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Morphology of appendix bursae as an auxiliary criterion in the determination of females of genus *Eupithecia* Curtis, 1825 (Lepidoptera, Geometridae)

Vladimír Kubín & Josef Procházka

Abstract

The authors examined 79 species of females of the genus *Eupithecia* Curtis, 1825 and divided the material studied into 10 groups according to the shape of appendix bursae. This classification can be used for determination purposes when examining the genus and/or group specificity. Predominantly, the study has been carried out with the scanning electron microscope. In this case, the use of a stereoscopical light microscope with appropriate lighting is quite sufficient.

Zusammenfassung

Unsere Untersuchung umfasst 79 Arten der Weibchen der Gattung *Eupithecia* Curtis, 1825. Nach der Form und Größe des Appendix bursae wird dieses Material in 10 Gruppen geteilt. Diese Einteilung kann für die Determination der Weibchen der Gattung *Eupithecia* Verwendung finden. Die Form und die Größe des Appendix bursae ist für einzelne Arten beziehungsweise Artengruppen ganz spezifisch. Die Studie wurde überwiegend mit dem Rasterelektronenmikroskop durchgeführt, wobei für Determinationszwecke die Verwendung des Lichtstereoskopischen Mikroskopes auch geeignet war.

Introduction

The morphological diversity of the genitalia of various genera and species does not enable to universalize successfully the attributes of a larger group of genera of Lepidopters generally (Williams 1943, Dougdale 1974). In our study, therefore, we have paid attention only to the situation of females of the genus *Eupithecia*. When determining the females of this genus, the morphology of bursa copulatrix with its internal structures serves as the basic criterion (Mironov 2003, Petersen 1910, Bolte 1990, Weigt 1987, 1988, 1990, 1991, 1993). With genus *Eupithecia*, a protuberance of appendix bursae leaving bursa copulatrix at various locations of its surface (Bolte 1990, Mc Dunnough 1949, Scoble 1995, Hausmann 2001, Kristensen 2003) forms a typical part of its anatomy. Exceptionally, the appendix bursae is connected with ductus bursae (group 10). Ductus seminalis, a hair-thin tube, is attached to the peripheral end of the appendix.

Material and methods

Information on the material used can be found in the list of localities and collectors included in an earlier study (Kubín & Procházka 2012). After the preparation of the female abdomens with a hot solution of NaOH and subsequent intensive washing, the bursae were separated mechanically from the genitalia (Bennet 1929, Kubín & Procházka 2014) and fixed with 70% ethanol. After dehydration with an ascending line of aceton (90%, 96%, 100%) they were dried out in the POLARON apparatus in the CO_2 atmosphere. The coating was accomplished with a gold or palladium electrode (Kubín 2003). For photographs, the FOMA 100 film was used in the TESLA BS300 scanning electron microscope. After an analogous preparation, some photos were taken digitally with a HITACHI SU 8010 scanning electron microscope. In the optical stereoscopical microscope, the appendices bursae were very clearly visible when a matte black of the microscope table and oblique lighting in an almost horizontal level with the surface of the table were used.

Results

Our study includes 79 females of genus *Eupithecia* species. The length, shape and thickness of appendices bursae were compared. According to its morfology, the females were sorted into ten groups. Quite comfortably, the appendices were discernible not only in the scanning electron microscope, but – after a necessary adjustment of lighting – also in the light stereoscopical microscope. The linking of appendix bursae into ductus seminalis was characteristic generically – either fully continuous, or ductus seminalis was variously immersed into the body of the appendix (Figs 2-5). The form of the linking did not influence the group classification in any way. In some genus, the site of the link was determined in a larger magnification (1000-2000). In the captions of the report proper, to simplify describing individual photographs we do not state specifically that this particular detail is dealt with.

Further identification of the species in individual groups must be performed conventionally by means of a morphological analysis of bursa copulatrix. The method was verified on 92 specimens of 18 species of the genus *Eupithecia*. Intentionally, the check – up was performed with a light stereoscopical microscope (see Methods). In all cases, the species

in question could be classified in one of the ten described groups, defined according to the morphology of bursa copulatrix.

Discussion

The anatomical structure of the genitalia of females of the genus *Eupithecia* used for determination is simpler than with the males, where there exist a number of main and affiliated signs, the combination of which enables a reliable determination of the species. In females, such signs are substantially less numerous (Mironov 2003). Most frequently, the determination is limited to the shape of the bursa and layout or size of spins and the shape of apophysae or ostium bursae, respectively. Our study draws attention to the possibility of using the length, shape and thickness of appendix bursae (Bolte 1990) to the categorization into ten groups containing different numbers of species. The final determination of species involves using conventional techniques. A scanning electron microscope was used to depict the appendices bursae. For most entomologists, however, this technique is hard – to – get – at, and – on top of that – rather lengthy. The similar results may be achieved with a light stereoscopical microscope (see Methods). The morphology of appendices bursae is relatively very constant, representing a reliable secondary sign in determining females. As with all natural objects, a certain degree of variability must be taken into account. The same stands also for appendices bursae, but potential variability seems to be negligible and does not affect the group determination. Classification into groups was not influenced by the variability of linking. The physiological significance of appendix bursae has not been clarified in detail yet, and its examination was beyond our possibilities.

Classification according to the size of appendix bursae

1. Long appendix bursae Figs 5-14

E. cocciferata Milliőre, 1864
E. denticulata (Treitschke, 1828)
E. icterata (Villers, 1789)
E. orphnata W. Petersen, 1909
E. pauxillaria Boisduval, 1840
E. semigraphata Bruand, 1850
E. subfuscata (Haworth, 1809)
E. subumbrata (Denis & Schiffermüller, 1775)
E. succenturiata (Linnaeus, 1758)

2. Medium bent appendix bursae Figs 15-33

E. absinthiata (Clerck, 1759)
E. antalica Mironov, 2001
E. cauchiata (Duponchel, 1831)
E. denotata (Hübner, 1813)
E. dodoneata Guenée, 1857
E. exiguata (Hübner, 1813)
E. expallidata Doubleday, 1856
E. insigniata (Hübner, 1790)
E. intricata (Zetterstedt, 1839)
E. millefoliata Rössler, 1866
E. reisserata Pincer, 1976
E. satyrata (Hübner, 1813)
E. tantillaria Boisduval, 1840
E. valerianata (Hübner, 1813)

3. Medium straight appendix bursae Figs 34-39

E. assimilata Doubleday, 1856
E. thalictrata (Püngeler, 1902)
E. vulgata (Haworth, 1809)

4. Medium spirally coiled appendix bursae Figs 40-47

E. egenaria Herrich-Schäffer, 1848
E. immundata (Lienig & Zeller, 1846)
E. pimpinellata (Hübner, 1813)
E. plumbeolata (Haworth, 1809)
E. quercetica Prout, 1938
E. selinata Herrich-Schäffer, 1861

5. Short thick appendix bursae Figs 48-57

E. gelidata Möschler, 1860
E. innotata (Hufnagel, 1767)
E. nanata (Hübner, 1813)
E. ochridata Schütze & Pinker, 1968
E. trisignaria Herrich-Schäffer, 1848
E. veratraria Herrich-Schäffer, 1850

6. Short bent appendix bursae Figs 58-69

E. abbreviata Stephens, 1831
E. abietaria (Goeze, 1781)
E. breviculata (Donzel, 1837)
E. ericeata (Rambur, 1833)
E. graphata (Treitschke, 1828)
E. *indigata* (Hübner, 1813)
E. irriguata (Hübner, 1813)
E. oxycedrata (Rambur, 1833)
E. pusillata (Denis & Schiffermüller, 1775)
E. simpliciata (Haworth, 1809)

7. Short straight appendix bursae Figs 70-82

E. actaeata Walderdorff, 1869
E. gemellata Herrich-Schäffer, 1861
E. haworthiata Doubleday, 1856
E. scalptata Christoph, 1885
E. schiefereri Bohatsch, 1893
E. unedonata (Mabille, 1868)
E. venosata (Fabricius, 1787)

8. Short tumid appendix bursae Figs 83-93

E. centaureata (Denis & Schiffermüller, 1775)
E. distinctaria Herrich-Schäffer, 1848
E. extraversaria Herrich-Schäffer, 1852
E. impurata (Hübner, 1813)
E. silenicolata Mabille, 1867
E. spissilineata (Metzner, 1846)

9. Filamentary – very small appendix bursae Figs 94-117

E. alliaria Staudinger, 1870
E. analoga Djakonov, 1926
E. dissertata (Püngeler, 1905)
E. gueneata Milliőre, 1862
E. inturbata (Hübner, 1817)
E. lanceata (Hübner, 1825)
E. laquaearia Herrich-Schäffer, 1848
E. linariata (Denis & Schiffermüller, 1775)
E. phoeniceata (Rambur, 1834)
E. pulchellata Stephens, 1831
E. pyreneata Mabille, 1871
E. scopariata (Rambur, 1833)
E. silenata Assmann, 1848
E. sinuosaria (Eversmann, 1848)
E. virgaureata Doubleday, 1861

10. Outlet from ducrus bursae Figs 118-124

E. lariciata (Freyer, 1841)
E. *tenuiata* (Hübner, 1813)
E. tripunctaria Herrich-Schäffer, 1852

List of the species in aphabetical classification

Species	Group	Figs
E. abbreviata Stephens, 1831	6	58
E. abietaria (Goeze, 1781)	6	59 – 60
E. absinthiata (Clerck, 1759)	2	15
E. actaeata Walderdorff, 1869	7	70 – 71
E. alliaria Staudinger, 1870	9	94
E. analoga Djakonov, 1926	9	95 – 96
E. antalica Mironov, 2001	2	16
E. assimilata Doubleday, 1856	3	34 – 35
E. breviculata (Donzel, 1837)	6	61
E. cauchiata (Duponchel, 1831)	2	17 – 18
E. centaureata (Denis & Schiffermüller, 1775)	8	83 – 84
E. cocciferata Milliőre, 1864	1	6
E. denotata (Hübner, 1813)	2	19
E. denticulata (Treitschke, 1828)	1	7
E. dissertata (Püngeler, 1905)	9	97
E. distinctaria Herrich-Schäffer, 1848	8	85 – 86
E. dodoneata Guenée, 1857	2	20 – 21
E. egenaria Herrich-Schäffer, 1848	4	42
E. ericeata (Rambur, 1833)	6	62
E. exiguata (Hübner, 1813)	2	22
E. expallidata Doubleday, 1856	2	23
E. extraversaria Herrich-Schäffer, 1852	8	87 – 88
E. gelidata Möschler, 1860	5	48
E. gemellata Herrich-Schäffer, 1861	7	72 – 73
E. graphata (Treitschke, 1828)	6	63 – 64
E. gueneata Milliőre, 1862	9	98 – 99
E. haworthiata Doubleday, 1856	7	74 – 75
E. icterata (Villers, 1789)	1	8
E. immundata (Lienig & Zeller, 1846)	4	40

Species	Group	Figs
E. impurata (Hübner, 1813)	8	89 – 90
E. indigata (Hübner, 1813)	6	65
E. innotata (Hufnagel, 1767)	5	50 – 51
E. insigniata (Hübner, 1790)	2	24 – 25
E. intricata (Zetterstedt, 1839)	2	26
E. inturbata (Hübner, 1817)	9	100 – 101
E. irriguata (Hübner, 1813)	6	66
E. lanceata (Hübner, 1825)	9	102
E. laquaearia Herrich-Schäffer, 1848	9	103 – 104
E. lariciata (Freyer, 1841)	10	118 – 119
E. linariata (Denis & Schiffermüller, 1775)	9	105 – 106
E. millefoliata Rössler, 1866	2	27
E. nanata (Hübner, 1813)	5	52 – 53
E. ochridata Schütze & Pinker, 1968	5	54 – 56
E. orphnata W. Petersen, 1909	1	9
E. oxycedrata (Rambur, 1833)	6	67
E. pauxillaria Boisduval, 1840	1	10
E. phoeniceata (Rambur, 1834)	9	107 – 108
E. pimpinellata (Hübner, 1813)	4	41
E. plumbeolata (Haworth, 1809)	4	44 – 45
E. pulchellata Stephens, 1831	9	109
E. pusillata (Denis & Schiffermüller, 1775)	6	68
E. pyreneata Mabille, 1871	9	110 – 111
E. quercetica Prout, 1938	4	43
E. reisserata Pincer, 1976	2	28
E. satyrata (Hübner, 1813)	2	29 – 30
E. scalptata Christoph, 1885	7	76 – 77
E. scopariata (Rambur, 1833)	9	112
E. selinata Herrich-Schäffer, 1861	4	46 – 47

Species	Group	Figs
E. semigraphata Bruand, 1850	1	11
E. schiefereri Bohatsch, 1893	7	78 – 79
E. silenata Assmann, 1848	9	113 – 115
E. silenicolata Mabille, 1867	8	91 – 92
E. simpliciata (Haworth, 1809)	6	69
E. sinuosaria (Eversmann, 1848)	9	116
E. spissilineata (Metzner, 1846)	8	93
E. subfuscata (Haworth, 1809)	1	12
E. subumbrata (Denis & Schiffermüller, 1775)	1	13
E. succenturiata (Linnaeus, 1758)	1	14
E. tantillaria Boisduval, 1840	2	31
E. tenuiata (Hübner, 1813)	10	120 – 121
E. thalictrata (Püngeler, 1902)	3	36 – 37
E. tripunctaria Herrich-Schäffer, 1852	10	122 – 124
E. trisignaria Herrich-Schäffer, 1848	5	49
E. unedonata (Mabille, 1868)	7	80
E. valerianata (Hübner, 1813)	2	32 – 33
E. venosata (Fabricius, 1787)	7	81 – 82
E. veratraria Herrich-Schäffer, 1850	5	57
E. virgaureata Doubleday, 1861	9	117
E. vulgata (Haworth, 1809)	3	38 – 39

