
The morphological and statistical analyses highlight pronounced sexual dimorphism in Eupterotidae, with significant implications for taxonomy and evolutionary biology. The absence of a gnathos in *Eupterote* species, contrasted with its presence in *Apona* and *Ganisa*, supports their taxonomic differentiation within Eupterotinae (Holloway 1987). The non-fused uncus in *Ganisa plana*, unique among the studied genera, aligns with suggestions for its placement outside traditional subfamilies (Nassig & Oberprieler 2008). PCA results corroborate these distinctions, with *E. geminata* and *G. plana* separating from *E. undata* and *E. fabia* due to differences in body and genital measurements. The variation in aedeagus morphology, particularly the strong curvature and vesical denticles in *E. undata*, suggests species-specific copulatory mechanisms that may reduce interspecific mating (Mutanan 2006). The absence of vesical armature in *E. geminata* and *E. fabia* indicates simpler mating structures, potentially reflecting different reproductive strategies (Eberhard 1985). In females, the larger corpus bursae in *E. geminata* (2.0 ± 0.3 mm) compared to *E. undata* (1.5 ± 0.2 mm) may indicate greater sperm storage capacity, influencing mating frequency and reproductive success (Raha et al. 2017). The presence of a submarginal band in *A. cashmirensis*, absent in *Eupterote* species, combined with genital differences, reinforces their diagnostic utility (Pugaev & Du 2011). ANOVA and PCA results highlight significant morphometric variation, with female wing expanses consistently larger, likely linked to reproductive demands. The limited availability of female *G. plana* specimens highlights a research gap, necessitating further collections to characterize its female genitalia. Integrating molecular data with morphological analyses could further resolve phylogenetic relationships, particularly for *Ganisa*, and clarify subfamily classifications within Eupterotidae.

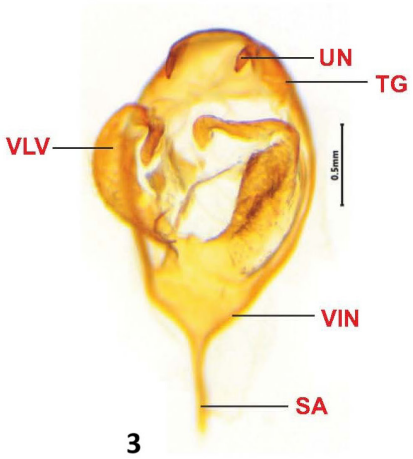
CONCLUSION

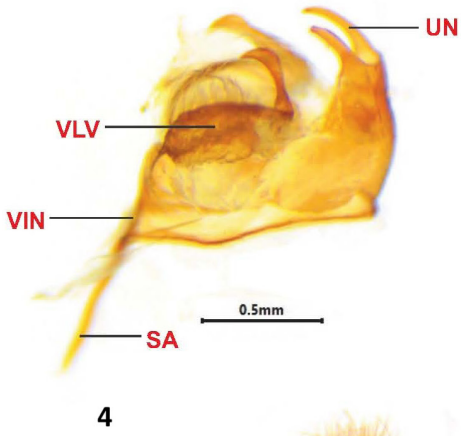
This study provides a comprehensive morphological and statistical analysis of sexual dimorphism in the genitalic structures of Eupterotidae species within *Eupterote*, *Apona*, and *Ganisa*. Morphological differences in male (uncus, valva, aedeagus) and female (corpus bursae, apophyses) genitalia, supported by ANOVA and PCA, highlight their taxonomic and evolutionary significance. The absence of a gnathos in

