Morphology and taxonomy of tortricid moth pests attacking fruit crops in South Africa

by Monique Rentel

Thesis presented in partial fulfilment of the requirements for the degree Master of Sciences in Agriculture (Entomology) in the Faculty of AgriScience at the University of Stellenbosch

Supervisor: Dr P. Addison (Department of Conservation Ecology and Entomology)

Co-Supervisors: Prof H. Geertsema (Department of Conservation Ecology and Entomology) and
Dr J. W. Brown (United States Department of Agriculture)

Stellenbosch University http://scholar.sun.ac.za

Declaration

By submitting this thesis/dissertation electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof (save to the extent explicitly otherwise stated), that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Date: March 2013

Copyright © 2013 Stellenbosch University

All rights reserved

Abstract

Cydia pomonella (codling moth), Thaumatotibia leucotreta (False codling moth), Thaumatotibia batrachopa (Macadamia nut borer), Grapholita molesta (Oriental fruit moth), Cryptophlebia peltastica (Litchi moth), Epichoristodes acerbella (Pear leafroller/Carnation worm) and Lozotaenia capensana (Apple leafroller) are the most economically important tortricids affecting various crops in South Africa. The correct identification of these species, especially of the larval stage, is of great importance in pest management. Using available literature, augmented by additional morphological studies, an interactive identification key (Lucid key) for larval and adult stages of the seven species was developed. The colour and markings of the head, characteristics of the prothoracic and anal shields, the position of the prespiracular setae (L-group) relative to the spiracle on the prothoracic segment, the position of the spiracle on the eighth abdominal segment and L-group on the ninth abdominal segment, as well as the presence or absence of the anal comb are key characteristics for larval identification. For adult identification, wing pattern and genitalia are the most important features. However, the use of genitalia for moth identification might be difficult for the lay user, as the dissection and mounting of these structures requires certain skills and specialized equipment. Thus, genitalia have not been included in the Lucid Key. Differences in the morphological characteristics of most pupae were so minute that this stage was also not included in the Lucid key. However, the pupae of E. acerbella and L. capensana are easily distinguished from those of the other species by the presence of acremaster. This study also included the first morphological description of the pupa of L. capensana, which can be distinguished from that of E. acerbella by various features of the cremaster, antennae, spiracle shape, number of setae on abdominal segments A5-7, the size of spines on A3-7, and the presence/absence of spines on A9. A previous study by Timm (2005) indicated that geographically isolated populations of T. leucotreta tend to be genetically distinct. This raised the question of whether speciation/subspeciation has occurred or is occurring. Male moth genitalia are thought to evolve rapidly and are often the only features that can reliably distinguish similar species. Hence, variation in the shape of the valvae of T. leucotreta was used to determine whether divergence has occurred between populations of T. leucotreta. Elliptical Fourier analysis was used to analyze the valvar variation in three different populations. Although some variation in valvar shape was detected among mean population values for certain traits, no clear pattern emerged. Principle component analysis also showed no distinct clustering of valvae shape among populations, providing no evidence for divergence in male genitalia and therefore no morphological evidence of incipient speciation.

Opsomming

Cydia pomonella (Kodlingmot), Thaumatotibia leucotreta (Valskodlingmot), *T*. batrachopa (Makadamianeutboorder), Grapholita molesta (Oosterse vrugtemot), Cryptophlebia peltastica (Lietsjiemot), Epichoristodes acerbella (Peerbladroller/Angelierrusper) en Lozotaenia capensana (Appelbladroller) is die mees ekonomies belangrike tortrisiede van die vrugtebedryf in Suid-Afrika. Die juiste identifikasie van hierdie spesies, veral van hulle larwale stadium, is van groot belang by plaagbestuur. Deur gebruik te maak van beskikbare literatuur, aangevul deur bykomstige morfologiese studies, is 'n interaktiewe uitkenningssleutel ("Lucid key") vir die larwale en volwasse stadia van die sewe spesies ontwikkel. Die kleur en tekening van die kop, kenmerke van die prothorakale en anale skild, die ligging van die prespirakulêre setae (L-groep) relatief tot die spiraculum op die prothorakale segment, die ligging van die spirakulum op die agste abdominale segment en L-groep op die negende abdominale segment, asook die aan- of afwesigheid van die anale kam is sleutel kenmerke vir larwale uitkenning. Vir die volwassenes is die vlerktekening en genitalia die mees belangrike kenmerke. Die gebruik van die genitalia vir motuitkenning kan egter vir die leek gebruiker moeilik wees omdat die disseksie en montering van hierdie strukture bepaalde vaardighede en gespesialiseerde toerusting vereis. Vir die rede is die genitalia nie in die Lucid-sleutel ingesluit nie. Verskille in die morfologiese kenmerke van meeste papies is klein en die stadium is gevolglik ook nie in die sleutel ingesluit nie. Die papies van E. acerbella en L. capensana kan egter maklik van die ander spesies onderskei word deur die aanwesigheid van 'n cremaster. Hierdie studie sluit ook die eerste morfologiese beskrywing van die papie van L. capensana in, wat van dié van E. acerbella onderskei kan word deur gebruik te maak van kenmerke van die cremaster, antennae, spirakulêre vorm, aantal setae op abdominale segmente A5-7, die grootte van stekels op A3-7, en die aan- of afwesigheid van stekels op A9. 'n Vroeëre studie (Timm 2005) het aangedui dat geografies geïsoleerde bevolkings van T. leucotreta neig om geneties verskillend te wees. Dit het die vraag laat ontstaan of spesiasie/subspesiasie moontlik plaasgevind het of steeds plaasvind. Manlike mot genitalië word geag om vinnig te ontwikkel en is dikwels die enigste kenmerke wat betroubaar tussen soortgelyke spesies kan onderskei. Dus is die variasie in die vorm van die valvae van T. leucotreta gebruik om te bepaal of divergensie wel tussen bevolkings van T. leucotreta plaasgevind het. Elliptiese Fourier ontleding is gebruik om die valvae se variasie by drie verskillende bevolkings te ontleed. Alhoewel enkele variasie in die vorm van die valvae bespeur is by die gemiddelde bevolkingswaardes vir bepaalde eienskappe, kon geen duidelike patroon bespeur word nie. Hoofkomponentontleding het ook geen duidelike groepering van valvae se vorm tussen bevolkings getoon nie, wat geen bewys lewer van divergensie in die manlike genitalia en dus geen morfologiese bewys van beginnende spesiasie.

Acknowledgements

I wish to express my sincere appreciation to the following person and institutions:

My supervisors, Dr P. Addison, Prof. H. Geertsema and Dr J. W. Brown for their guidance, interest and constructive criticism during this research.

Citrus Research Industry (CRI), the Deciduous Fruit Producers Trust, trading as FruitGro Science and THRIP for funding this research project. Stellenbosch University for awarding me the Merit Bursary for 2011 and 2012.

S. Moore (CRI), T.M Gilligan (Colorado State University), M.F Addison, Dr K. Mitchell and L. Boardman for advice and guidance throughout the research.

Dr J. W. Brown and T.M. Gilligan and their families for taking me up in their homes during my stay in the USA.