Figures

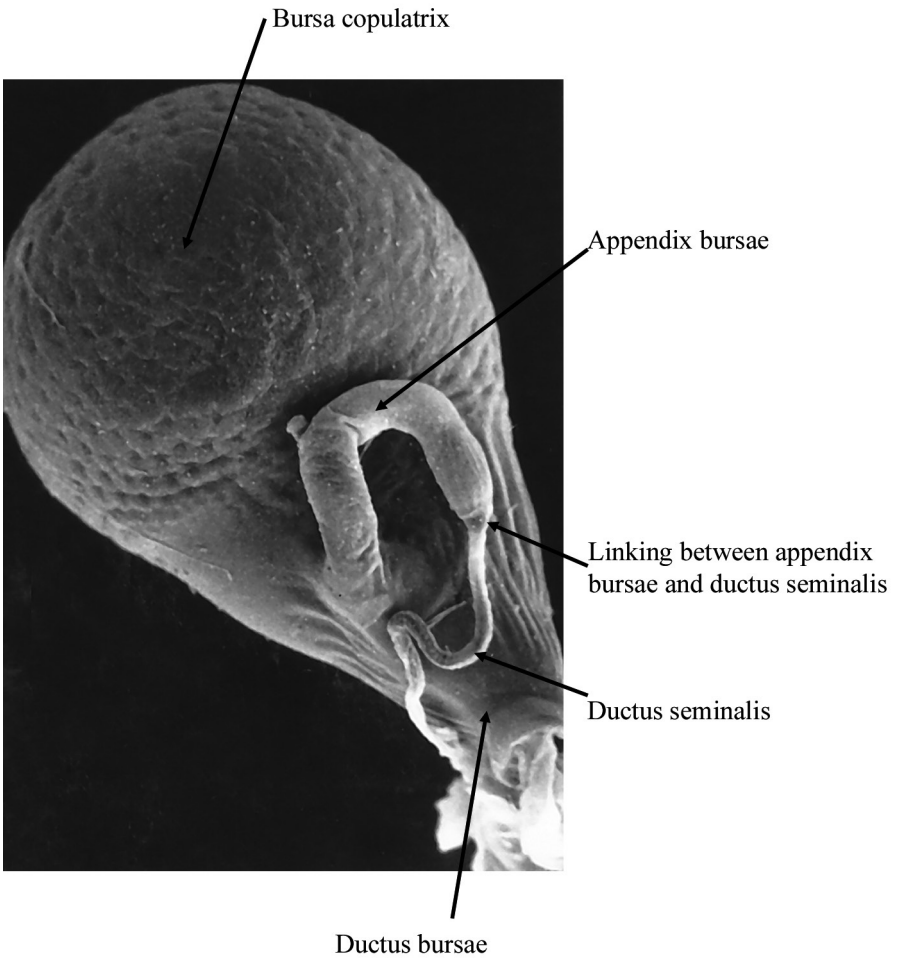
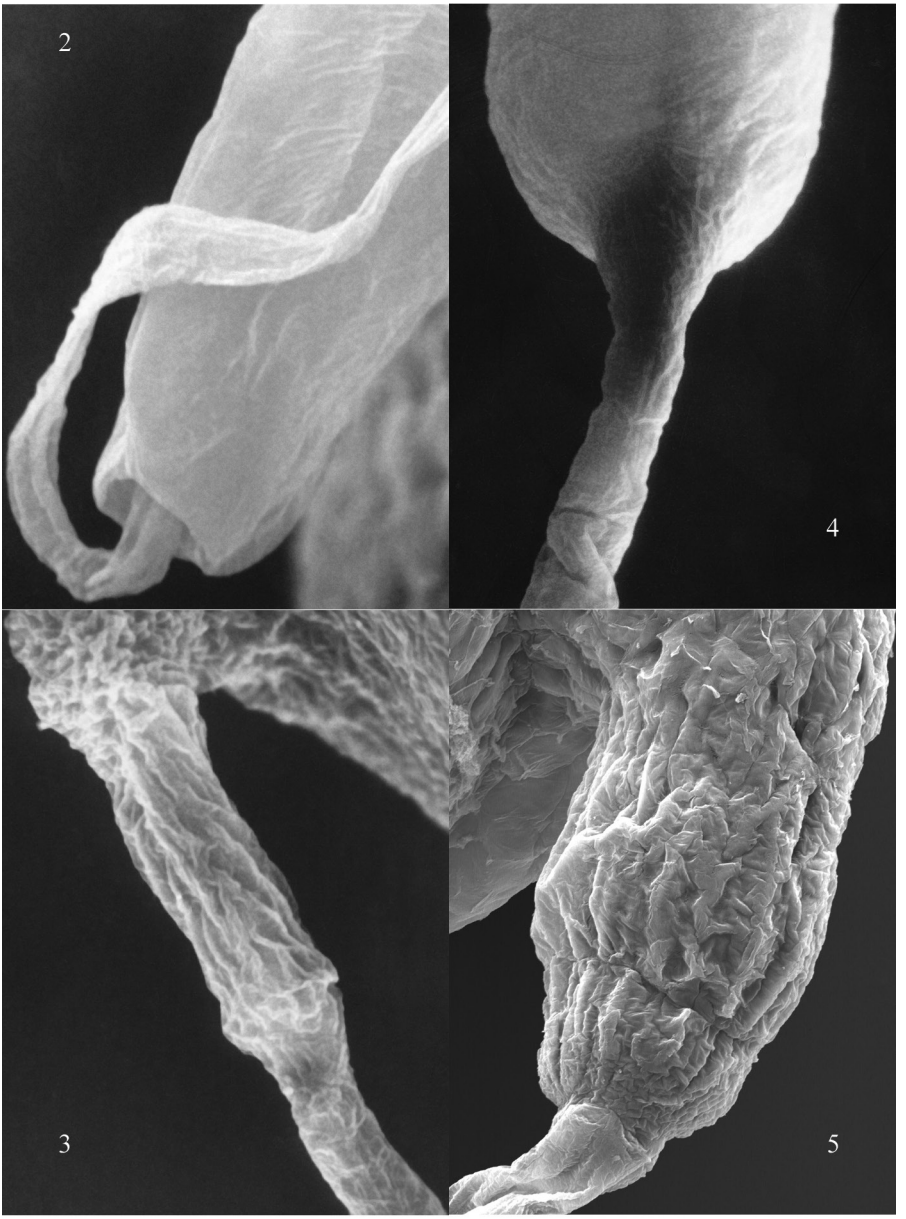
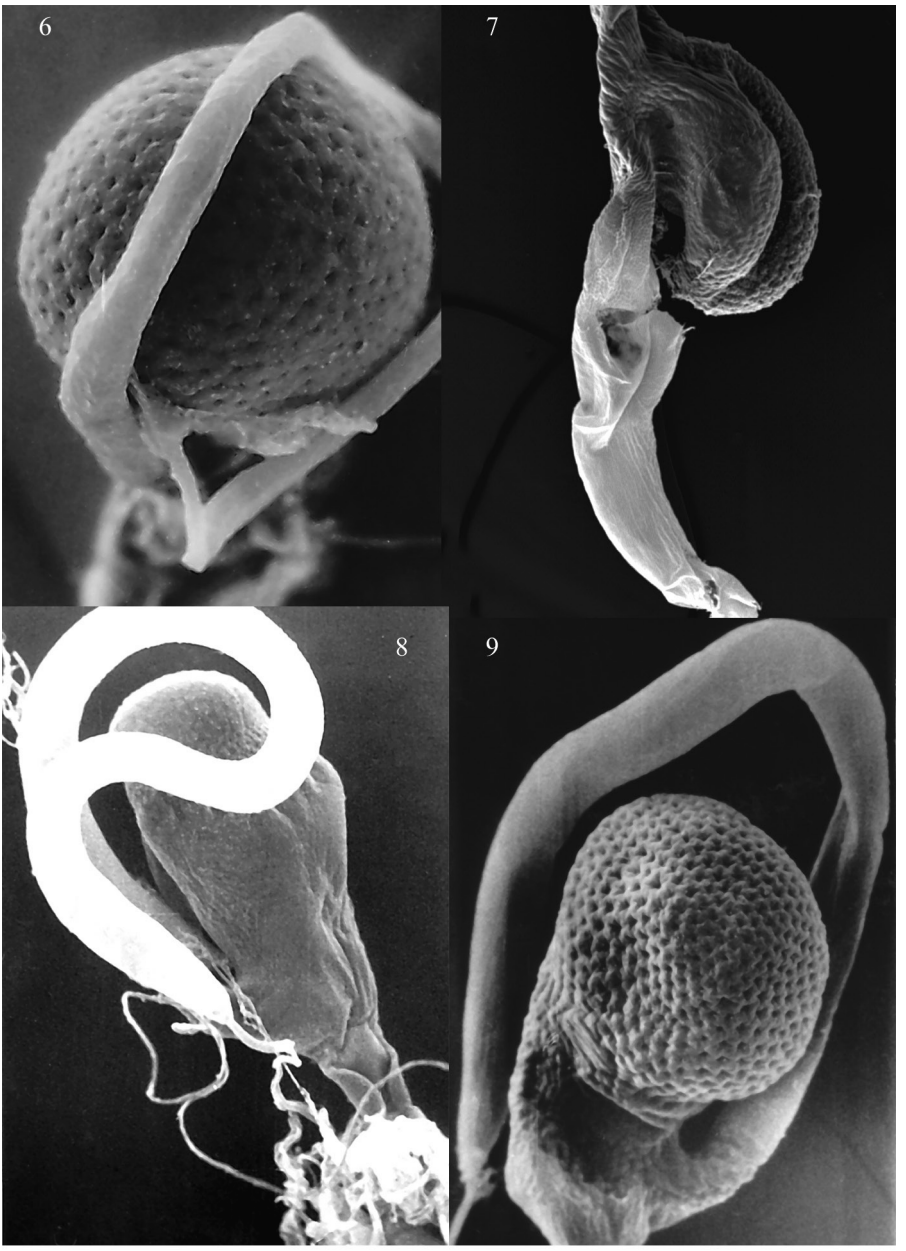


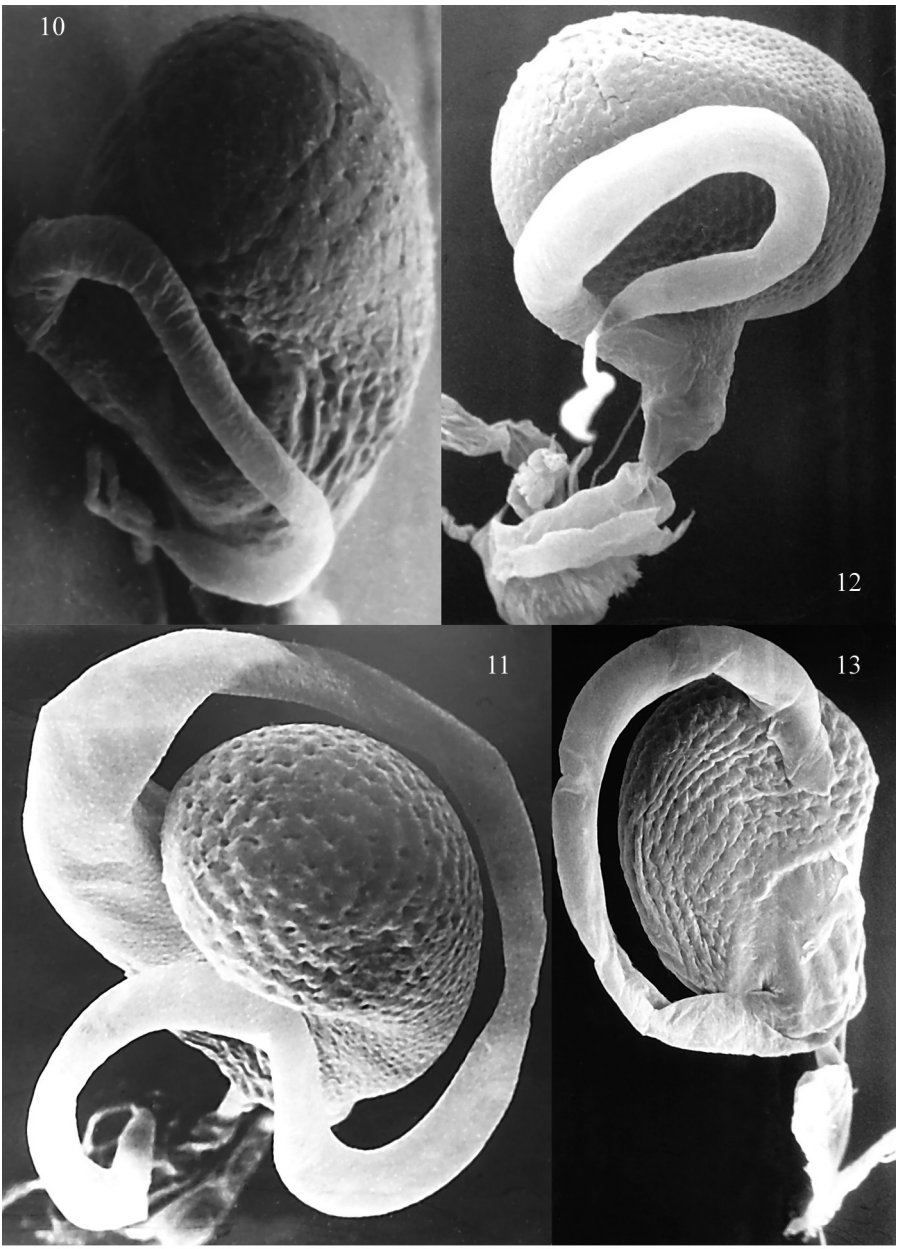
Fig. 1: General view for anatomical orientation.