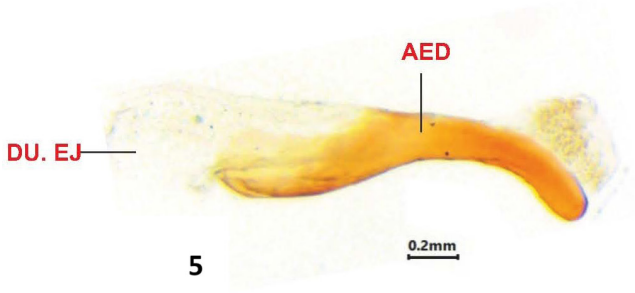












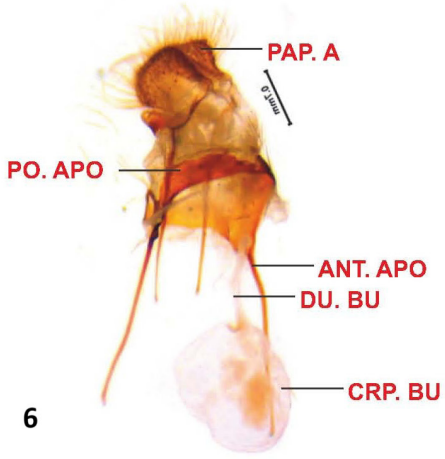
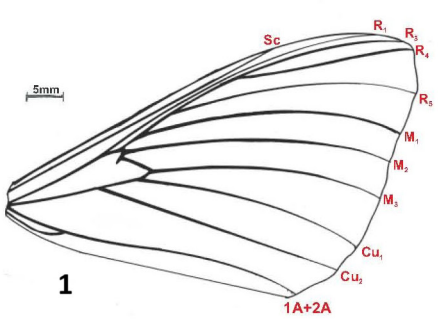
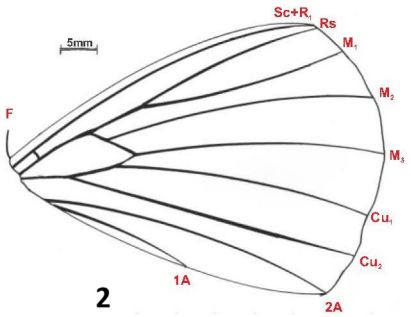
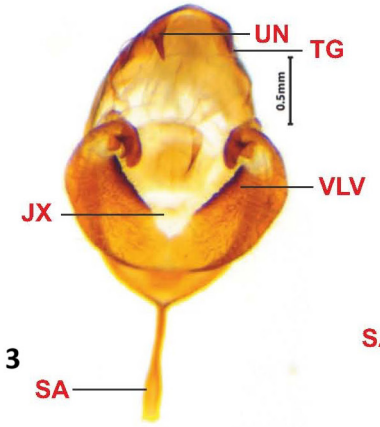


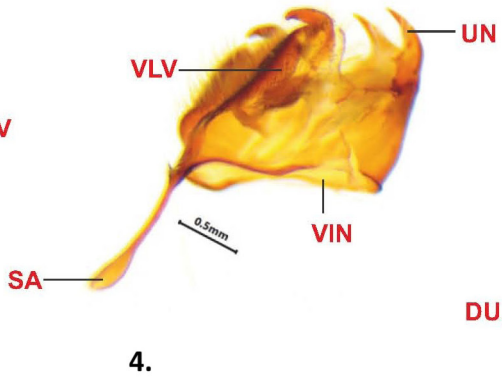
Image 1. ***Eupterote geminata*** **Walker: 1—Forewing | 2—Hindwing | 3—Male genitalia-ventral view | 4—Male genitalia-lateral view | 5—Aedeagus | 6—Female genitalia.**