Dr. D. Mazzi (Eidgenössische Technische Hochschule, Zürich, Switzerland), Dr. M. Botton (Embrapa Grape and Wine, Brazil), Dr D. Steenkamp (Entomon), Dr K.L. Pringle, Dr. J.M. Heunis, J. K. Opoku-Debrah, R. Stotter (XSIT), C. Chambers (River Bioscience), A. Burger, G. Nel (Wynkelderberg), F. Chidawanyika, J. Groenewald, G. Morland, R. Schoombie, M. Strydom, R. Rentel, G. Wilckens, J. Liebenberg, Z.M de Jager, S. Faure, A.J. Bam, A. Brinkhuis, S. Rosenberg and Plant Quarantine (Department of Agriculture, Forestry and Fisheries) for assistance in obtaining specimens or technical assistance.

T.W. Walters and his team (USDA, Fort Collins) for hosting me and for valuable knowledge exchange.

The landowners of the following farms for allowing me to set up traps and provide infested fruit material: Wynkelderberg, De Hoof, Frankenhof, Brandrivier, Nietvoorby (ARC Elgin), Killkewyn, Excelsior Boerdery, The Grange, Wolwehoek Boerdery, Somerslus, Glen Oak, Goosen Bourdery, Rooihoogte, Timberlea, Vergenoegd, Upotn farm and The Rest 38.

My family and friends for their support throughout.

Presentations at conferences:

- Rentel, M., De Wet, PPH., Addison, P. & Geertsema, H. (2011). Morphology of two frequently confused moth pests of citrus and pomegranates: a quick identification guide. Poster, Entomological Society of southern Africa Conference, Bloemfontein.
- Rentel, M., Addison, P., Geertsema, H. & Brown, J.W. (2012). Improved taxonomic understanding and the development of a LUCID key for tortricid moth pests in South Africa. 7th Citrus Research Symposium, Drakensberge.

Table of contents

Declar	ration	I
Abstra	nct	II
Opson	nming	III
Ackno	owledgements	IV
Table	of contents	V
List of	f Figures	X
List of	f tables	XIII
СНАР	TER 1	1 -
Introd	uction	1 -
1.1.	Classification and systematics	2 -
1.2.	General morphology and characteristics of Tortricidae	4
1.2.1.	Adult	4
1.2.2.	Egg	11
1.2.3.	Larva	12
1.2.4.	Pupa	13
1.3.	Pest status	14
1.4.	Aims and objectives	17
1.5.	References	17
СНАР	TER 2	24
Morph	nological study and development of a taxonomic key for the larval stages of economic imp	portance
tortrici	ids in South Africa	24
2.1.	Abstract	24
2.2.	Introduction	24
2.3.	Material and methods	25
2.3.1.	Insect material	25
2.3.2.	Preparation of specimens	25
2.3.3.	Preparation of images	25

2.3.4.	Nomenclature	26
2.3.5.	Key development	27
2.4.	Results	27
2.4.1.	Thaumatotibia leucotreta (Figure 13)	27
2.4.2.	Cydia pomonella (Figure 14)	29
2.4.3.	Grapholita molesta (Figure 15)	31
2.4.4.	Cryptophlebia peltastica (Figure 16)	33
2.4.5.	Thaumatotibia batrachopa (Figure 17)	35
2.4.6.	Epichoristodes acerbella (Figure 18)	37
2.4.7.	Lozotaenia capensana	38
2.5.	Summary of literature and morphological study	38
2.6.	Discussion and conclusion	43
2.7.	References	44
2.8.	Appendix 2.1	46
2.8.1	Thaumatotibia leucotreta	46
2.8.2	Cydia pomonella	49
2.8.3	Grapholita molesta	52
2.8.4	Cryptophlebia peltastica	57
2.8.5	Thaumatotibia batrachopa	60
2.8.6	Epichoristodes acerbella	62
СНАР	PTER 3	66
Morph	nological study for the pupal stages of economic importance tortricids in South Africa	66
3.1	Abstract	66
3.2	Introduction	66
3.3	Material and methods	66
3.3.1	Insect material	66
3.3.2	Preparations of specimens	67
3.3.3	Preparation of images	67

3.3.4	Nomenclature	67
3.3.5	Key development	68
3.4	Results	68
3.4.1	Thaumatotibia leucotreta	68
3.4.2	Cydia pomonella	70
3.4.3	Grapholita molesta	73
3.4.4	Cryptophlebia peltastica	75
3.4.5	Thaumatotibia batrachopa	77
3.4.6	Epichoristodes acerbella	79
3.4.7	Lozotaenia capensana	81
3.5	Summary of literature and morphological study	82
3.6	Discussion and Conclusion.	86
3.7	References	86
3.8	Appendix 3.1	88
3.8.1	Thaumatotibia leucotreta	88
3.8.2	Cydia pomonella	89
3.8.3	Grapholita molesta	90
3.8.4	Cryptophlebia peltastica	93
3.8.5	Thaumatotibia batrachopa	94
3.8.6	Epichoristodes acerbella	96
СНАР	TER 4	98
Morph	nological study and development of a taxonomic key for the adult stages of economic impor-	ance
tortric	ids on fruit crops in South Africa	98
4.1	Abstract	98
4.2.	Introduction	98
4.3.	Material and methods	98
4.3.1.	Insect material	98
4.3.2.	Preparations of specimens	99
4.3.3.	Preparation of Genitalia	99

4.3.4.	Preparation of images	99
4.3.5.	Nomenclature	99
4.3.6.	Key development	99
4.3.7.	Field trapping	99
4.4.	Results	101
4.4.1.	Thaumatotibia leucotreta (Figs 52-53)	101
4.4.2.	Cydia pomonella (Figs 54-55)	102
4.4.3.	Grapholita molesta (Figs 56-57)	104
4.4.4.	Cryptophlebia peltastica (Figs 58-59)	105
4.4.5.	Thaumatotibia batrachopa (Figs 60-61)	106
4.4.6.	Epichoristodes acerbella (Figs 62-63)	108
4.4.7.	Lozotaenia capensana (Figs 64-65)	108
4.4.8.	Field trapping	110
4.5.	Discussion and Conclusion	110
4.5.1.	Diagnostic characters	110
4.5.2.	Trapping	111
4.6.	References	111
4.7.	Appendix 4.1	113
4.7.1.	Thaumatotibia leucotreta	113
4.7.2.	Cydia pomonella	113
4.7.3.	Grapholita molesta	113
4.7.4.	Cryptophlebia peltastica	114
4.7.5.	Thaumatotibia batrachopa	115
4.7.6.	Epichoristodes acerbella	115
4.7.7.	Lozoteinia capensana	115
СНАР	PTER 5	116
Deterr	mining intraspecific variation in <i>Thaumatotibia leucotreta</i> (Lepidoptera: Tortricidae) mal	es by using
shape	analysis of genitalia	116
5.1	Abstract	116

Stellenbosch University http://scholar.sun.ac.za

5.2	Introduction	116
5.3	Material and methods	117
5.3.1	Study organism	117
5.3.2	Preparation of material	117
5.3.3	SHAPE and Data analysis	117
5.4	Results	118
5.4.1	Principle components	118
5.4.2	Analysis of Variance	120
5.5	Discussion and Conclusion	122
5.6	References	124
Concl	usion	126
Appen	ndix 1	129