Figs 2-5: Linking of appendix bursae with ductus seminalis.
2. embedded *E. vulgata*. 4. direct *E. satyrata*
3. gradual *E. silenata.* 5. constricted *E. tripunctaria.*

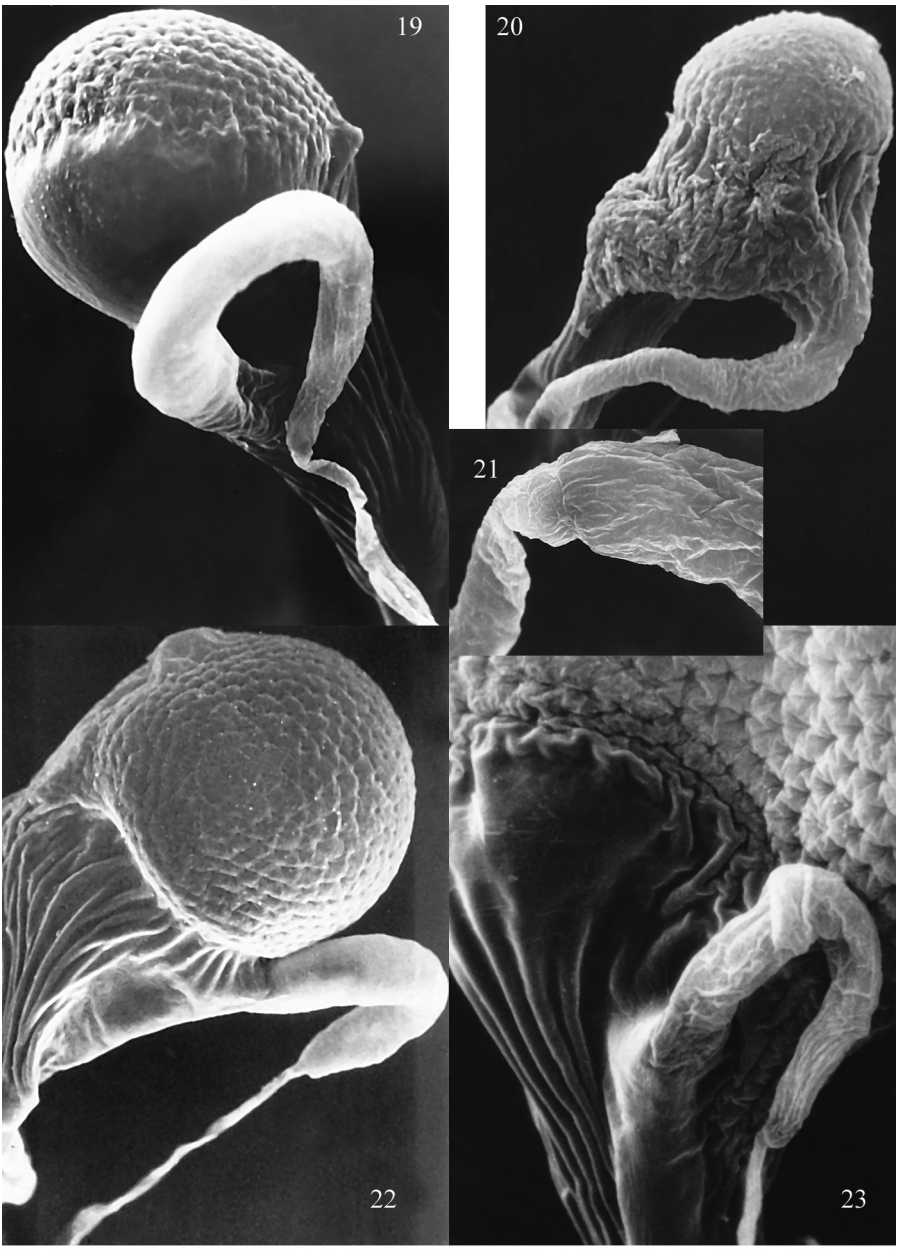


Figs 6-9: **Group 1.** 6. *E. cocciferata*. 7. *E. denticulata.* 8. *E. icterata.* 9. *E. orphnata.*

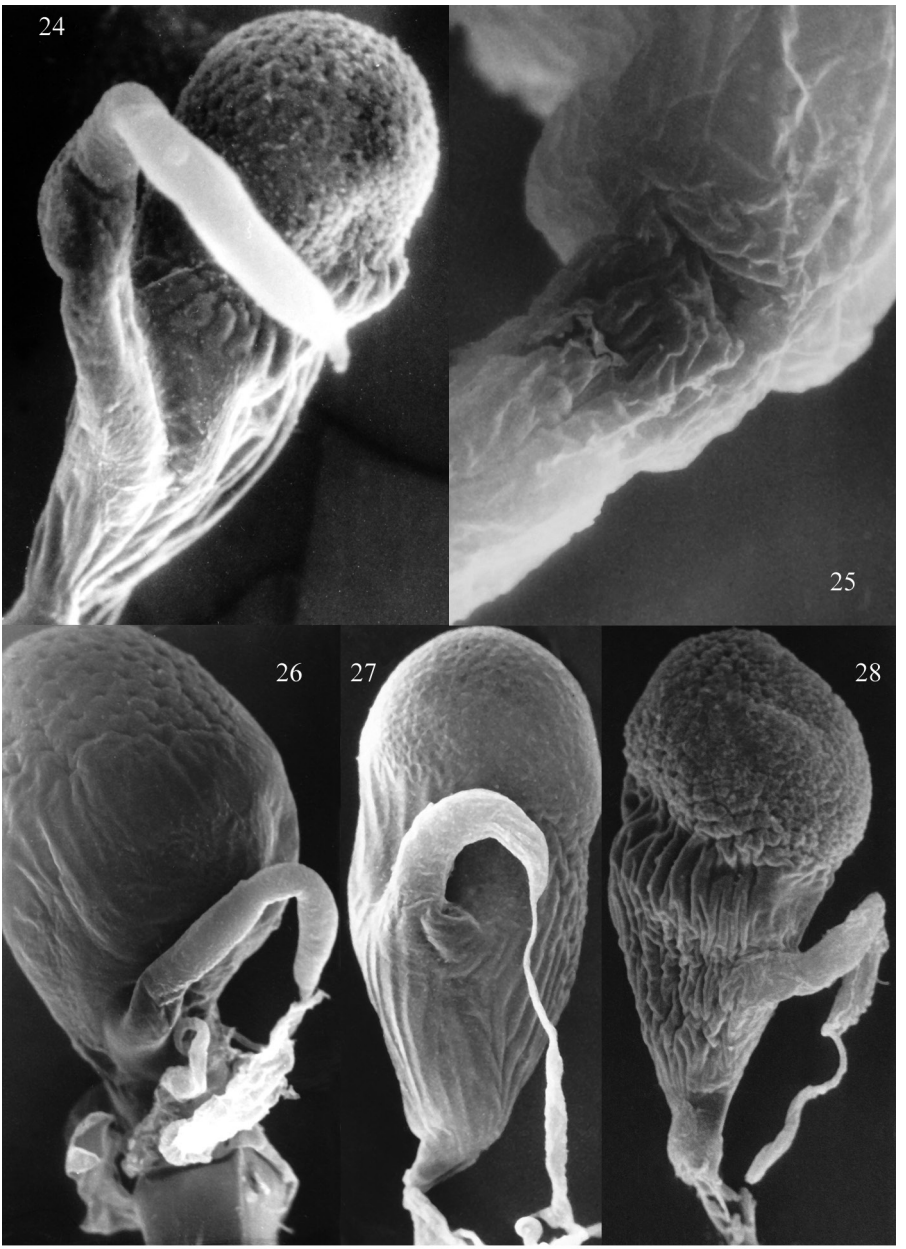


Figs 10-13: **Group 1.** 10. *E. pauxillaria*. 11. *E. semigraphata.* 12. *E. subfuscata.* 13. *E. subumbrata.*

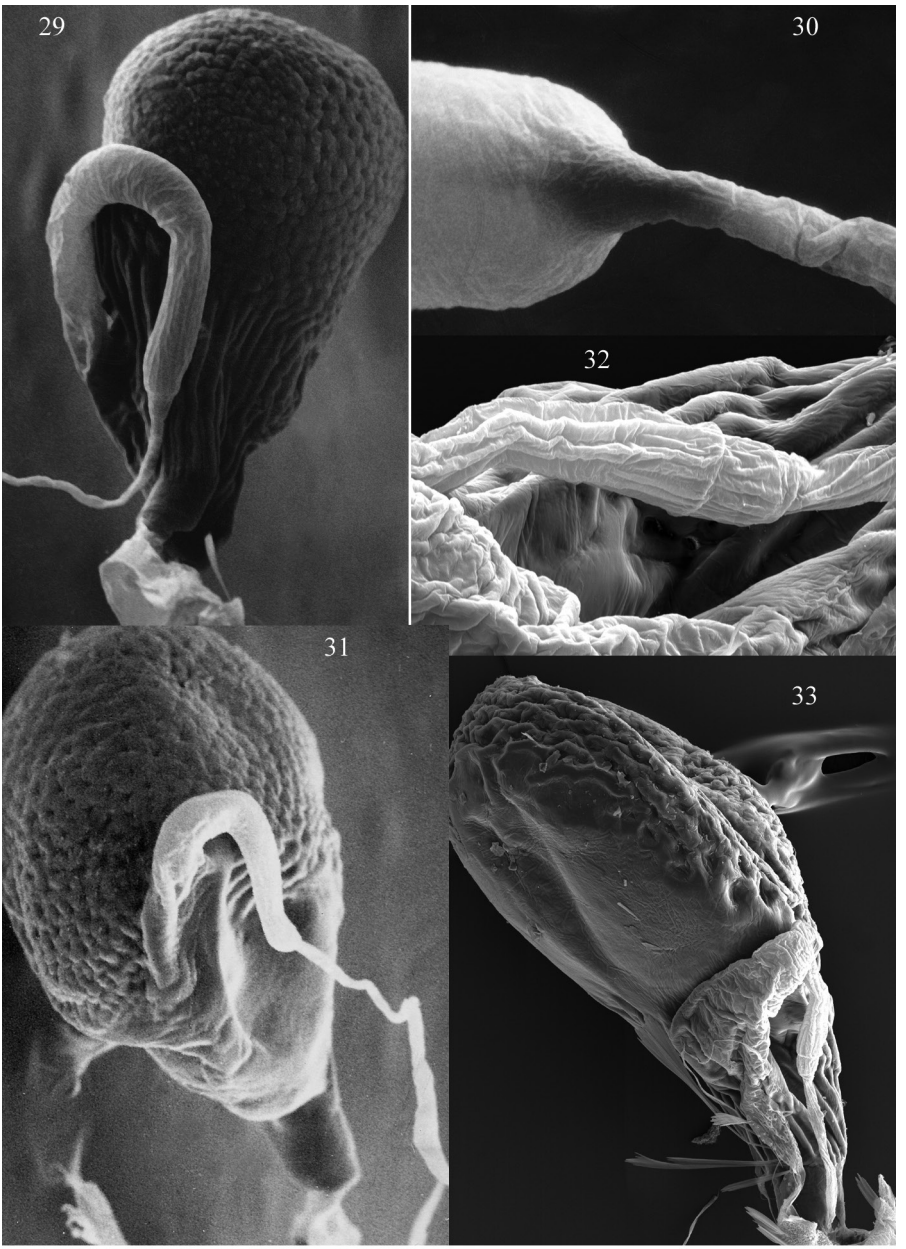
Figs 14 - 18: **Group 1.** 14. *E. succenturiata*.
Group 2. 15. *E. absinthiata.* 16. *E. antalica.* 17 - 18. *E. cauchiata.*



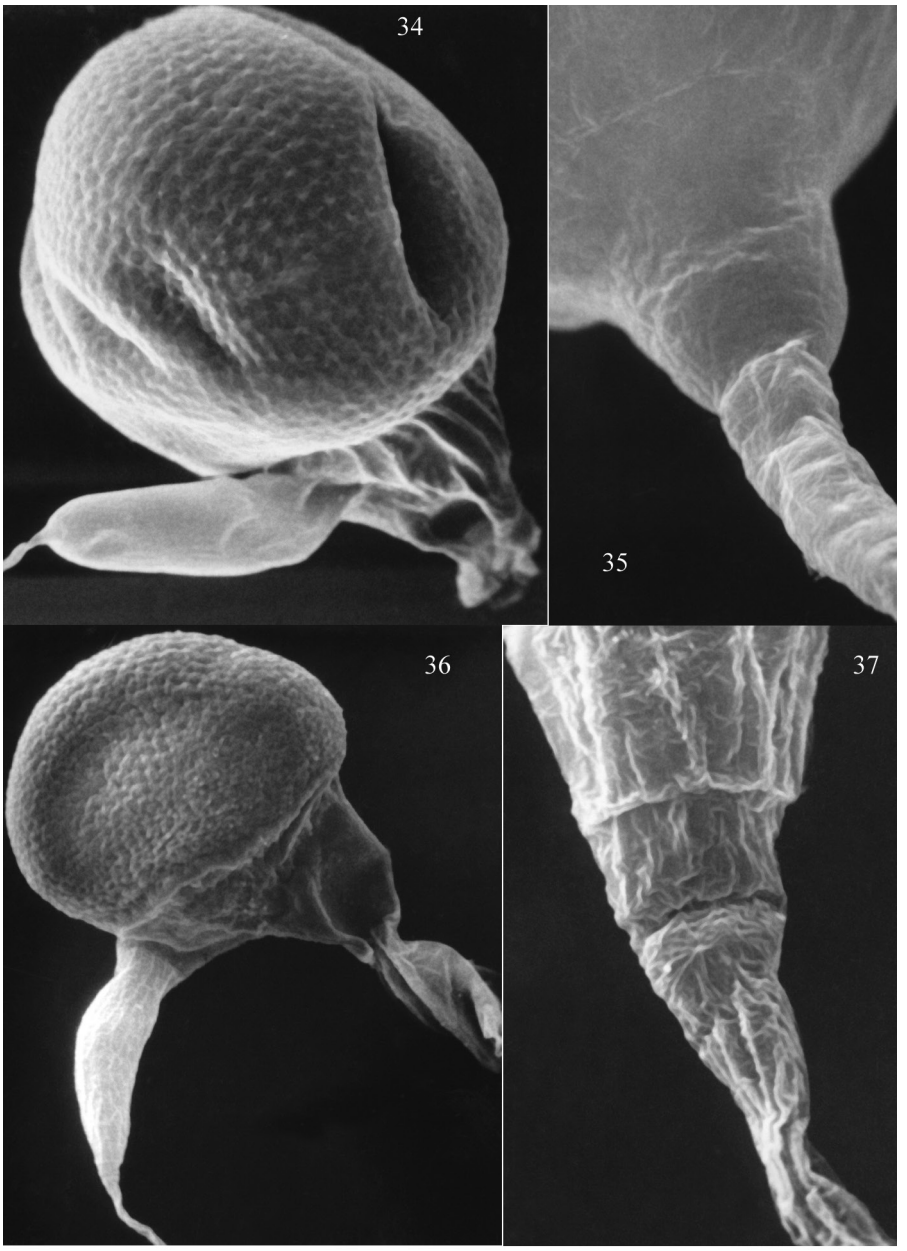
Figs 19-23: **Group 2.** 19. *E. denotata*. 20 - 21. *E. dodoneata.* 22. *E. exiguata. 23. E. expallidata.*



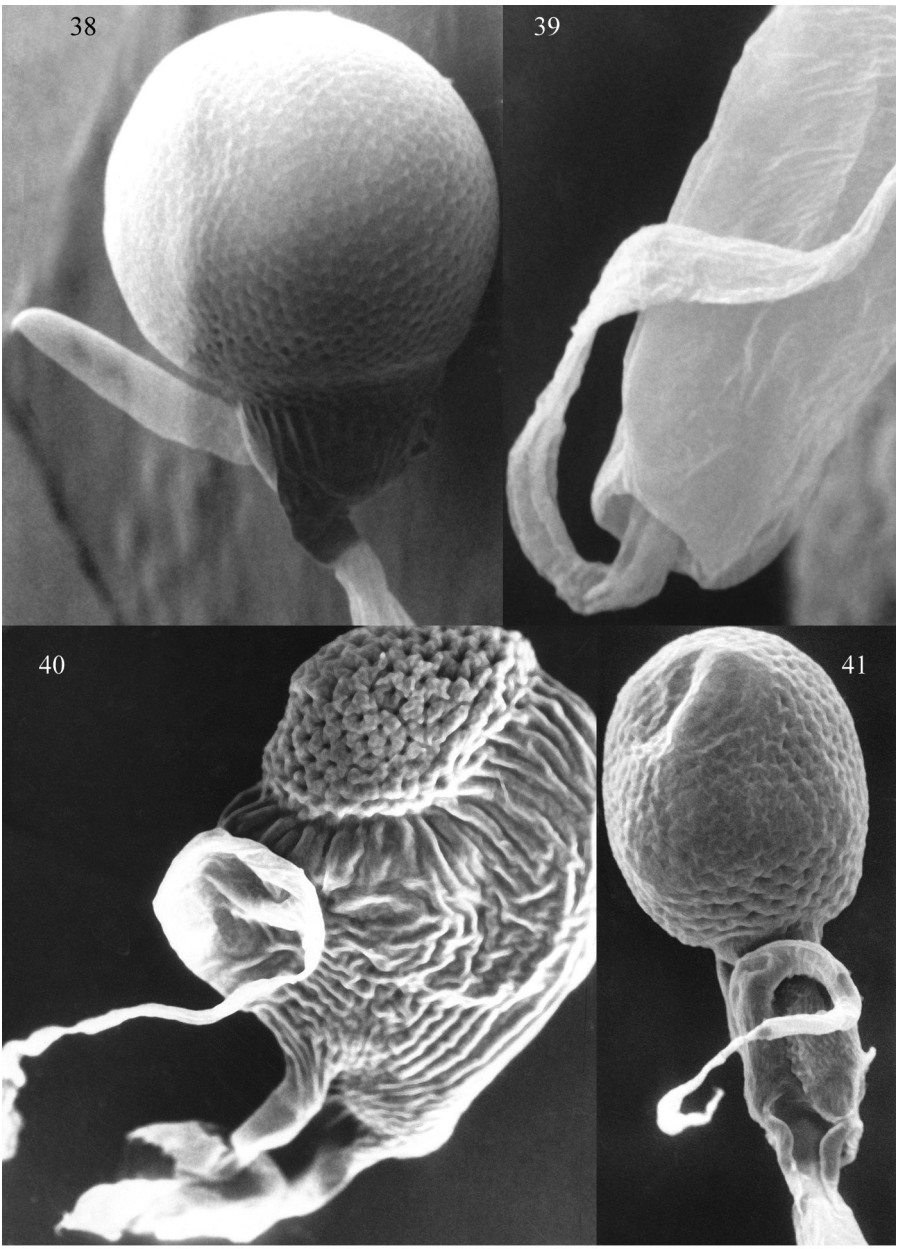
Figs 24-28: **Group 2.** 24 - 25. *E. insigniata*. 26. *E. intricata*. 27. *E. millefoliata*. 28. *E. reisserata*.