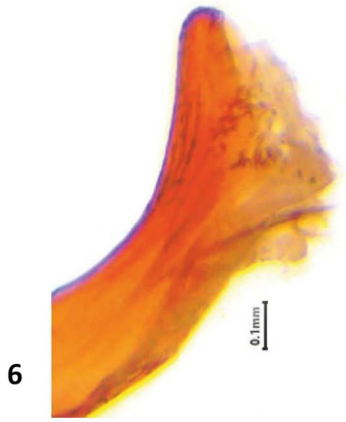
Eupterote undata Blanchard











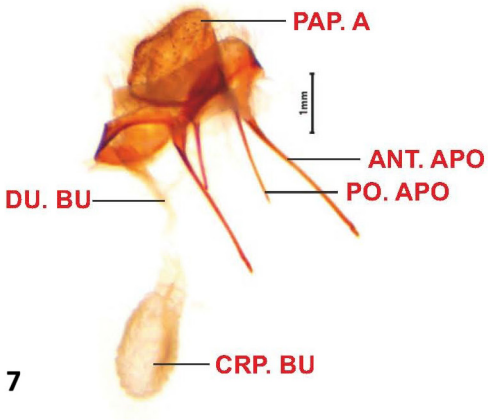
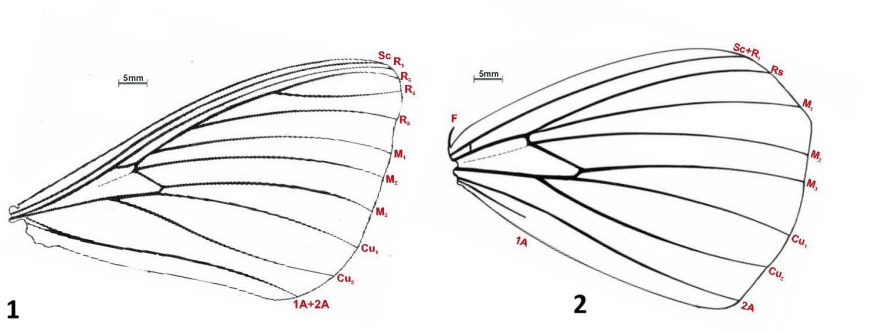
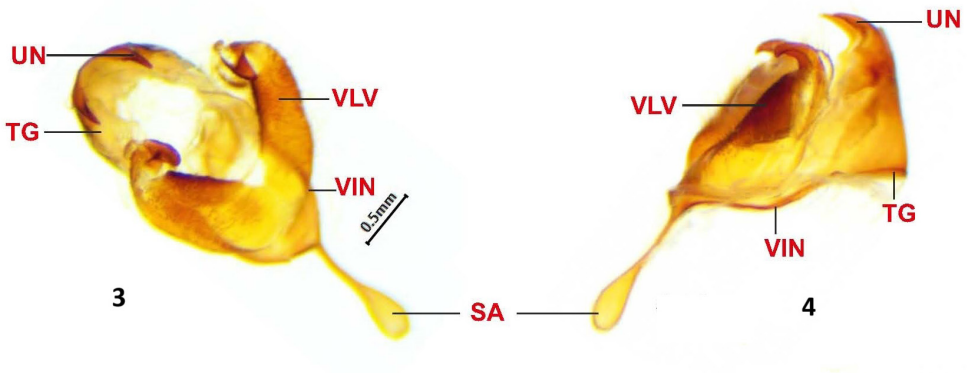


Image 2. ***Eupterote undata*** **Blanchard: 1—Forewing | 2—Hindwing | 3—Male genitalia-ventral view | 4—Male genitalia-lateral view | 5—Aedeagus-distal end | 6—Female genitalia.**