List of Figures

Figure 1: Head of Tortricid moths (Bradley et al., 1973)	4
Figure 2: Mouthparts of the Tortricini (Razowski, 2002).	5
Figure 3: Legs of Tortricini: A) fore leg, B) middle leg, C) hind leg (Bradley et al., 1973)	6
Figure 4: Frenular bristles at base of hind wing, A) males, B) females (Bradley et al., 1973)	7
Figure 5: Wing venation of Tortricidae (Razowski, 2002)	7
Figure 6: Wing pattern of Tortricidae: A) & B) Tortricini, C) & D) Archipini (Bradley et al. 1973)	8
Figure 7: Male genitalia of Oletreutinae (Gilligan et al., 2008)	10
Figure 8: Female genitalia of Oletreutinae (Gilligan et al., 2008)	11
Figure 9: Thaumatotibia leucotreta eggs	12
Figure 10: Head capsule, frontal and lateral view (Stehr, 1958, adjusted with Swatschek, 1958)	26
Figure 11: Lepidoptera setal maps (Stehr, 1958 adapted from MacKay, 1959)	26
Figure 12: A8-A10 dorsal view (MacKay,1959)	27
Figure 13: Thaumatotibia leucotreta larvae	28
Figure 14: Cydia pomonella larvae	30
Figure 15: Grapholita molesta larvae	32
Figure 16: Cryptophlebia peltastica larvae	34
Figure 17: Thaumatotibia batrachopa larvae	36
Figure 18: Epichoristodes acerbella larvae	38
Figure 19: Thaumatotibia leucotreta final instar larva (Timm et al., 2007;)	48
Figure 20: Cydia pomonella head, (Swatschek, 1958)	49
Figure 21: Cydia pomonella prothoracic and anal shield (Swatschek, 1958; Figs, 89, 90)	50
Figure 22: Cydia pomonella larva (MacKay, 1959)	51
Figure 23: Cydia pomonella larva, (Brown, 1987)	52
Figure 24: Grapholoita. molesta larva (Garmen, 1917)	53
Figure 25: Grapholita molesta larva (Wood & Selkregg, 1918)	54
Figure 26: Grapholita molesta larva (MacKay, 1959).	56
Figure 27: Grapholita molesta larva(Brown, 1987)	57
Figure 28: Cryptophlebia peltastica final instar larva. (Timm et al., 2007)	59

Figure 29: Thaumatotibia batrachopa final instar larva (Timm et al., 2007)	61
Figure 30: Epichoristodes acerbella, dorsal head chaetotaxy (Nuzzaci, 1973)	63
Figure 31: Epichoristodes acerbella head (Nuzzaci, 1973).	63
Figure 32: Epichoristodes acerbella setal map (Nuzzaci, 1973)	64
Figure 33: Epichoristodes acerbella final instar larva (Timm et al., 2008)	65
Figure 34: General pupae (Patočka & Turčáni, 2005)	68
Figure 35: Thaumatotibia leucotreta pupa.	70
Figure 36: Cydia pomonella pupa.	72
Figure 37: Grapholita molesta pupa	74
Figure 38: Cryptophlebia peltastica pupa.	76
Figure 39: Thaumatotibia batrachopa pupa	78
Figure 40: Epichoristodes acerbella pupa	80
Figure 41: Lozotaenia capensana pupa.	82
Figure 42: Thaumatotibia leucotreta pupa (Timm et al. 2007)	89
Figure 43: Cydia pomonella pupa (Patočka & Turčáni, 2005)	90
Figure 44: Grapholita molesta pupa (Garmen, 1917)	91
Figure 45: Grapholita molesta pupa (Wood & Selkregg, 1918	92
Figure 46: Grapholita molesta pupa (Patočka & Turčáni, 2005)	93
Figure 47: Cryptophlebia peltastica pupa (Timm et al., 2007)	94
Figure 48: Thaumatotibia batrachopa pupa (Timm et al., 2007)	95
Figure 49: Epichoristodes acerbella pupa (Nuzzaci, 1973; Fig 17)	96
Figure 50: Epichoristodes acerbella pupa (Timm et al., 2008).	97
Figure 51: Google Earth map with trapping locations in the Western Cape	101
Figure 52: Thaumatotibia leucotreta adults	102
Figure 53: Thaumatotibia leucotreta genitalia.	102
Figure 54: Cydia pomonella adults	103
Figure 55: Cydia pomonella genitalia.	103
Figure 56: <i>Grapholita molesta</i> adult	104
Figure 57: Grapholita molesta genitalia	104

Figure 58: Cryptophlebia peltastica adults.	105
Figure 59: Cryptophlebia peltastica genitalia	106
Figure 60: Thaumatotibia batrachopa adult	107
Figure 61: Thaumatotibia batrachopa genitalia.	107
Figure 62: Epichoristodes acerbella adult	108
Figure 63: Epichoristodes acerbella genitalia.	108
Figure 64: Lozotaenia capensana adult wing pattern variations	109
Figure 65: Lozotaenia capensana genitalia.	109
Figure 66: Outlines of left and right valva of <i>T. leucotreta</i> used for shape analysis	118
Figure 67: Reconstructed shape contours of the principle components analysis and corresponding percentages for left valva	119
Figure 68: Reconstructed shape contours of principle components analysis and corresponding percentator for right valva.	-
Figure 69: Plot PC1 vs. PC 2 for both left and right valvae.	120
Figure 70: Mean of PC1 vs. mean of PC 2 for each population, indicating the centre of distribution	120
Figure 71: Means (± SE) for left and right valvae to determine significant differences in PC values be populations.	
Figure 72: A single species can split into two lineages over time (grey zone) as they acquire different properties (horizontal SC lines) (de Queiroz, 2007)	123
Figure 73: <i>Epiphyas postvittana</i> A) Forewing pattern; B) Pupa; C) Early instar larva; D) Final instar la (Gilligan & Epstein, 2012)	
Figure 74: <i>Lobesia botrana</i> A) Adult (male); B) Pupa; C & D) Larvae (Gilligan & Epstein, 2012)	130

List of tables

Table 1: Classification of the seven important tortricids in South Africa 3 -
Table 2: Wing venation systems according to Razowski (2002) (MS) and Hampson's system (HS)
Table 3: Main cultivated host plants for seven tortricid pests damaging fruit crops in South Africa
Table 4: Important morphological characteristics for identifying and distinguishing between the six economically important tortricid larvae
Table 5: Important and distinct morphological characteristics for identifying and distinguishing between the six economically important tortricid pupae
Table 6: Pheromones used in field surveys to trap tortricid moths associated with deciduous fruit in the Western Cape
Table 7: Trap locations to surveys for tortricid moths in Western Cape fruit orchards and adjoining natural habitats (veld)
Table 8: Total results from field survey conducted in deciduous fruit orchards and vineyards of the Western Cape for various tortricid moths from March to end of April 2012
Table 9: Analysis of variance in <i>T. leucotreta</i> male valvae
Table 10: Summarized larval morphological characteristics for <i>E. postvittana</i> and <i>L. botrana</i> (Venette <i>et al.</i> , 2003; Brown <i>et al.</i> 2010; Gilligan et al. 2011).
Table 11: Summarized pupal morphological characteristics for <i>E. postvittana</i> and <i>L. botrana</i>
Table 12: Summarized adult morphological characteristics for <i>E. postvittana</i> and <i>L. botrana</i>

CHAPTER 1

Introduction

Tortricidae, commonly known as leafrollers or leaftwisters, are the largest family of microlepidoptera with more than 5000 species (Powell, 1964; Pinhey, 1975; Horak & Brown, 1991). The family includes some of the most economically important pests of agriculture, forest trees, and ornamental plants (Powell, 1964; MacKay 1959; Holloway et al., 1987; Razowski, 2002; Timm, 2005). The family is worldwide in distribution but reaches its greatest species-richness in temperate and tropical regions (Common, 1990; Horak & Brown, 1991; Scoble, 1992). The common name, leafrollers, originates from the larval behaviour of spinning and/or rolling leaves of the host plant upon which they feed and develop (Pinhey, 1975; Timm, 2005). Seven major economically important tortricid species can be found in South Africa, all of which have great impact on the local fruit industry: Cydia pomonella (Linnaeus, 1758) (Codling moth), Thaumatotibia leucotreta (Meyrick, 1913) (False codling moth), Grapholita molesta (Busck, 1916) (Oriental fruit moth), Cryptophlebia peltastica (Meyrick, 1921) (litchi moth), Thaumatotibia batrachopa (Meyrick, 1908) (Macadamia nut borer), Epichoristodes acerbella (Walker, 1864) leafroller/Carnation worm) and Lozotaenia capensana (Walker, 1863) (Apple leafroller) (Table 1). The larvae of all these species feed on a range of cultivated crops causing extensive damage and losses to the fruit industry (Powell, 1964; Timm, 2005).