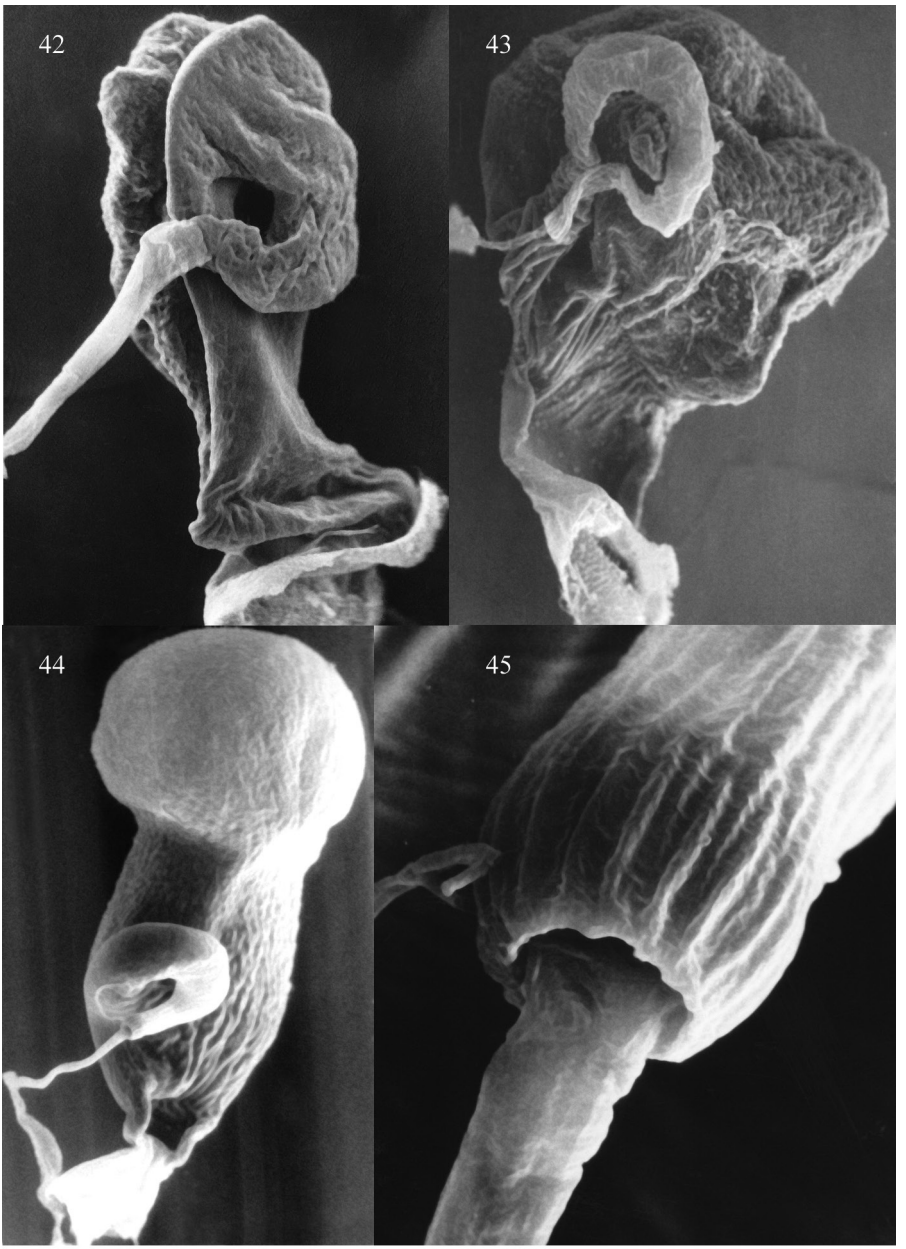
Figs 29-33: **Group 2.** 29 - 30. *E. satyrata*. 31. *E. tantillaria.*
32 - 33. *E. valerianata.*



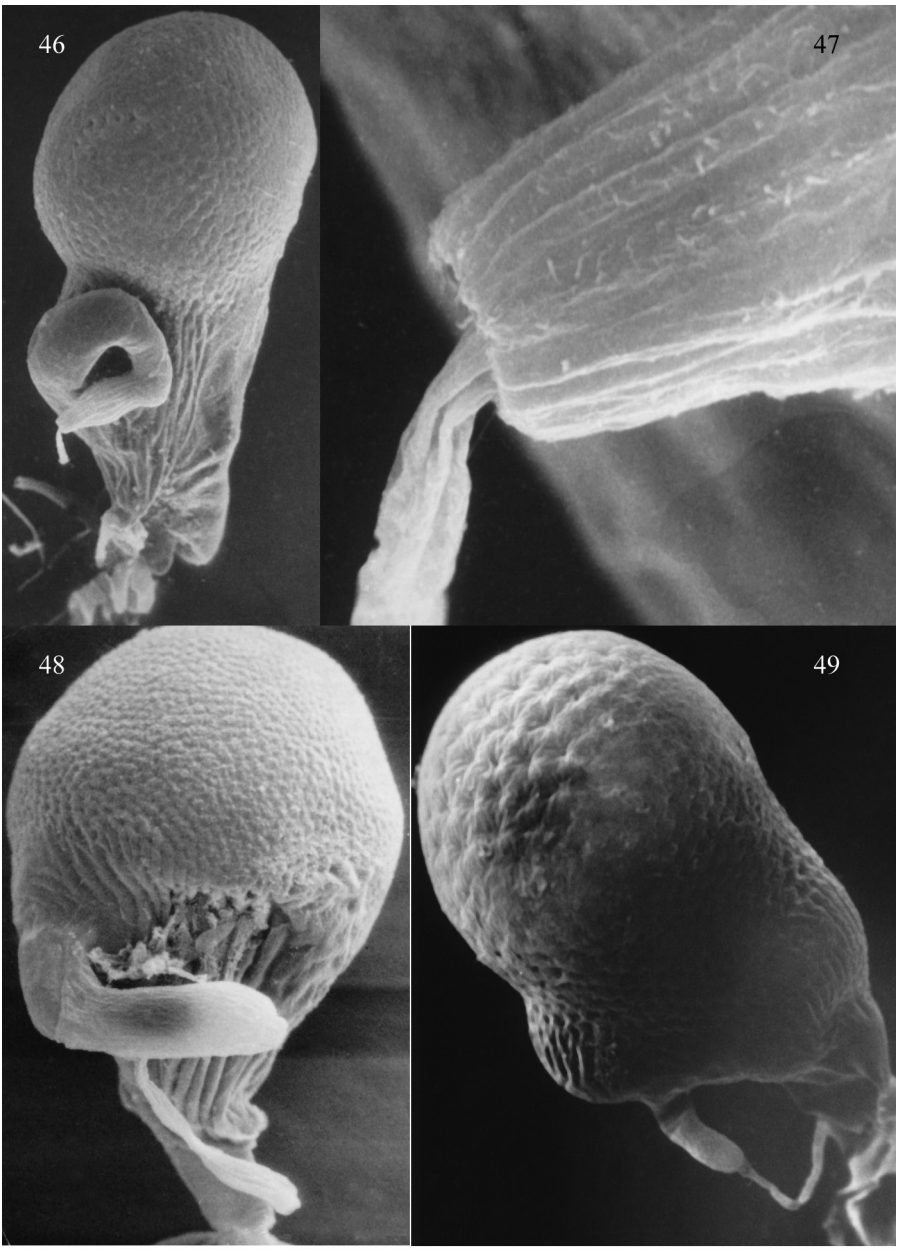
Figs 34 - 77: **Group 3.** 34 - 35. *E. assimilata*.
36 - 37. *E. thalicrata.*



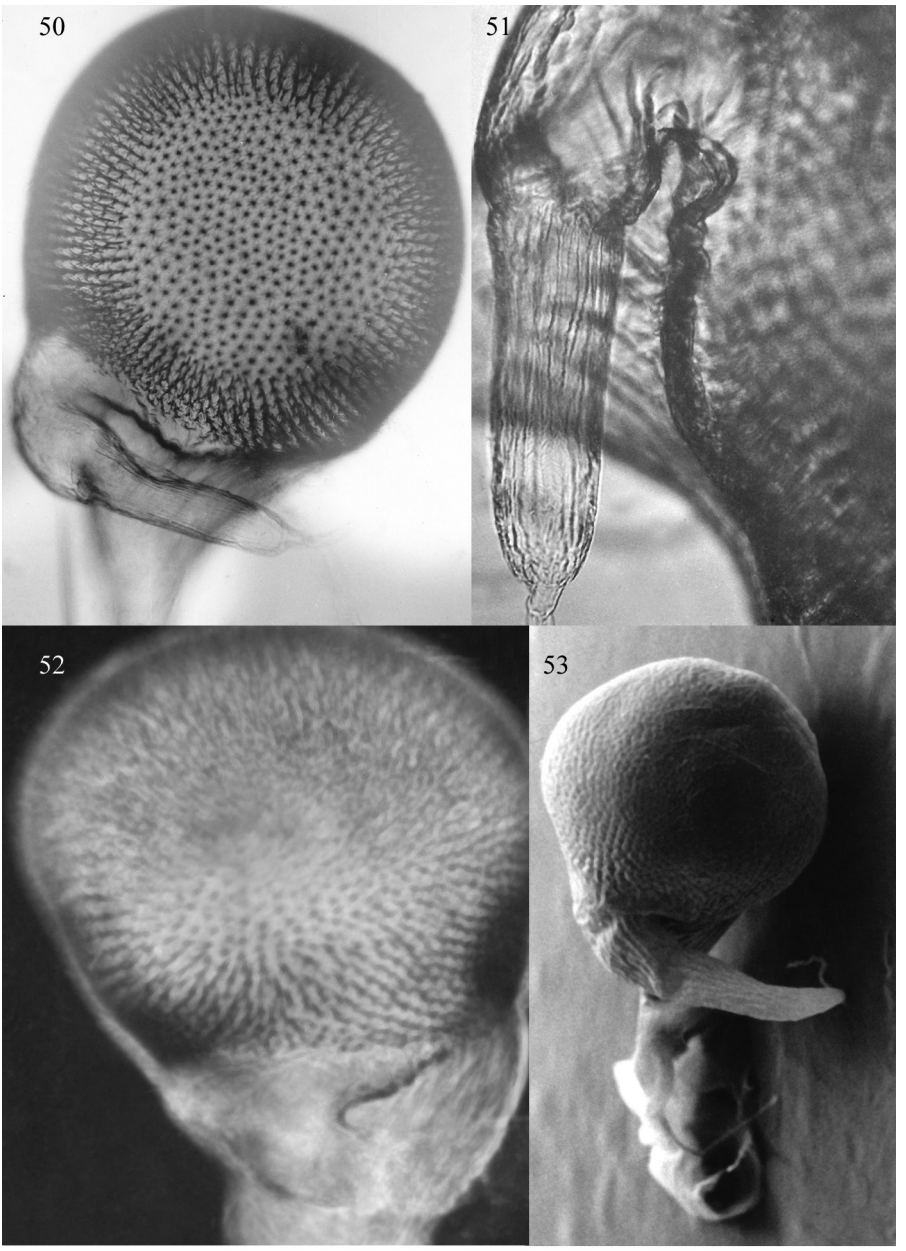
Figs 38 - 41: **Group 3.** 38 - 39. *E. vulgata.*
Group 4. 40. *E. immundata.* 41. *E. pimpinellata.*