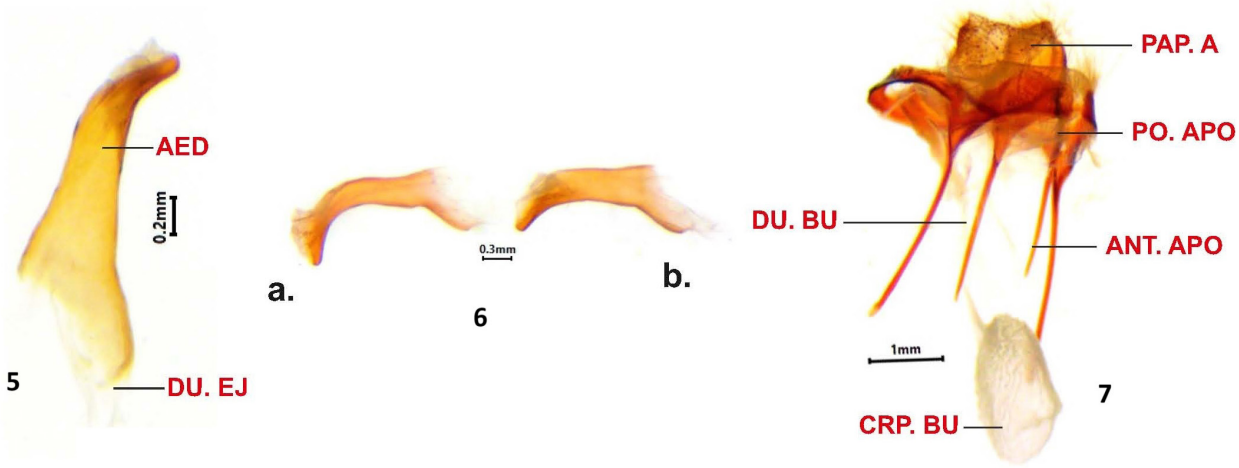
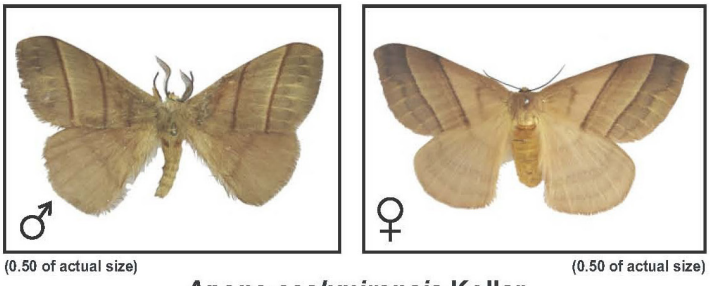
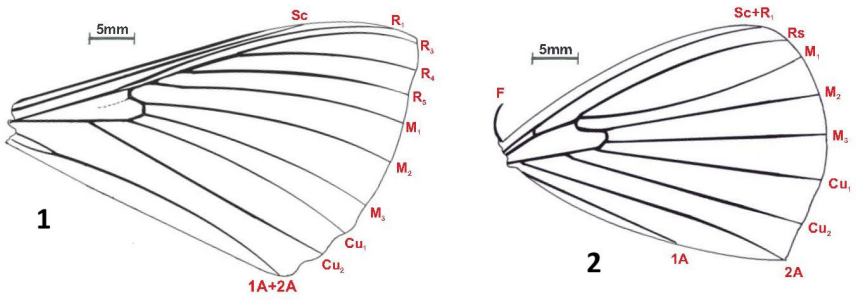
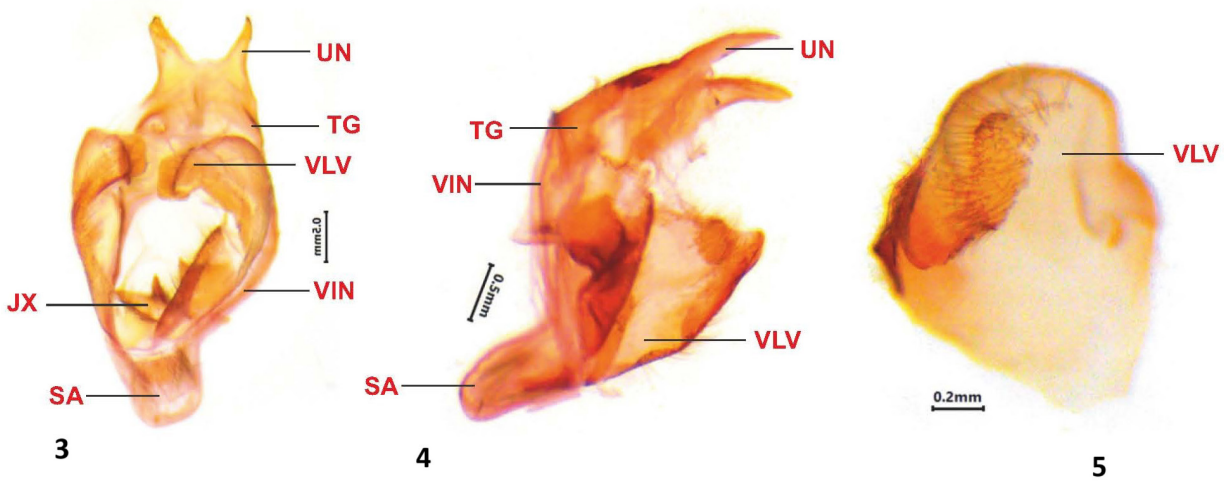


Image 3. *Eupterote fabia* Cramer: 1—Forewing | 2—Hindwing | 3—Male genitalia-ventral view | 4—Male genitalia-lateral view | 5—Aedeagus | 6—Adeagus of (a) *E. undata* and (b) *E. fabia* | 7—Female genitalia.