Correct identification, especially of the immature stages, is important because misidentifications can lead to ineffective pest management. Current keys for tortricid species in South Africa are unsatisfactory because they are mostly incomplete. McGeoch & Krüger (1994) developed a key for identifying moth larvae associated with *Ravenelia* galls on *Acacia karroo*. One important tortricid larval species, *C. peltastica*, found on *Acacia karroo* galls, was included in their study. Krüger (1998) subsequently developed a key for identifying adult moths associated with *Ravenelia* galls on *Acacia karroo*, which included larvae of two economically important tortricid species, *C. peltastica* and *T. leucotreta*. Yet, in both McGeoch & Krüger (1994) and Krüger (1998), no description is provided for either *C. peltastica* or *T. leucotreta*. Timm *et al.* (2007, 2008) produced a dichotomous key to distinguish among six economically important tortricid larvae and their pupae present in South Africa, but for some of these species, especially *L. capensana*, a complete description for the immature life stages is lacking.

Species identification is the basis of traditional taxonomy, also known as alpha taxonomy, which relies on subjective visual evaluations (Mutanen & Pretorius 2007). Traditional taxonomy, which also included the describing of species based on morphology, is facing a serious challenge in that there is a lack of time, funding, and expertise (taxonomist have become a dying breed), and that relevant information available is often inaccessible (Walters & Winterton, 2007). This principle could be assisted by using more integrative taxonomy. Integrative taxonomy combines the use of traditional taxonomy together with multiple disciplines and modern identification techniques such as DNA barcoding, interactive identification keys, and morphometrics.

Walter & Winterton (2007) discussed how searches on "identification keys" and "insects keys" increased dramatically based on the number of "hits" on Google over a one-year period from March 2005 to March 2006. The search "Identification keys" increased over the one year period by almost 100 000 hits and "insects keys" by 19 000 hits.

Dichotomous keys have been, and are still used for identification purposes (Osborne, 1963). In dichotomous keys, each "question" has a couplet with two possible contrasting characters (Osborne, 1963; Amante & Norton, 2003; Walters & Winterton, 2007). Depending on the answer chosen, the user is either redirected to another couplet or to an endpoint providing an identity (Amante & Norton, 2003; Walters & Winterton, 2007). Dichotomous keys frequently present one major problem, the unanswerable couplet, for which a user is not able to decide on one statement, and hence, is unable to continue with the key (Amante & Norton, 2003; Walters & Winterton, 2007). Matrix keys, such as LUCID keys, are more interactive and enable the user to select more than one character to examine or to skip characters which are not conspicuous to them, and still reach a possible identification (Amante & Norton, 2003; Walters & Winterton, 2007).

Morphometrics is the "measurement and analysis of a form" (Daly, 1985) and was traditionally based on size, ratios, and linear measurements (Daly, 1985; Mutanen & Pretorius, 2007). Geometric morphometrics has become more and more popular; enabling users to quantify shapes (Mutanen & Pretorius, 2007).

Timm et al. (2010) found no evidence of specific host races in a population of T. leucotreta population, but evidence was presented for population structure on a fine-scale, indicating that populations of different geographic origins were genetically distinct. Timm et al. (2010) suggested that the reason for this divergence could be due to limited dispersal. This begs the question of whether speciation within T. leucotreta has occurred. To answer this question, the male genitalia were studied and analyzed using shape morphometrics. Meyrick (1895), the first person to introduce the use of genitalia in tortricid taxonomy, however, later opposed their use and Kennel (1908), although receiving criticism, concluded that "genitalia are so strongly diverse" that they may be useful for separating related taxa but should not be used in higher classification (Horak, 1984). Dampf (1908) proved Kennel (1908) wrong in a comparative study analyzing the genitalia of Rhopobota naevana (Hübner, 1817) (Horak, 1984). Pierce & Metcalfe (1922) were the first to carry out a comparative study of tortricid male and female genitalia of the British Islands. Powell (1964) mentioned that the "male genitalia in tortricids form the basis for classifications," and that genitalia alone can be used to determine the identity of a species. Various other authors have proven the importance and taxonomic value of genitalia (Horak, 1984). The shape and size of the genitalia play an important role in identification, and by using geometric morphometrics one can identify quantitative evidence of the difference between different species.

1.1. Classification and systematics

The classification of the seven important tortricids in South Africa is presented in Table 1.

Table 1: Classification of the seven important tortricid species in South Africa (Pinhey, 1975; Karisch, 2003; Brown, 2005)

	Codling moth	False codling moth	Macadamia nut borer	Oriental fruit moth	Litchi moth	Pear leafroller	Apple leafroller	
Order				Lepidoptera				
Family				Tortricidae				
Subfamily		(Tortr	ricinae			
Tribe		(Grapholitini			Arch	ipinif	
Genus	Cydia	Thaumate	otibia	Grapholita	Cryptophlebia	Epichoristodes	Lozotaenia	
Species	pomonella	leucotreta	batrachopa	molesta	peltastica	acerbella	capensana	
Synonyms	Phalaena (Tortrix) pomonella (Linnaeus, 1758) Phalaena nitens (Fourcroy, 1785) Pyralis pomona (Fabricius, 1775) Phalaena (Tortrix) aeneana (Villers, 1789) Tortrix pomonana (Denis & Schiffermüller, 1775) Laspeyresia pomonella (Hübner,1825) Carpocaspa putaminana (Staudinger, 1859) Carpocaspa glaphyrana (Rebel, 1941)	Natal codling moth Carpocapsa sp. (Fuller, 1901) Orange codling moth Enarmonia batrachopa (Howard, 1909) Agryroploce leucotreta (Meyrick, 1913) Cryptophlebia leucotreta Thaumatotibia roerigii (Zacher, 1915)	Cryptophlebia batrachopa Enarmonia batrachopa (Meyrick, 1908) Argyroploce colivora (Meyrick, 1932)	Cydia molesta Laspeyresia molesta (Busck, 1916)	Agryroploce peltastica (Meyrick, 1921)	Depressaria acerbella (Walker, 1864) Epichorista galetata (Meyrick, 1921) Tortrix iocoma (Meyrick, 1908) Proselena ionephela (Meyrick, 1905) Epichorista ionephela (Meyrick, 1909)	Teras capensana (Walker, 1863) Teras (Tortrix) meridionana (Walker, 1863) Teras reciprocana (Walker, 1863) Parapandemis capensana (Walker, 1863) Tortrix capitana (Felder, 1875) Cacoeia adustana (Walsingham, 1881) Tortrix adustana (Walsingham, 1881) Cacoeia (Tortrix) dorsiplagana (Walsingham, 1881) Tortrix capensana (Walsingham, 1881) Tortrix capensana (Meyrick, 1937)	

1.2. General morphology and characteristics of Tortricidae

Tortricids are small to medium-sized (wingspan 8-35mm) moths, generally with dull or cryptic colours; however, a few species have spectacular colours and patterns (Common, 1990; Scoble, 1992). When the moths are at rest, the forewings form a somewhat bell shape, and many species possess tufts of scales on the forewings and thorax (Scoble, 1992). The following is a summary of available literature describing tortricid characteristics of all life stages.