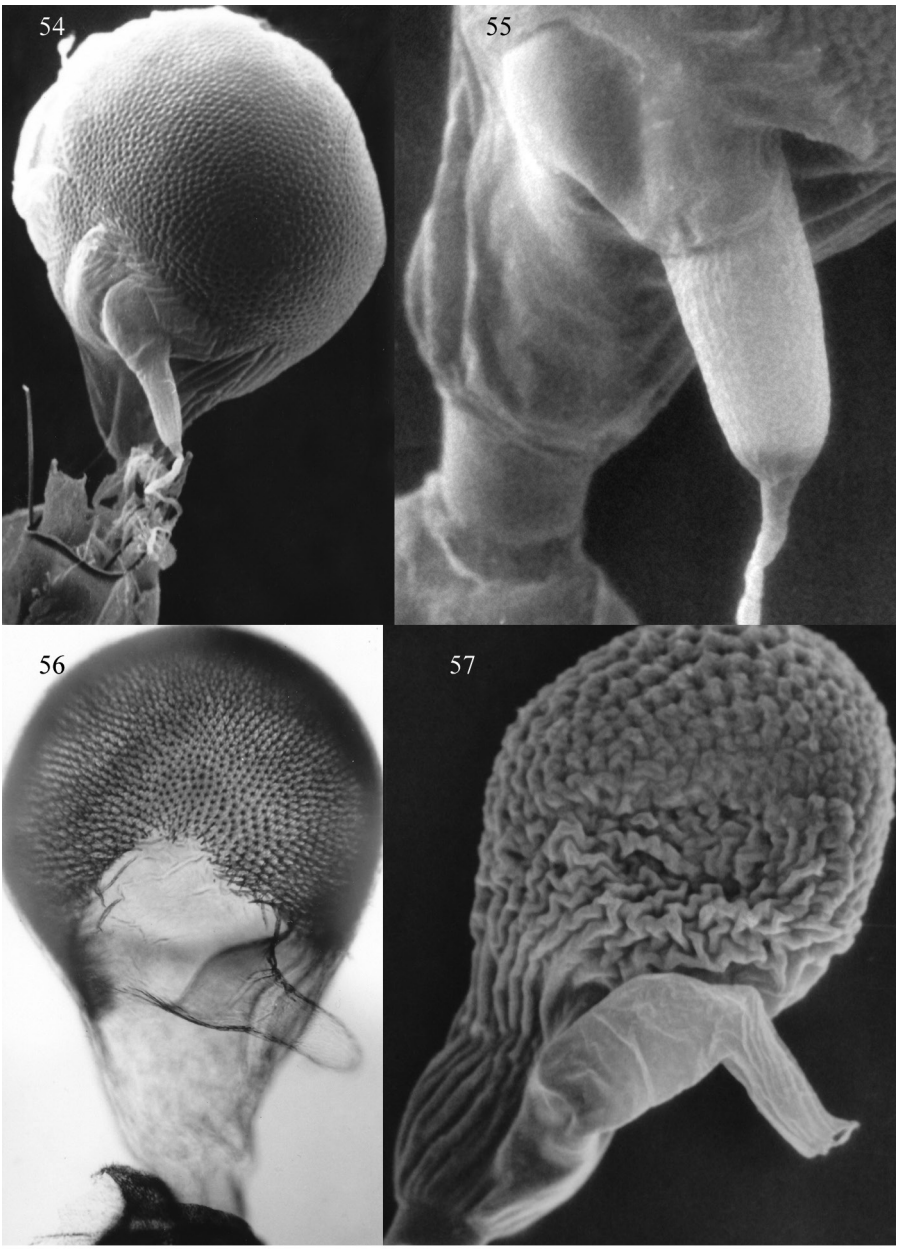
Figs 42 - 45: **Group 4.** 42. *E. egenaria*. 43. *E. quercetica.*
44 - 45. *E. plumbeolata.*



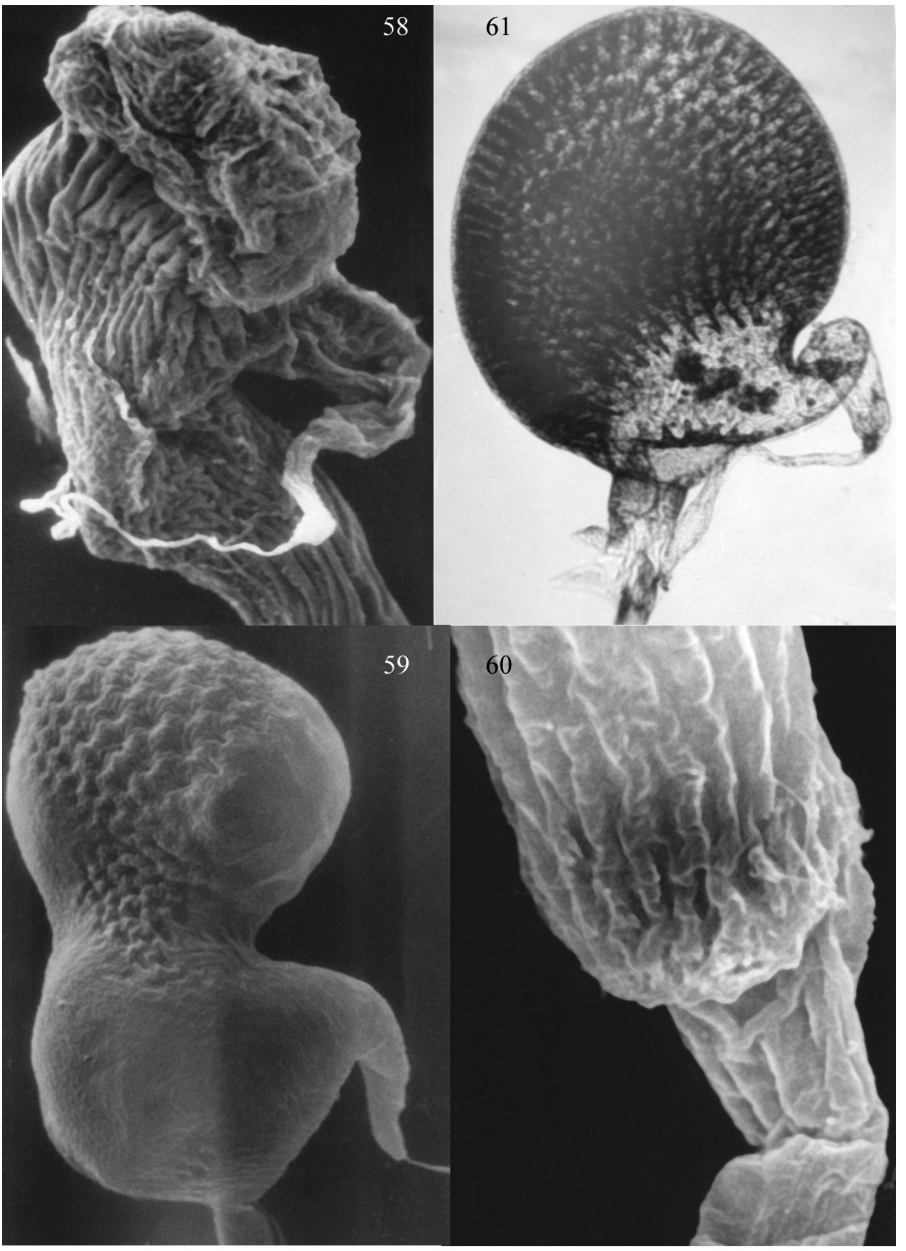
Figs 46-49: **Group 4.** 46 - 47. *E. selinata*.
Group 5. 48. *E. gelidata.* 49. *E. trisignaria.*



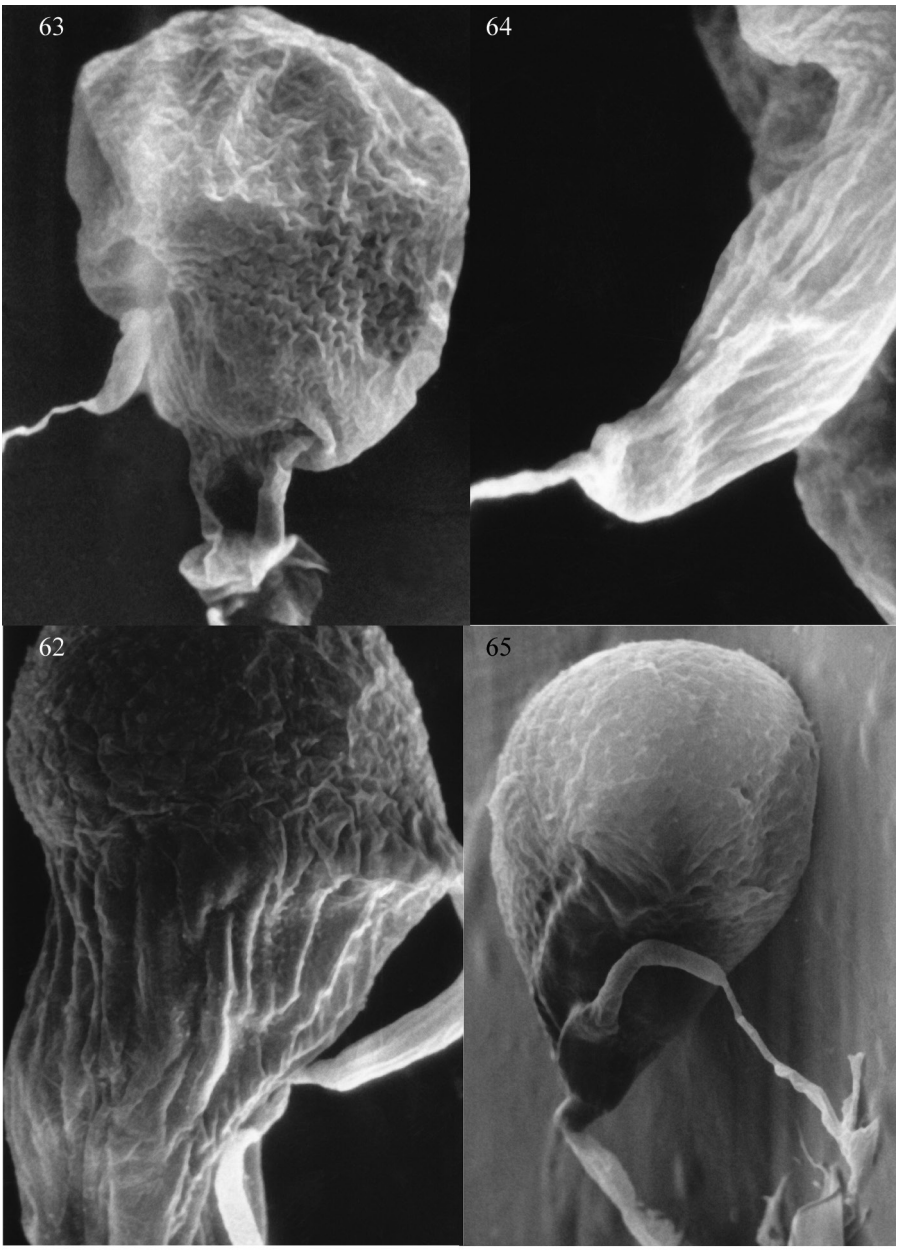
Figs 50 - 53: **Group 5.** 50 - 51. *E. innotata*. 52 - 53. *E. nanata.*



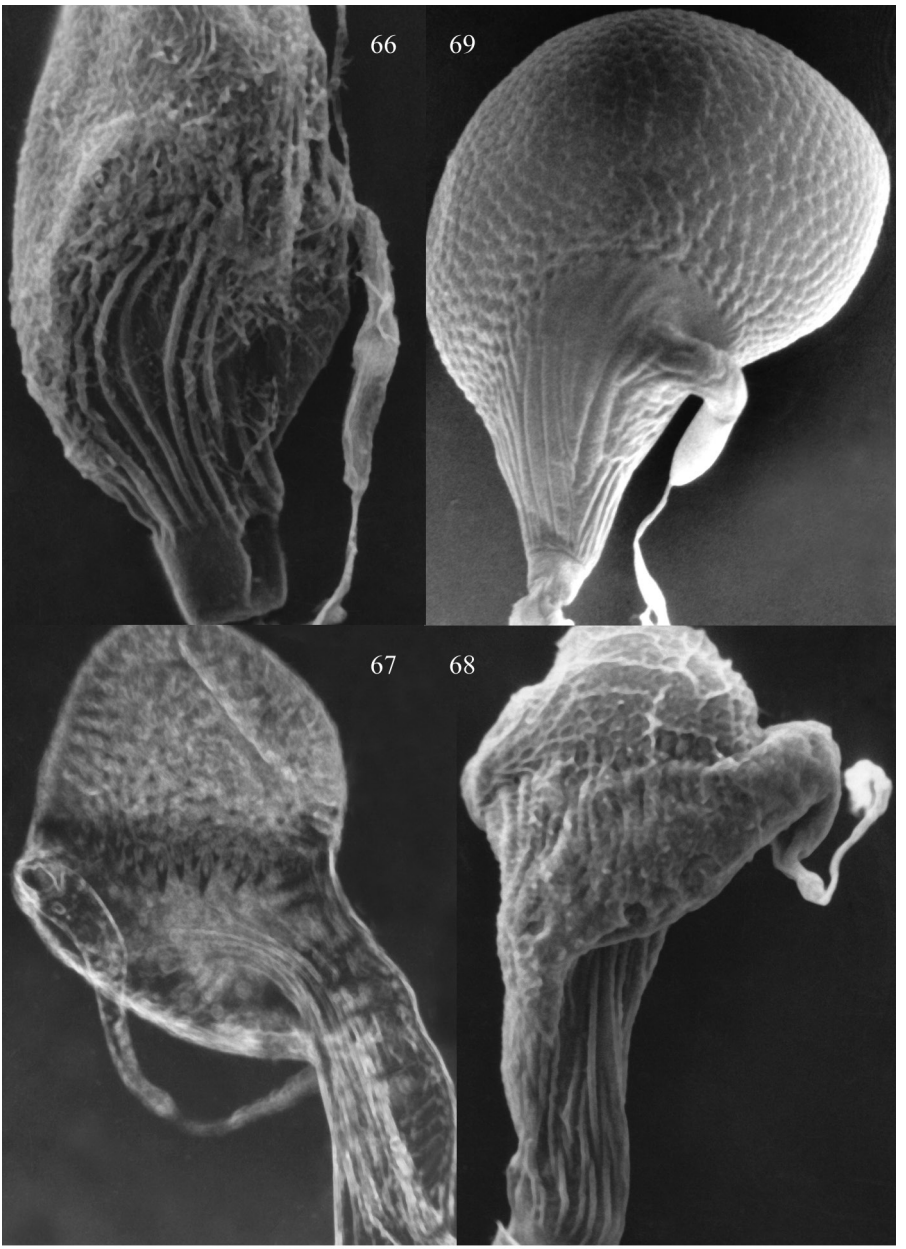
Figs 54 - 57: **Group 5.** 54 - 55 - 56. *E. ochridata*.
57. *E. veratraria.*