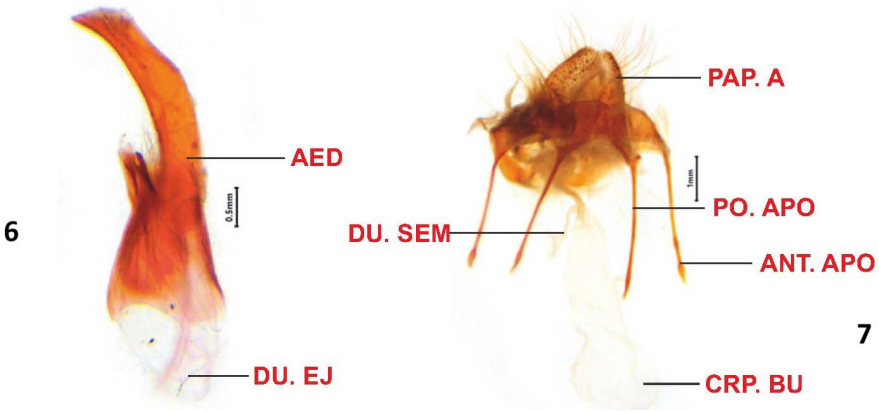
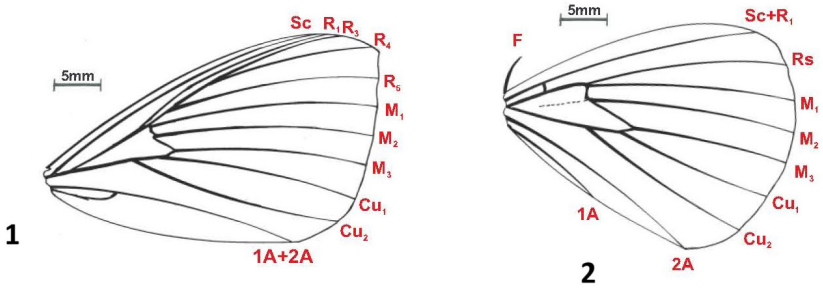
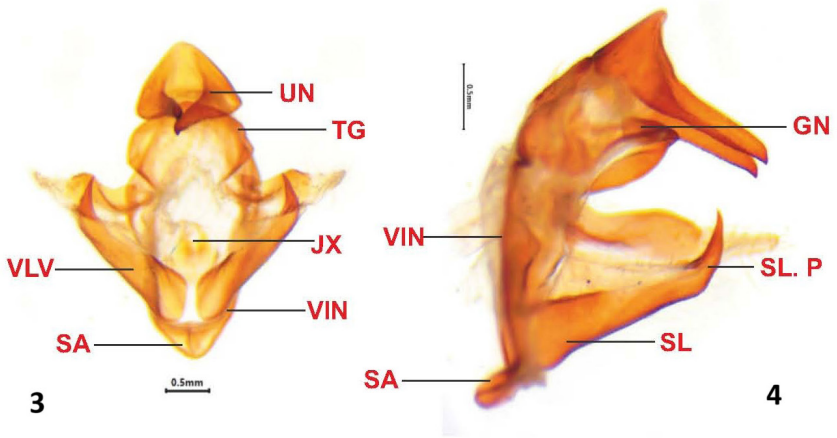


Image 4. ***Apona cashmirensis*** **Kollar: 1—Forewing | 2—Hindwing | 3—Male genitalia-ventral view | 4—Male genitalia-lateral view | 5—right valva | 6—Aedeagus | 7—Female genitalia.**

Ganisa plana Walker





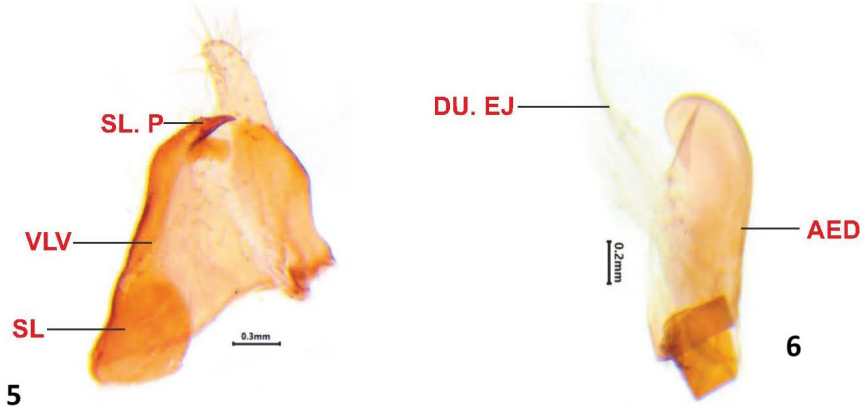


Image 5. *Ganisa plana* Walker: 1—Forewing | 2—Hindwing | 3—Male genitalia-ventral view | 4—Male genitalia-lateral view | 5—Right valva | 6—Aedeagus.

Eupterote, its presence in *Apona* and *Ganisa*, and the unique non-fused uncus in *Ganisa* underscore genitalic diversity. These findings enhance the understanding of reproductive isolation and phylogenetic relationships in Eupterotidae, with implications for their ecological roles as polyphagous pests. Future studies should combine morphological and molecular approaches to refine subfamily classifications and explore evolutionary drivers of genital dimorphism.

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