1.2.1. Adult

Head

Head anteriorly covered with rough, broad, long scales on vertex and upper frons, usually downward orientated and appressed, and with short, upwards orientated scales on lower frons (Bradley *et al.*, 1973; Common, 1990; Horak, 1991, 1999, 2006; Razowski, 2002; Gilligan *et al.*, 2008). Head capsule sutures reduced or absent (Horak, 1999; Razowski, 2002). Ocellus above each compound eye usually well developed, chaetosemata well-developed, pin-bristle-like, domed, adjacent to ocellus (Fig. 1) (Common, 1990; Horak, 1991, 1999, 2006; Scoble, 1992; Gilligan *et al.*, 2008). Compound eyes spherical with marginal band of microtrichia (Fig. 1) (Common, 1990; Horak, 1991, 1999, 2006; Scoble, 1992).

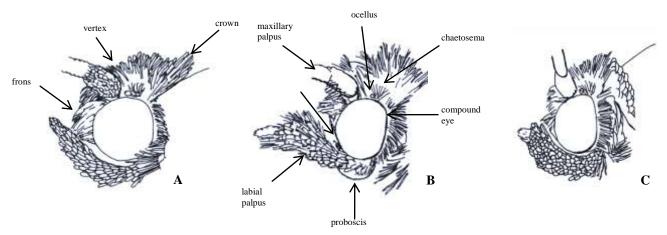


Figure 1: Head of Tortricid moths, a) Tortricinae: Archipini; b) Tortricinae: Tortricini; c) Olethreutinae: Grapholitini – *Cydia pomonella* (Redrawn from Bradley *et al.*, 1973; Figs 8, 11, 14)

Scape and pedicle of antenna densely covered with scales with small intercalary sclerite between basal two antennal segments (Horak, 1991, 2006). Scales on flagellum across dorsal surface, ventral surface unscaled with many sensilla (Common, 1990; Horak, 1991; Gilligan *et al.*, 2008). Scales arranged ventrally as interrupting rings, with one (Olethreutinae) or two (Tortricinae) rings per segment (Horak, 1991, 1999, 2006; Razowski, 2002; Gilligan *et al.*, 2008). Sensory setae (often referred to as cilia) either evenly distributed and short or grouped and longer, varying in size, usually more well developed in males (Horak, 1991, 2006; Razowski, 2002). Males of certain groups with secondary sexual modifications, e.g., a notched or expanded and flattened base of flagellum forming scent organ (Horak, 1991, 1999; Scoble, 1992; Razowski, 2002). Pilifers present and well developed (Horak, 1999; Razowski, 2002).

Proboscis well-developed and naked (unscaled) (Common, 1990; Scoble, 1992; Horak, 2006; Gilligan *et al.*, 2008). Maxillary palpi very small, varying from well-developed, scaled, with one to four segments, to reduced, naked and unsegmented remnants (Fig. 2) (Horak, 1991, 2006). Labial palpi three-segmented, varying in length from short (ca. equal to diameter of compound eye) to very long (ca. 3 times diameter of compound eye), porrect, or ascending (Fig. 2) (Bradley, 1973; Common, 1992; Razowski, 2002). First second (basal) short; second segment long, sinuate and distally widened; third segment short and angled forward and downwards, containing Rath's organ (Horak, 1991, 2006; Razowski, 2002), and frequently concealed by the expanded distal scaling of the second segment.

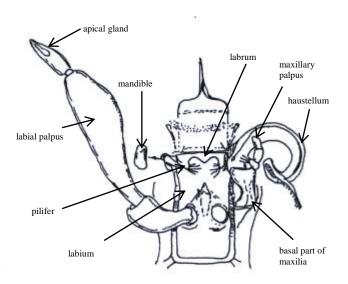


Figure 2: Mouthparts of the Tortricini (Redrawn from Razowski, 2002; Fig 1 – Mouth parts).

Thorax

Thorax mostly smooth scaled, sometimes with single or bipartite posterior crest of raised scales (Common, 1992; Razowski, 2002; Horak, 2006). Patagia (flap-like scales) present on prothorax, well-developed, parapatagia absent (Common, 1991; Horak, 1999).

Legs

Legs well-developed, densely covered with scales, characteristic for ditrysian Lepidoptera (Horak, 1991, 2006). Forecoxa free and undivided, tibia with epiphysis bristled along inner surface, comb-shaped (Bradley *et al.*, 1973; Horak, 1991; 2006; Razowski, 2002). Tibial spur formula 0-2-4 (Common, 1991; Scoble, 1992). Mid and hind coxae firmly fused to thorax, subdivided into anterior eucoxa and posterior meron (Horak, 1991). Apical spurs and specialized spiny scales present on mid tibia (Horak, 1999; Razowski 2002). Hind tibia with two types of spurs (Fig. 3), apical spurs and an additional median spur, scent scales present (Bradley *et al.*, 1973; Horak, 1991, 2006; Razowski, 2002). Males from various Olethreutine groups with modified hind tibiae with various scale modifications (Horak, 2006). Tarsus divided into five segments with spines near apex of segments 1-4, but reduced in some groups, pretarsus complete with two simple claws (Horak, 1991, 2006; Razowski, 2002).

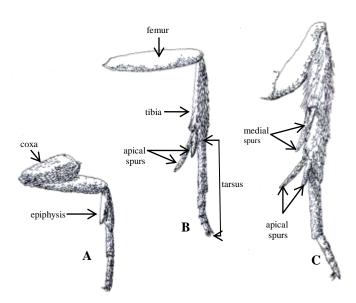


Figure 3: Legs of Tortricinii: A) fore leg, B) middle leg, C) hind leg (Redrawn from Bradley et al., 1973; Figs 15-17).

Wings

Wings well-developed (with some exceptions), shape of wings differing occasionally between sexes of conspecifics as a result of sexual modifications (Horak, 1991; Razowski, 2002). Fore and hind wings of males sometimes with folds associated with scent scales that distribute chemicals that presumably function in short-range courtship behaviour (Common, 1990; Scoble, 1992; Horak 1999, 2006; Razowski, 2002).

Fore wing shape variable: broadly triangular, fairly narrow-triangular, subrectangular, or subovate (Horak, 1999, 2006). Termen varying from nearly right-angled to costa to strongly oblique, sometimes more or less straight or variably sinuate with an indentation beneath apex (Horak, 2006). Pterostigma mostly absent, but with microtrichia, however, only in small dorso-basal area (Common, 1990; Horak, 1999; Razowski, 2002). Most olethreutine groups with smooth, flattened scales on fore wing, but in some groups with raised scale tufts of taxonomic significance (Horak, 2006).

Hind wings usually as broad as fore wing, slightly broader or narrower in different groups (Common, 1990; Horak, 1999). Olethreutinae (and some Tortricinae: Sparganothini) with cubital pecten, a row or tuft of hair scales, on base of hind wings (Common, 1990; Scoble, 1992; Horak, 1999, 2006). Males sometimes with modified anal margin on hind wing, often containing scent-producing scales (Horak, 1999, 2006). Infrequently with small, broad scales on anal margin, causing hind wing to be semi-translucent with areas of melanic scales and scale pencils (Horak, 2006). Cubital pecten, fine row of hair-like scales, present on hindwings of most Oletreutinae (Gilligan *et al.*, 2008).