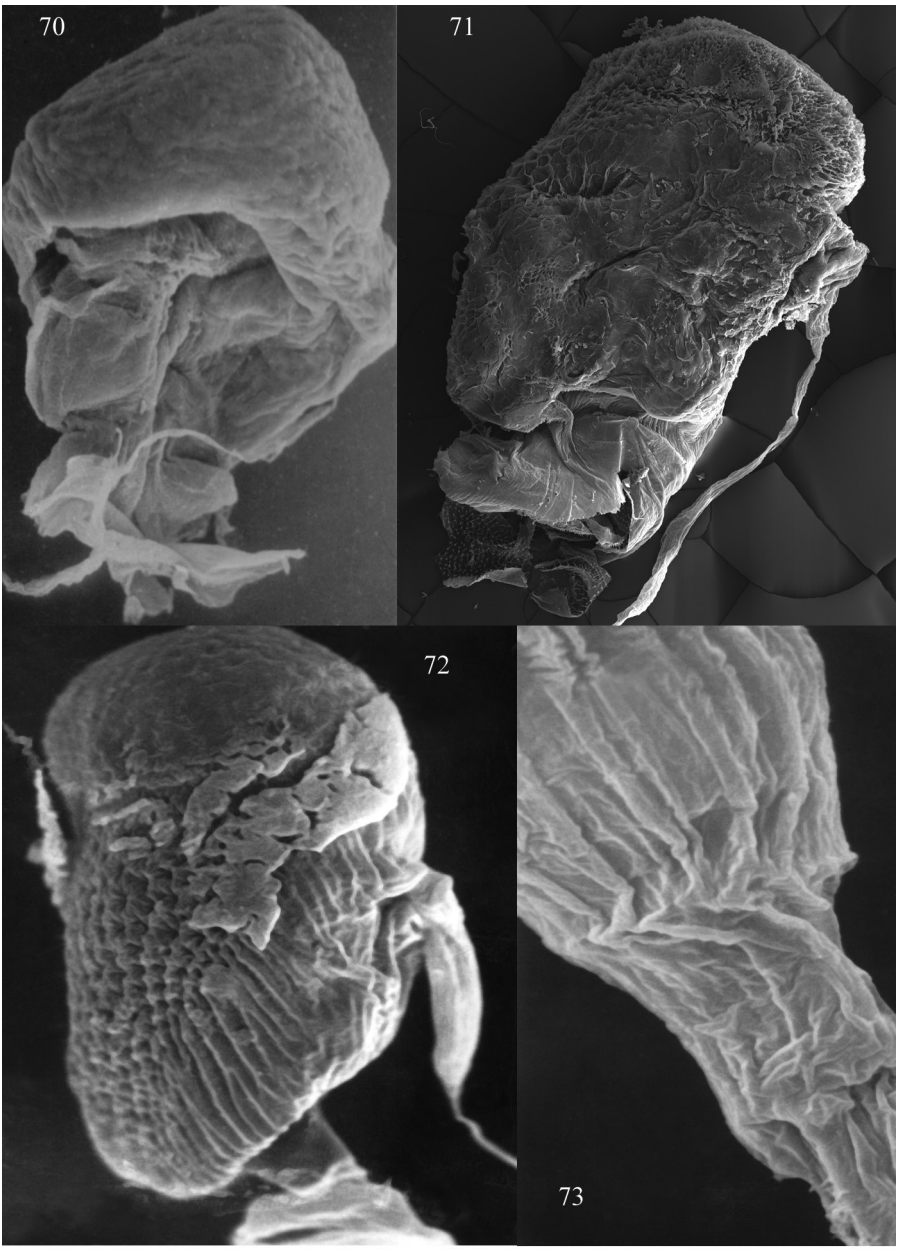
Figs 58 - 61: **Group 6.** 58. *E. abbreviata*. 59 - 60. *E. abietaria.*
61. *E. breviculata.*



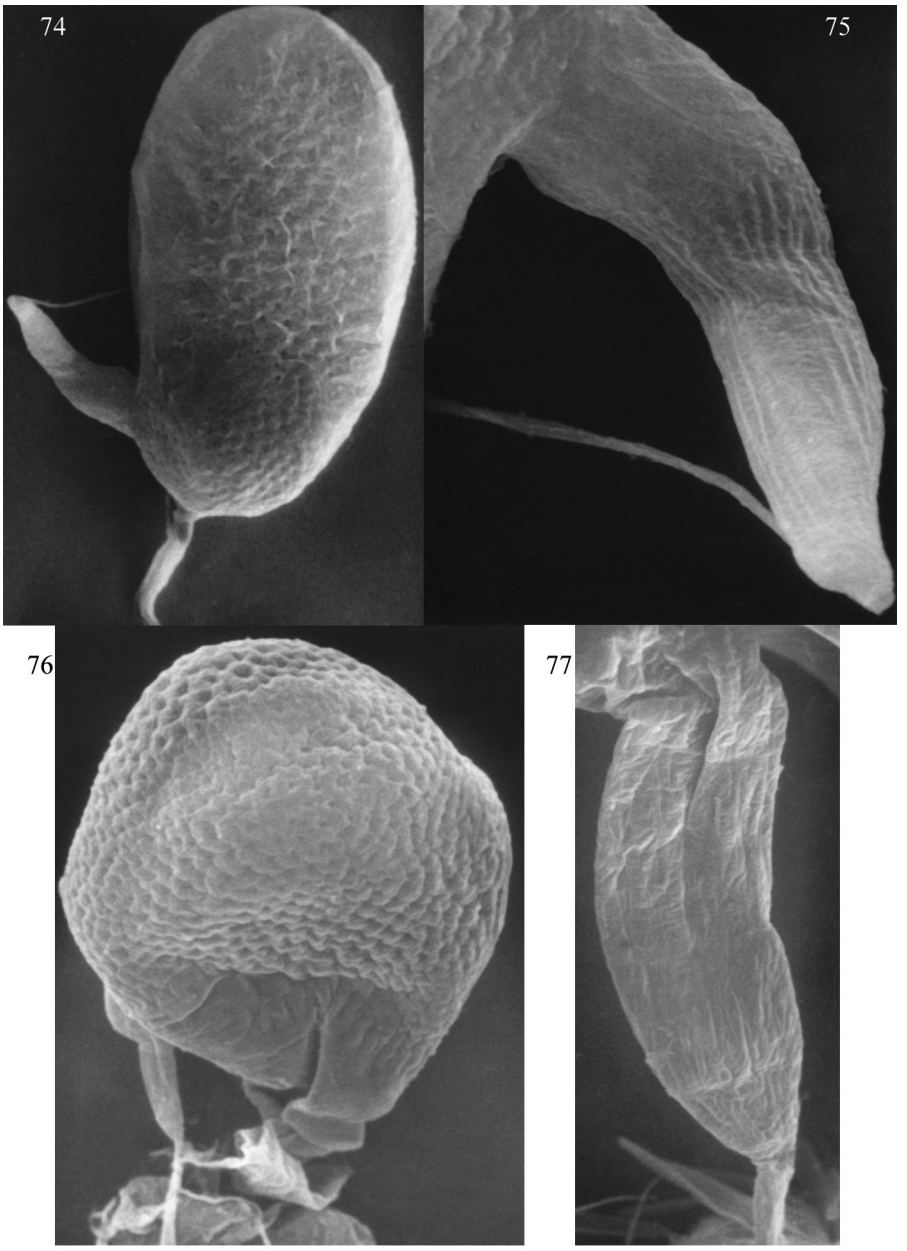
Figs 62 - 65: **Group 6.** 62. *E. ericeata*. 63 - 64. *E. graphata.*
65. *E. indigata.*



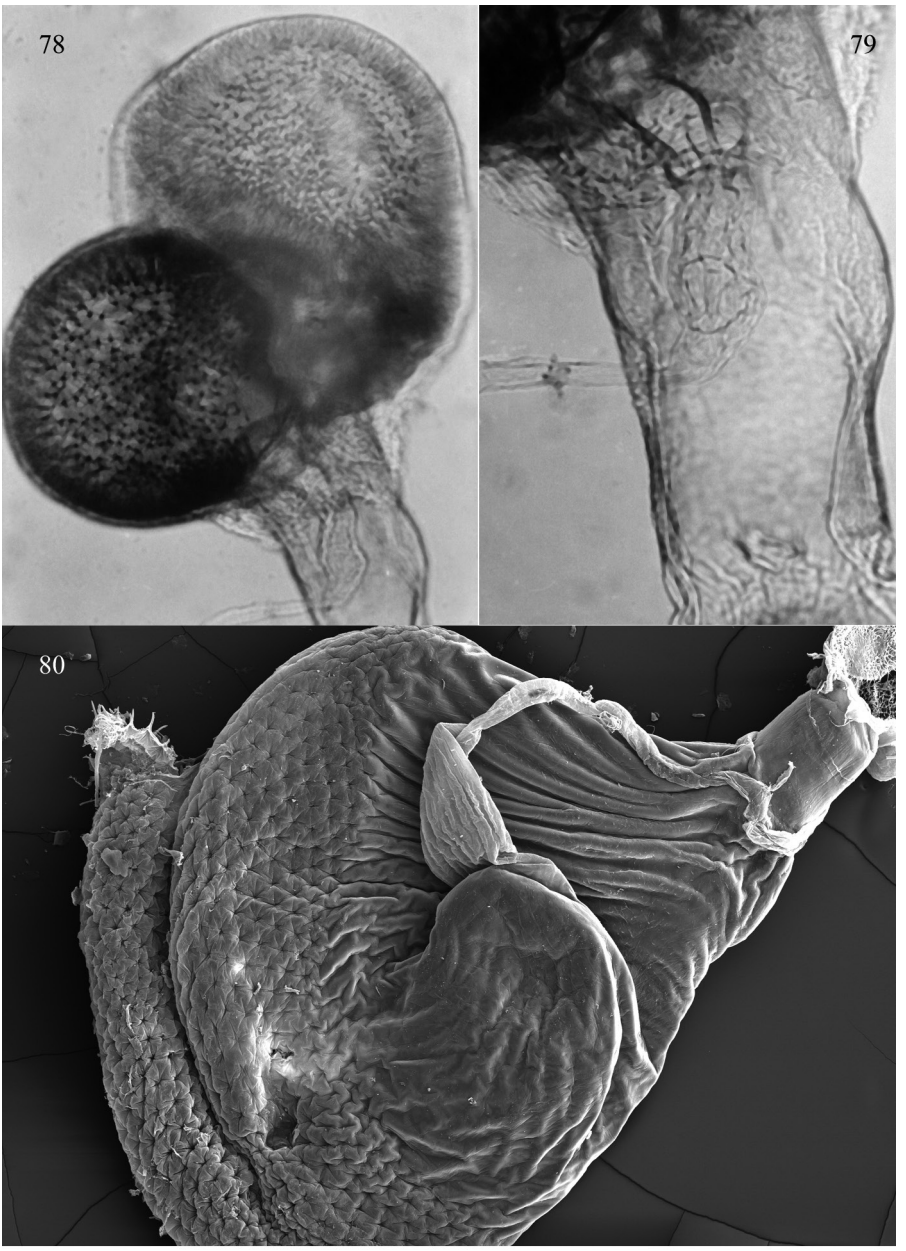
Figs 66-69: **Group 6.** 66. *E. irriguata*. 67. *E. oxycedrata.* 68. *E. pusillata.* 69. *E. simpliciata.*



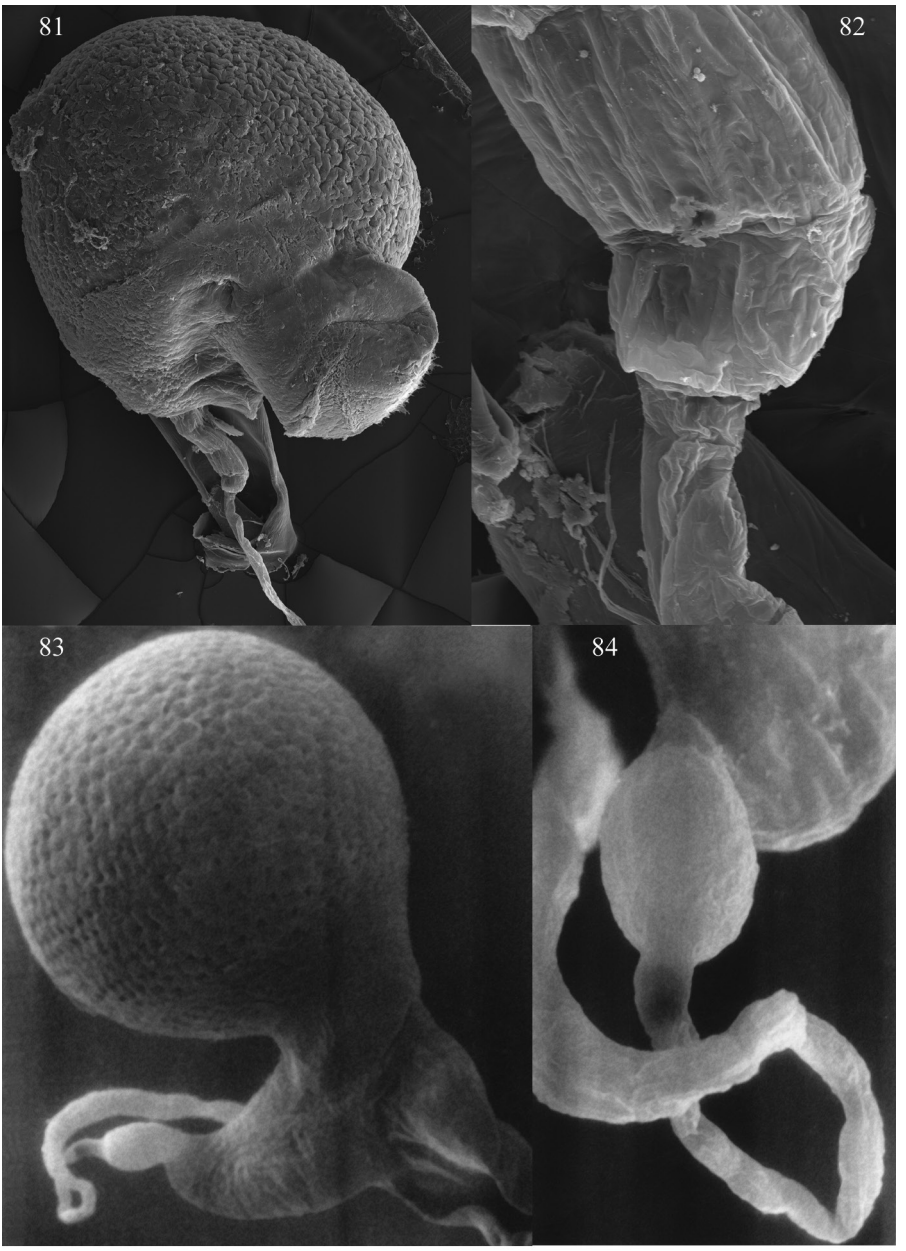
Figs 70 - 73: **Group 7.** 70 - 71. *E. actaeata*.
72 - 73. *E. gemellata.*