Wing coupling is accomplished by a frenular-retinacular system, with frenular bristles present at base of hind wing (Fig. 4) locking into a retinaculum (in males as a small membranous hook from subcosta, and in females as erect scales in a row behind base of cubitus) beneath forewing (Common, 1990; Scoble, 1992; Horak, 2006). Frenulum of males a single acanthus, that of females with multiple acanthi, variable from 2-6

(or more), frequently asymmetrical on the same individual (Fig. 4) (Bradley *et al.*, 1973; Common, 1999, Horak, 2006; Monsalve *et al.*, 2011).



Figure 4: Frenular bristles at base of hind wing, A) males, B) females (Redrawn from Bradley *et al.*, 1973; Figs 18, 19).

Wing venation of tortricids typically heteroneurous with hind wing venation reduced to eight veins or radial branches (R-branch) compared to the 12 veins in the fore wing (Fig. 5) (Bradley *et al.*, 1973; Horak, 1991, 2006). Cryptically coloured forewings in most species, bright-coloured in species active during the day (Razowski, 2002). Hindwings usually unicolourus (Bradley *et. al.* 1973).

Detailed descriptions and illustrations of wing venation and patterns are provided by Bradley *et al.*, 1973, Common, 1990; Horak, 1991; Scoble, 1992 and Razowski, 2002 (Figs 5-6, Table 2).

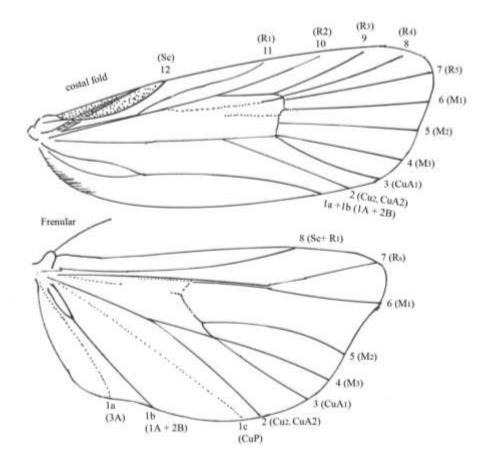


Figure 5: Wing venation of Tortricidae (Redrawn from Razowski, 2002; Fig. 2 Venation of Tortricidae)

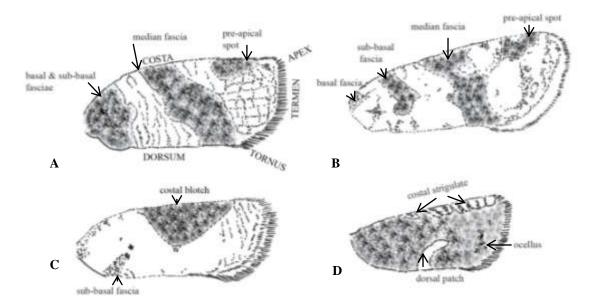


Figure 6: Wing pattern of Tortricidae: A) & B) Tortricini, C) & D) Archipini (Redrawn and adjusted from Bradley *et al.* 1973; Figs 3-5)

Table 2: Wing venation systems according to Razowski (2002) (MS) and Hampson's system (HS) (Bradley *et al.*, 1973).

		Fore	wing	Hind w	ing
Terminology		MS	HS	MS	HS
Subcosta	Sc	M_2	5	M_3	4
		M_1	6	M_2	5
		R_5	7	M_1	6
		R_4	8	Rs	7
		R_3	9	Sc+ R ₁	8
		R_2	10		
		R_1	11		
		Sc	12		
Radial branches	R	M_3	4	CuA ₁	3
Media stem	M	CuA ₁	3	CuA ₂	2
Cubitus anterior	CuA	CuA ₂	2	CuP	1c
Cubitus posterior	CuP	CuP		1A+1B	1b
Anal veins	A	1A+1B	1a+1b	3A	1a

Abdomen

Sternum of first segment absent, S2 with well-developed ventral tortricoid apodemes (Common, 1990; Horak, 1999; Razowski, 2002). Some genera with dorsal pits present on A2 or A3, either singly (fused) or in pairs (Common, 1990, Horak, 1991; Scoble, 1992; Brown & Miller, 1999; Razowski, 2002; Powell & Brown, 2012). Subgenital segments sometimes strongly modified to accommodate secondary sexual scales in males and specialized oviposition scales in females (Horak, 1991; Razowski, 2002). In males, segment A8 often supporting genitalia with distinct scent organs, e.g., coremata (Common, 1992. Horak, 1991,

1999; Razowski, 2002). In females, modified scales form the corethrogyne, a tuft of densely set scales that on segment A7 covers the eggs (Powell, 1976; Common, 1990; Horak, 1991; Razowski, 2002). Pheromone glands are situated in the intersegmental membrane as a dorsal invagination between terga 8-9 (Horak, 1999, Razowski, 2002). Dorsally unspined on segments A9-A11 in males and A8-11 in females (Horak, 1999; Razowski, 2002).

Genitalia

Male and female genitalia (Figs 7-8) are critically important for the identification of tortricids (Horak, 1991).

Male genitalia (Fig.7)

Dorsal tegumen and ventral vinculum originate from tergum and sternum of A9 to form a ring from which different paired processes of the genitalia arise (Horak, 1991; Gilligan et al., 2008). Pedunculi, the base of the tegumen, usually long, with subterminal, inner attachment points of tergal flexors of valva (m₄) (Horak, 1999; Razowski, 2002). Uncus, if present, slender and elongated, with or without processes, or bifid, welldeveloped ventral tuft or brush of setae in some groups (e.g., Archipini), modified by reduction or completely absent in others (Holloway et al., 1987; Common, 1990; Horak, 1991; Razowski, 2002). Socii, membranous to sclerotized setose, drooping (pendulous) lobes or sacs, often reduced, sometimes fused with gnathos, usually with dense setae; more specialized socii partially distinctly sclerotized and erect (Holloway et al., 1987; Common, 1990; Horak, 1999; Razowski, 2002; Gilligan et al., 2008). Gnathos, if present, a pair of lateral arms or bands fused distally, supporting the distal terminus of the anal tube; frequently reduced or absent (e.g. Tortricini), in latter, lobes formed by a distinct subscaphium (Common, 1990; Horak, 1999; Razowski, 2002; Gilligan et al., 2007). Valvae variable in shape, usually simple and symmetrical. Sclerotized costal margin (dorsal edge) and sacculus (ventral edge), in Olethreutinae distal part of valve specialized, densely hairy or spiny (=cucullus), in some groups with membranous pulvinus at inner base of valve (Holloway et al., 1987; Common, 1990; Razowski, 2002). Anellus as folded convex membrane forming transtilla dorsally and a sclerotized juxta ventrally (Razowski, 2002; Gilligan et al, 2008). Transtilla absent in Olethreutinae, present in Tortricinae as sclerotized, spined or membranous band connecting costal parts of valvae (Holloway et al., 1987; Horak, 1999; Razowski, 2002). Vinculum either U- or V-shaped, attached or fused to pedunculi, saccus developed in most Chlidanotini and Hilarographini, absent in most other groups, in Archipini broad flaps formed by laterally emarginated ventral part (Holloway et al., 1987; Horak, 1991; Razowski, 2002). Aedeagus variable, frequently short, curved, loosely attached to juxta by membrane and usually with well-developed coecum penis (lost in Olethreutinae) (Holloway et al., 1987; Common, 1990; Horak, 1999). Juxta a simple shield-shaped plate, aedeagus in Tortricinae attached to juxta by membrane and folded perpendicularly, in Olethreutinae juxta firmly folded and fused with anellus and aedeagus (Common, 1990; Horak, 1991, 1999; Razowski 2002). Vesica or endophallus variably shaped, often minutely spinose, frequently with fixed or deciduous internal spine-like or plate-like cornuti (Holloway et al., 1987; Horak, 1999; Razowski, 2002; Gilligan et al., 2008).