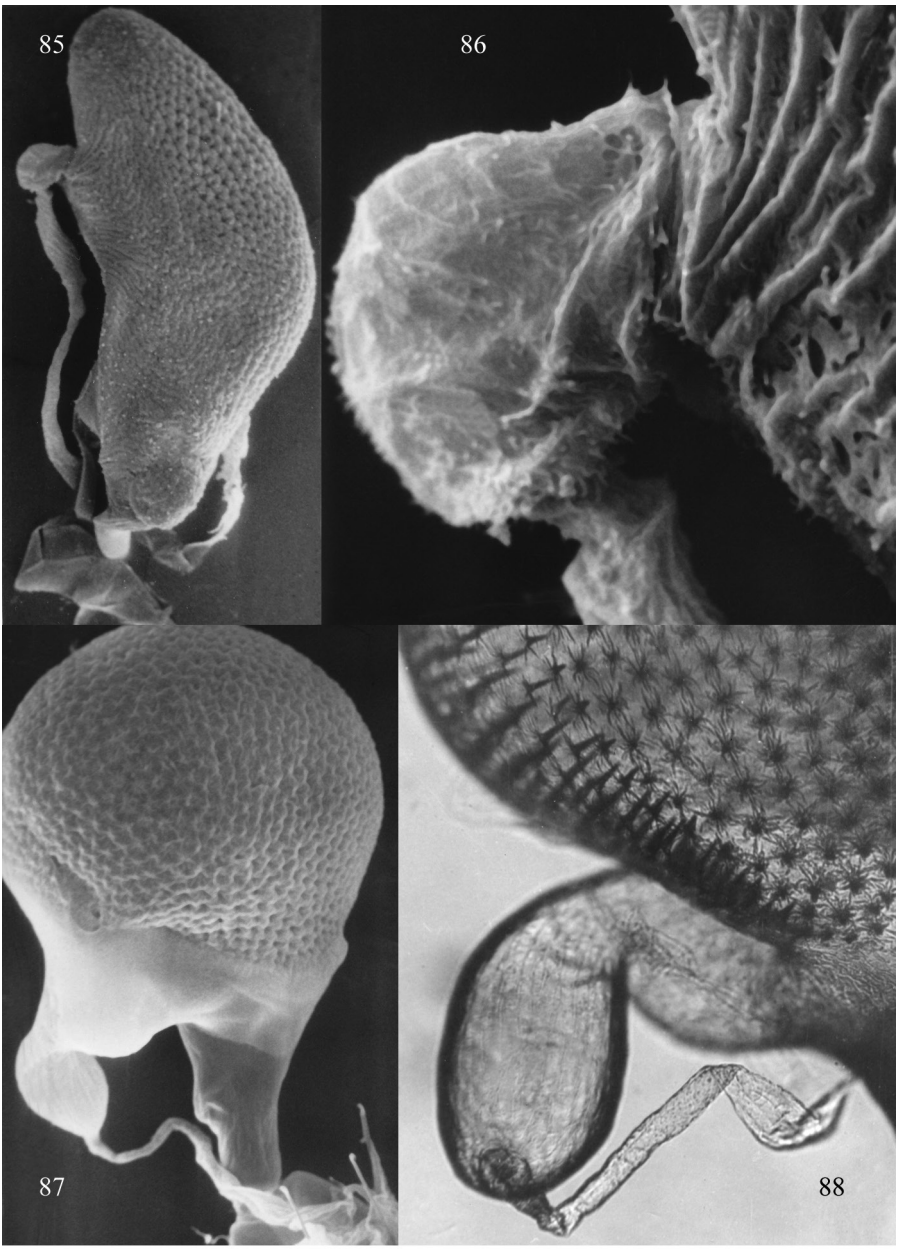
Figs 74 - 77: **Group 7.** 74 - 75. *E. haworthiata.*
76 - 77. *E. scalptata.*



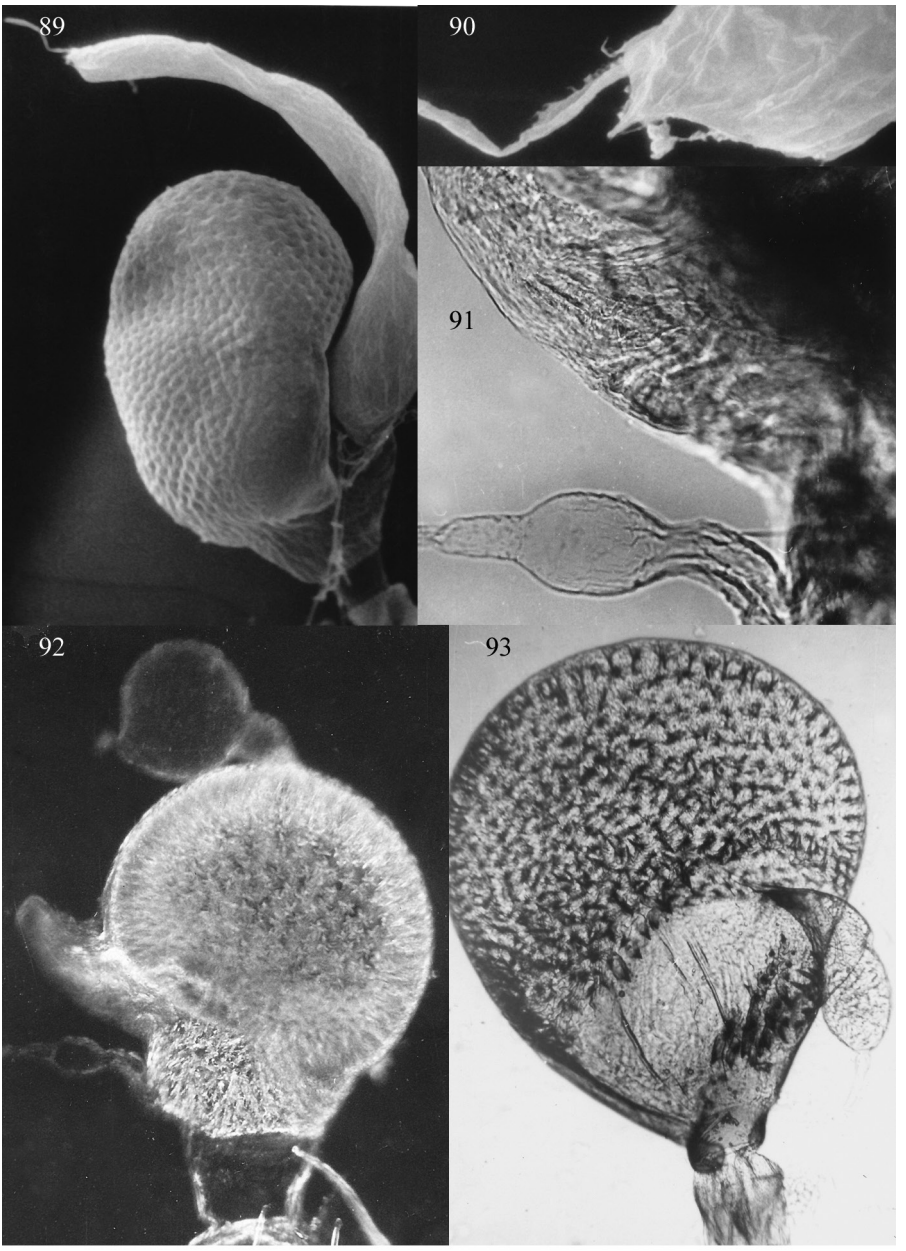
Figs 78-80: **Group 7.** 78 - 79. *E. schiefereri.*
80. *E. unedonata.*



Figs 81 - 84: **Group 7.** 81 - 82. *E. venosata.*
Group 8. 83 - 84. *E. centaureata.*

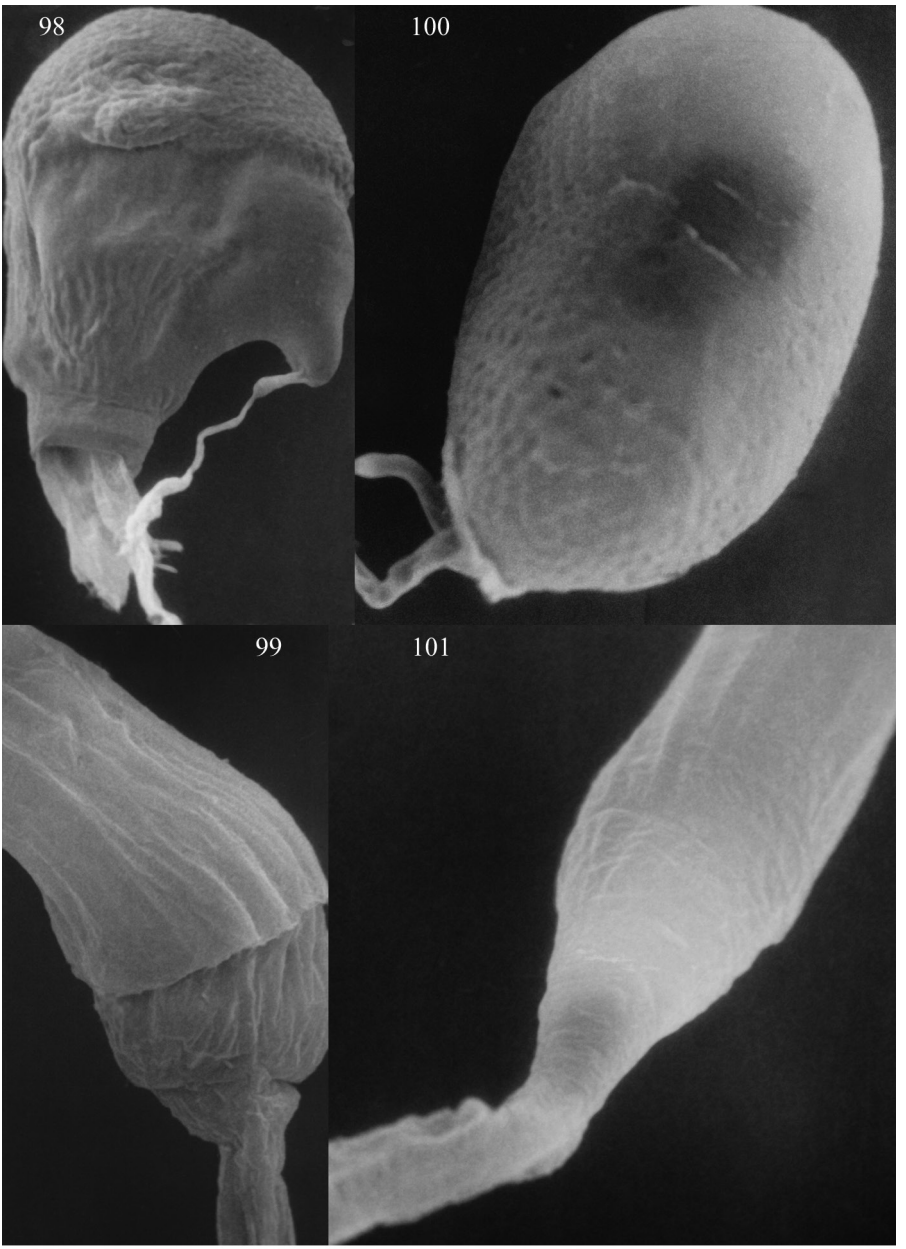


Figs 85 - 88: **Group 8.** 85 - 86. *E. distinctaria.*
87 - 88. *E. extraversaria.*

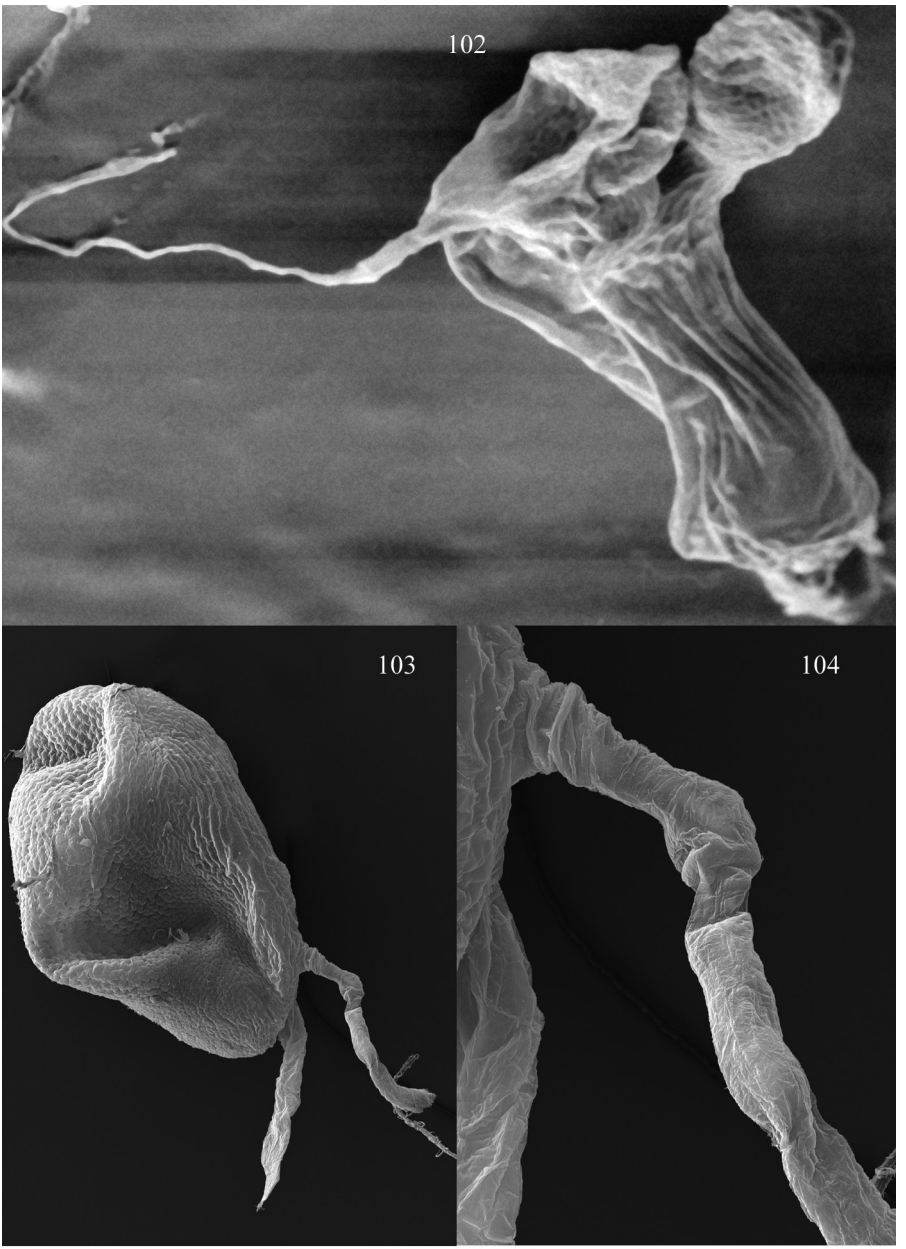


Figs 89-93: **Group 8.** 89 - 90. *E. impurata*. 91 - 92. *E. silenicolata.* 93. *E. spissilineata.*