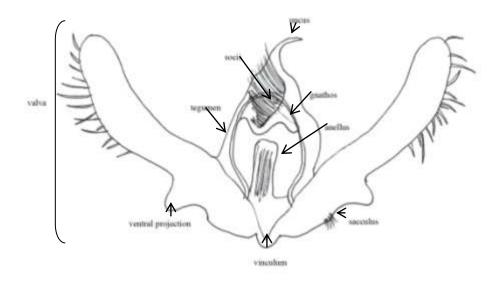


Figure 7: Male genitalia of Oletreutinae (Redrawn from Gilligan et al., 2008; Fig. 11).

Female genitalia (Fig. 8)

Ovipositor short or only slightly telescopic. Papillae anales soft, fleshy, setose (with papillate setae), flattened, lobe-like, more slender in groups adapted to deposit eggs into various crevices (Razowski, 2002); in a few groups (e.g. Tortricini and Cnephasiini) with nail-like setae (i.e. floricomous ovipositor) used to scrape debris over eggs (Holloway et al., 1987; Common, 1990; Scoble, 1992; Horak, 1999; Razowski, 2002, Gilligan et al., 2008). Apophyses ranging from short to very long, one pair connected to papillae anales (apophyses posteriors) and one pair to latero-posterior part of sterigma (Tortricinae) and tergum VIII (apophyses anteriores) (Common, 1990; Horak, 1999; Razowski, 2002, Gilligan et al., 2008). Connection between apophyses and sterigma well sclerotized in almost all Tortricinae (Horak, 1991). Sterigma simple, variously sclerotized, occasionally asymmetrical (anteostial part short and ventral or forming cup and tube with dorsal part), surrounding ostium bursae of sternum 8 (S8) (Common, 1999; Horak, 1999; Razowski, 2002). Sterigma rarely fused to S7 in modified ovipositor of Oletreutinae and membranous junctures with eighth tergite (Horak, 1999; Razowski, 2002, Gilligan et al., 2008). Ostium bursae opening in bursae copulatrix (important taxonomic characteristic) separated into slender ductus bursae and corpus bursae (Horak, 1991, 1999; Razowski, 2002). Corpus bursae usually with sclerotized signum (or signa): a single slender horn with prominent capitulum in Archipini (Tortricinae), a pair of thorn-like spines in Eucosmini and Grapholitini or absent in some Oletreutinae); ductus bursae with colliculum (distal part of ductus bursae, a sclerotized ring) below ostium, sometimes with cestum (inner part of ductus bursae, sclerotized ribbon) (Holloway et al., 1987; Horak, 1991, 1999; Common, 1999; Razowski, 2002). Ductus seminalis, with diverticulum (bulla seminalis, very distinct), connecting bursa to vagina and accessory bursa (corpus bursae with a variously developed diverticulum) variably situated (anterior or posterior, dorsal or ventral) (Horak, 1991; Razowski, 2002 Gilligan et al., 2008). Paired ovaries with four ovarioles (Horak, 1991). Spermatheca and accessory glands structure and arrangement as in higher Lepidoptera (Horak, 1991).

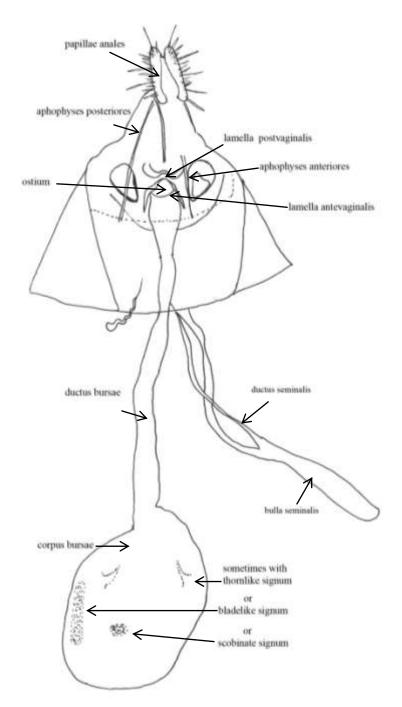


Figure 8: Female genitalia of Oletreutinae (Redrawn from Gilligan et al., 2008; Fig. 13)

1.2.2. Egg

Eggs oval or circular in outline and flat, scale-like, or sometimes slightly convex (Fig. 9) (Common, 1990), with a long horizontal axis and micropyle terminal (Common, 1990; Horak, 1991; Razowski, 2002). Surface reticulated with ridges or finely sculptured chorion (Common, 1990; Horak, 1999; Razowski, 2002). Eggs laid singly, in pairs, groups or overlapping, forming large imbricate masses (Archipini) (Bradley *et al.*, 1973; Powell & Common, 1985; Common, 1990). Eggs sometimes covered with female accessory gland secretion and/or scales from anal tuft or wings (Bradley *et al.*, 1973).



Figure 9: Thaumatotibia leucotreta eggs

1.2.3. <u>Larva</u>

External feeders generally slender, internal feeders stout, three pairs of thoracic legs, and pairs of prolegs each on abdominal segments 3-6 and 10 (Bradley *et al.*, 1973; Horak, 1991). Head semi-prognathous, dark in initial instars, lighter in later instars (Bradley *et al.*, 1973; Common, 1991). Adfrontals extending to epicranial notch (Common, 1990; Horak, 1999). Coronal suture short; six stemmata (often incorrectly referred to as ocelli) on each lateral side (Bradley *et al.*, 1973; Common, 1990; Horak., 1991, 1999; Scoble, 1992; Razowski, 2002). Stemmatal area rounded in most internal feeders (fruit borers), angular in most external feeders (leaf rollers) (Bradley *et al.*, 1973). Darker pigmentation around lateral indentation or towards stemmatal area (Dugdale *et al.*, 2007). Pinacula, anal and prothoracic shield well sclerotized (Bradley *et al.*, 1973; Common, 1990). Cuticle spinulose or granulose with only primary and subprimary setae (Common, 1990; Razowski, 2002).

Prothorax lateral (L-) group trisetose, with three prespiracular setae (Bradley *et al.*, 1973; Common, 1991; Scoble, 1992; Horak, 1999; Razowski, 2002). Subventral group (SV) on T1-3 usually 2:1:1 with two SV setae on prothorax, one on meso- and metathorax (Brown, 1987; Common, 1990; Horak, 1999; Razowski, 2002). Well-developed thoracic legs (Razowski, 2002).