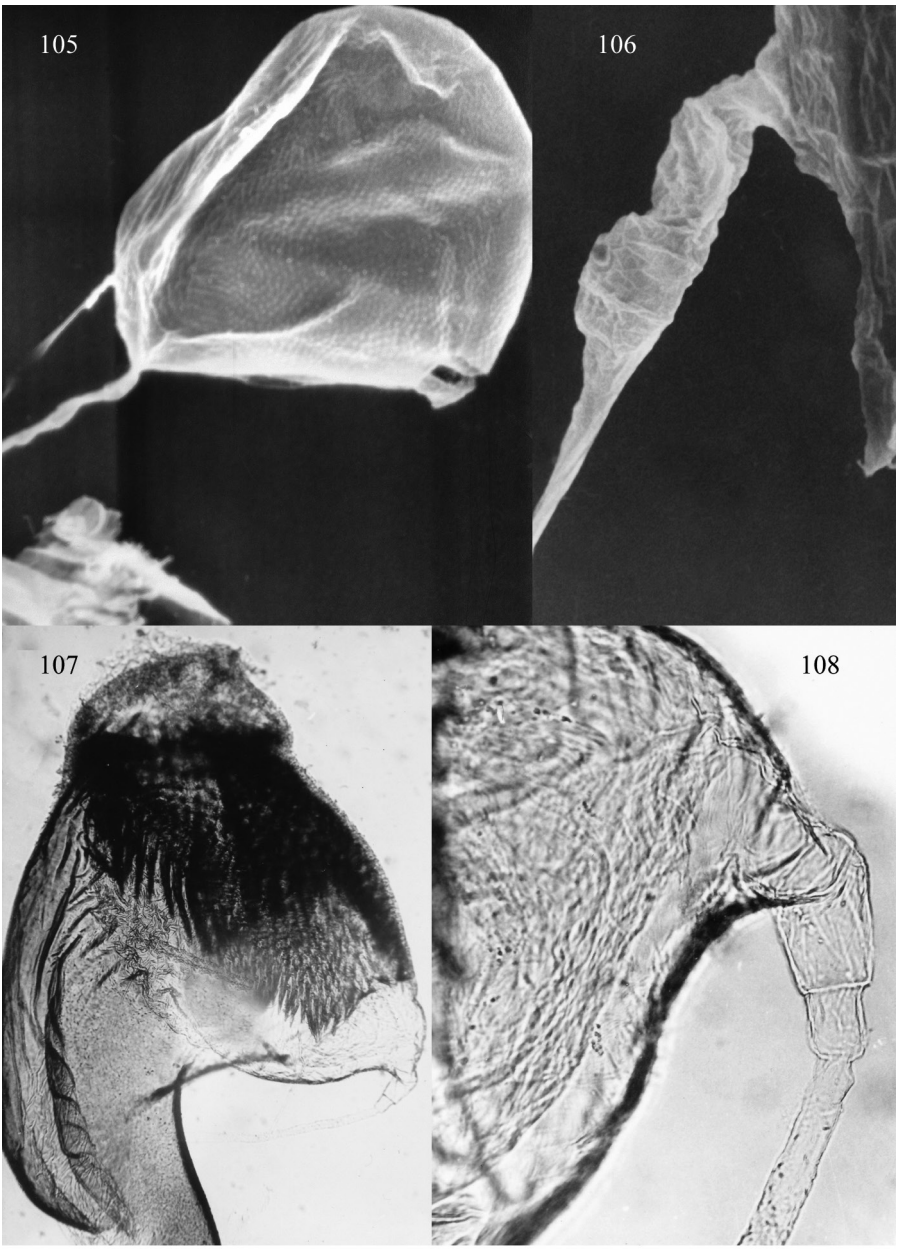
Figs 94-97: **Group 9.** 94. *E. alliaria*. 95 - 96. *E. analoga.*
97. *E. dissertata.*



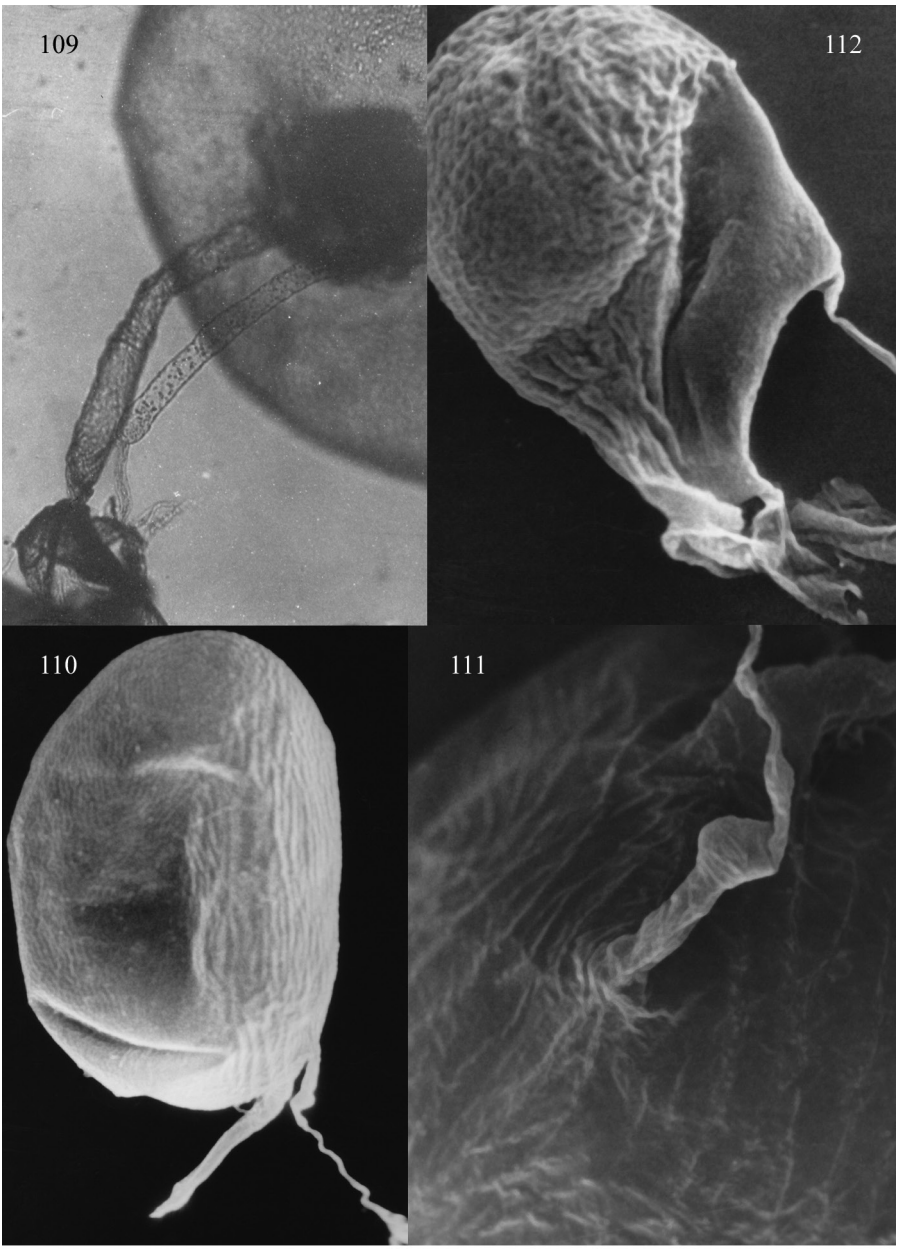
Figs 98 - 101: **Group 9.** 98 - 99. *E. gueneata.* 100 - 101. *E. inturbata.*



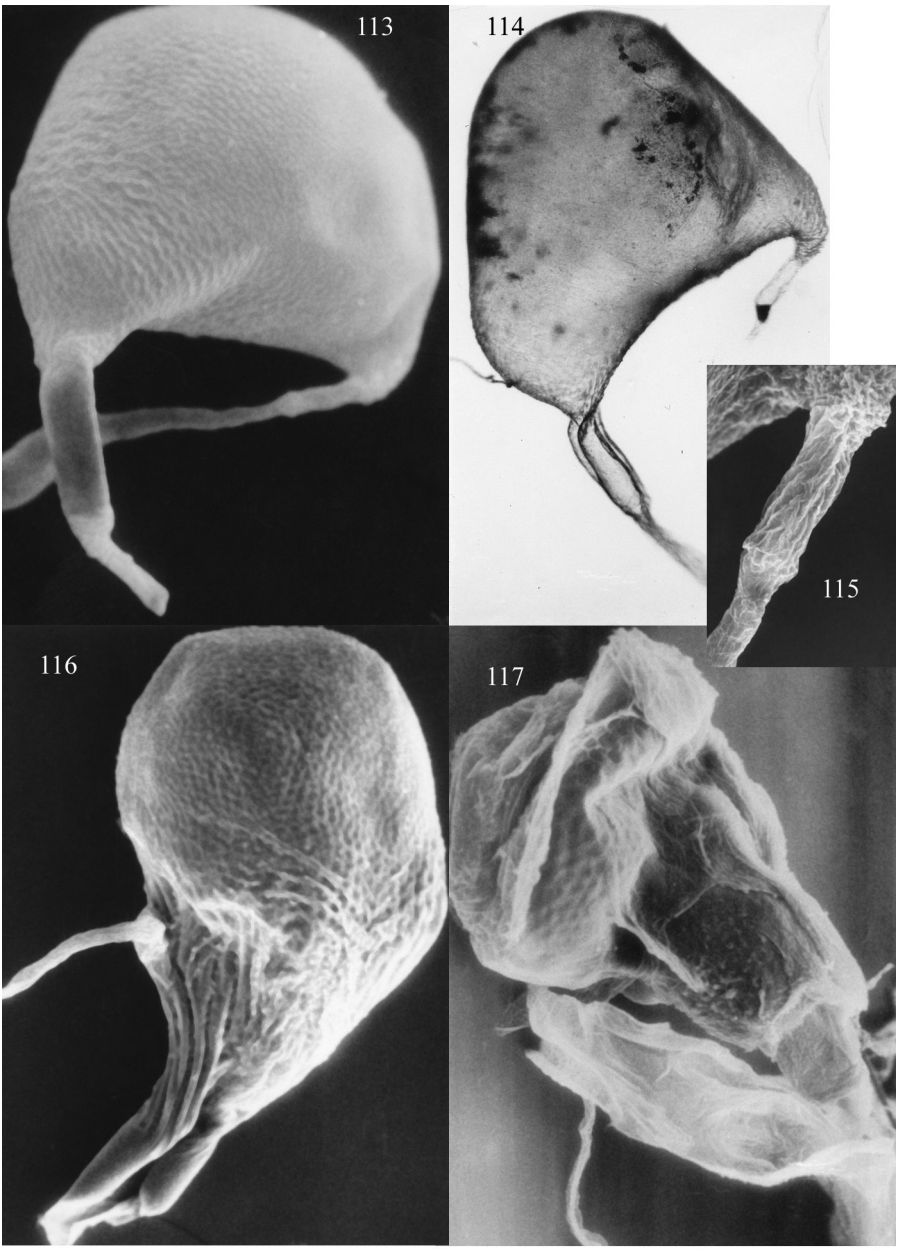
Figs 102-104: **Group 9.** 102. *E. lanceata.*
103 - 104. *E. laquaearia.*



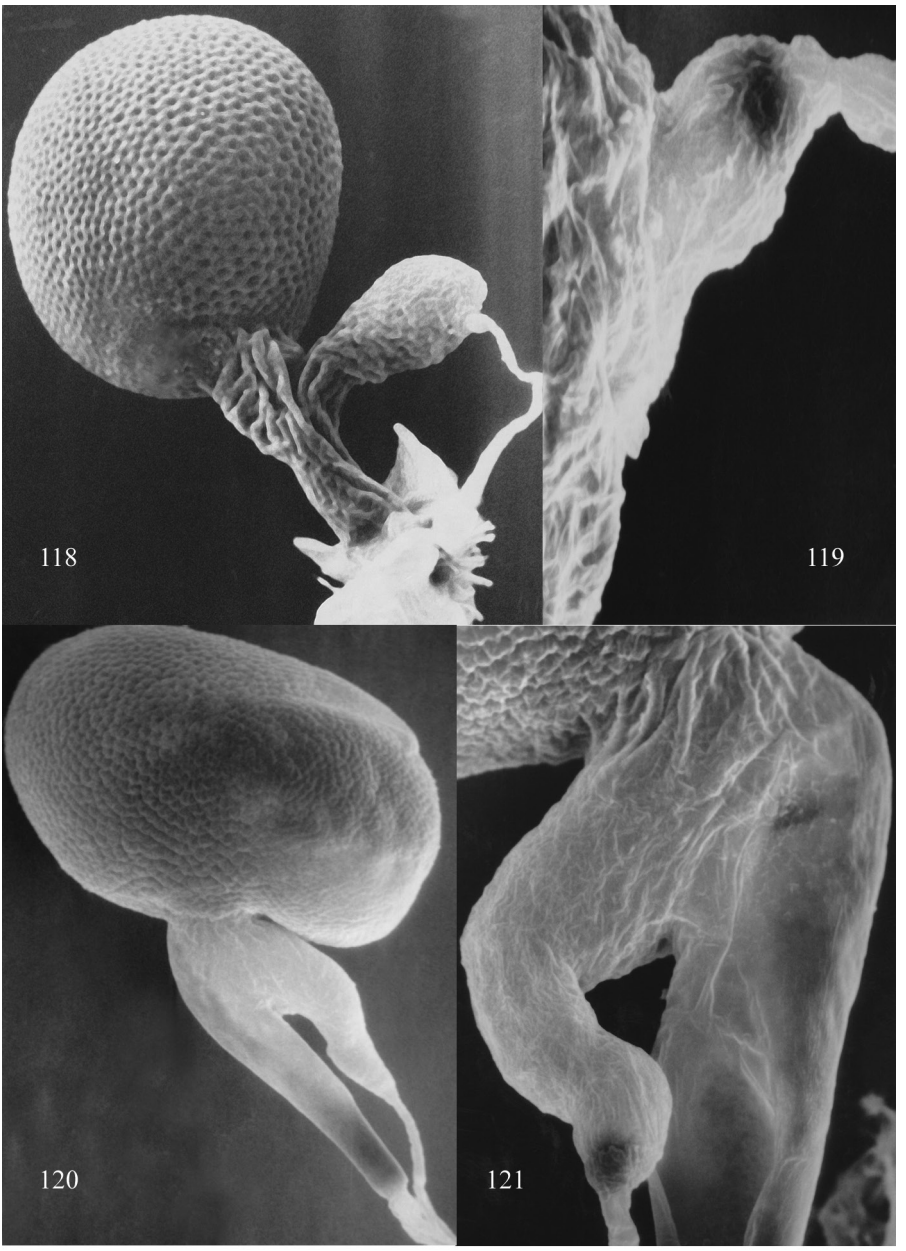
Figs 105-108: **Group 9.** 105 - 106. *E. linariata*.
107 - 108. *E. phoeniceata.*



Figs 109 - 112: **Group 9.** 109. *E. pulchellata*. 110 - 111. *E. pyreneata.* 112. *E. scopariata.*



Figs 113 - 117: **Group 9.** 113 -115. *E. silenata*. 116. *E. sinuosaria.* 117. *E. virgaureata.*



Figs 118-121: **Group 10.** 118 -119. *E. lariciata*.
120 - 121. *E. tenuiata.*

Figs 122 - 124: **Group 10.** 122 - 124. *E. tripunctaria.*

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