Abdominal integument granulated, usually without markings and secondary setae and usually with strongly sclerotized pinacula (Bradley *et al.*, 1973). Spiracles round to oval, peritreme sclerite well-defined (Bradley *et al.*, 1973). Eight abdominal segments with L¹ and L² setae adjacent, arranged vertically or at an angle on single pinaculum (Bradley *et al.*, 1973; Common, 1990; Scoble, 1992; Horak, 1999; Razowski, 2002). Anal shield sclerotized; anal comb or fork (3-8 straight prongs) on the last abdominal segment (A10) dorsal to anus, lost in a few internal feeders (Bradley *et al.*, 1973; Common, 1990; Scoble 1992; Razowski, 2002). Four pairs of ventral (A3-6) and one pair of anal prolegs (Razowski, 2002). Crotchets of prolegs either circular or eliptical shaped; uni-, bi-, or triordinal (Bradley *et al.*, 1973; Common, 1990; Scoble, 1992; Razowski, 2002). Crotchets on anal prolegs in semi-circle (Scoble, 1992). SV-group trisetose on A3-6

(Horak, 1999). Subdorsal seta (SD¹) directly anterior to spiracle on A8 (Bradley *et al.*, 1973, Common, 1990; Horak, 1999; Razowski, 2002). Dorsal setae D² on A9 usually sharing a single pinaculum forming a "saddle" (Common, 1990; Horak, 1999; Razowski, 2002). D¹ closer to SD¹ (often found on same pinaculum) than D² (Horak, 1999; Dugdale *et al.*, 2005). SD² absent on A9 (Dugdale *et al.*, 2005). According to Dugdale *et al.* (2005), "Anal shield with D² usually anterior to level of SD¹, or at the same level in endophytic larvae with shortened anal shield and with setae D² directed horizontally, not strongly directed dorsally or ventrally with anal combs sclerotized structure present above anus and secondary setae absent."

1.2.4. **Pupa**

Small to medium in size (length 4-15 mm), cylindrical, somewhat rounded-blunt anteriorly, tapered caudad (Patočka & Turčáni, 2005). Distinct setae on clypeus and frons. Hook-shaped caudal and perianal setae on A10 (Patočka & Turčáni, 2005). Adectious, obtect, well sclerotized, fused appendages (wings, legs, etc.), with some moveable abdominal segments (Common, 1990; Scoble, 1992; Horak, 1991, 1999; Gilligan et al., 2008). Head rounded apically and produced to anterior point, or rarely with large spine on frons (Common, 1990; Horak, 1999; Razowski, 2002). Vertex short (Patočka & Turčáni, 2005). Proboscis half as long as distance to wing tips, maxillary palpi present, but alar furrows rarely present (Common, 1990; Horak, 1999; Dugdale et al., 2005). Labial palpi, prothoracic femora and sometimes mesothoracic coxae exposed (Common, 1990; Patočka & Turčáni, 2005). Antenna tapered, shorter than wings, reaching nearly to apices of the forewings (Bradley et al., 1973; Common, 1990; Patočka & Turčáni, 2005). Forewings broad, rounded, extending to A4, and hindwings extending slightly past forewings (Bradley et al., 1973; Common, 1990; Patočka & Turčáni, 2005). Metatarsus visible slightly (Patočka & Turčáni, 2005). Pronotum short, mesonotum sometime with subdorsal longitudinal furrow or dorsal ridge (alar furrow) (Patočka & Turčáni, 2005). Two transverse rows of spines on mid-abdominal segments, the basal uni- or multiseriate and the caudal uniseriate (Patočka & Turčáni, 2005). Abdominal segments (A) fused: A1 and A2 in both male and female, A8-10 in males, and A7-10 in females (Bradley et al., 1973; Horak, 1999; Razowski, 2002; Gilligan et al., 2008). Abdominal segments A4-7 movable in males and A4-6 in females (Komai, 1999; Razowski, 2002). Two rows of dorsal transverse spines on tergites A3-7, variably developed on A2 and A8-10 (Bradley et al., 1973; Common, 1990; Scoble, 1992; Komai, 1999; Razowski, 2002). Along anterior margin of A2 and A3 paired dorsal pits between the anterior dorsal row of spines and anterior margin segments A2 and A3 in a few Archipini and Sparganothini (function unknown) (Scoble, 1992; Horak, 1991, 1999). Terminal segments A7-A10 on ventral side hold the genital pore (on A8 or A9) and anal opening (on A10) (Gilligan et al., 2008). The genital pore is located on A8 in females and A9 in males (Gilligan et al., 2008). A10 often with dorsal spines or transverse row (Patočka & Turčáni, 2005). Cremaster (caudal end) variably developed, short, round or extended with hooked setae or bristles in Tortricinae, usually reduced or absent in Oletreutinae (Bradley et al., 1973; Common, 1991; Scoble, 1992; Komai, 1999; Razowski, 2002).

1.3. Pest status

The main cultivated host plants for the seven tortricid pests that are the focus of this study are listed in Table 3. Basic information on each tortricid pest species is summarized below.

Table 3: Main cultivated host plants for seven tortricid pests damaging fruit crops in South Africa (Summer, 1966; Annecke & Moran, 1982; Barnes, 1991; Victor *et al.*, 1991; Begemann *et al.*, 1998; Newton, 1998; Van den Berg, 2001; Timm *et al.*, 2007, 2008; Stotter 2009).

Host Plants	CM	FCM	MNB	OFM	LM	PLR**	ALR**
Acorn		✓					✓
Almond	√			✓			
Apple	✓	?		✓		✓	✓
Apricot	√	✓		✓		✓	✓
Avocado		✓					
Banana		✓					
Bean		✓					
Cherry				✓			
Coffee		✓					
Cotton		✓					
Flowers						✓	
Grape		✓				✓	
Grapefruit		✓					
Guava		✓					
Lemon		✓					
Litchi		✓	✓		√		
Macadamia		✓	✓		✓		√ *
Maize		✓					
Mandarin		✓					
Mango		✓			✓		
Nectarine	√			✓			
Olive		✓					
Orange		✓					✓
Peach	✓	✓		✓		✓	✓
Pear	✓	✓		✓		✓	
Pea							✓
Persimmon		✓		✓			
Pineapple		✓					
Plum	✓	✓		✓		✓	✓
Pomegranate	√	✓					
Quince	√			✓	√		
Sweet Cherry	✓						
Tangelo		✓					
Tangerine		✓					
Tea		✓					
Walnut	√	✓					

^{*} Unpublished data from van den Berg, 1999, in van den Berg, 2001

^{**} Leafroller feeding mainly on foliage and sometimes on fruit

False codling moth, Thaumatotibia leucotreta

Fuller (1901) was the first to describe the false codling moth (FCM) as a pest of citrus, and he referred to it as the "Natal codling moth" (Gunn, 1921; Aschenborn, 1978; Catling & Aschenborn, 1978; Schwartz, 1981). FCM is indigenous to southern Africa (Stofberg, 1954; Annecke & Moran, 1982). Although initially absent from the Western Cape, it appeared in 1974 in the Clanwilliam district (Newton, 1998). FCM has an extensive host plant range that includes more than 50 cultivated and wild host plants (Gunn, 1921; Aschenborn, 1978; Catling & Aschenborn, 1978; Van der Geest *et al.*, 1991; Bloem *et al.*, 2003; Stotter, 2009). FCM attacks citrus, deciduous subtropical and tropical fruit, and is known as a pest of acorns, walnuts, olives, tea, almonds, and cotton (Table 3) (Gunn, 1921; Catling & Aschenborn, 1978; Bloem *et al.*, 2003; Stotter, 2009). In 1996, Bell & McGeoch (1996) ranked FCM as the ninth worst agricultural pest compared to Moran's (1983) ranking of 14thmost damaging pest. The USA regards an accidental introduction by FCM as the "worst of the worst" threats to the agricultural industry of the United States (ESA, 2003).

Codling moth, Cydia pomonella

Codling moth (CM), an invasive fruit pest to South Africa, originated most likely from Eurasia and was first recorded in Graaff-Reinet around 1885 (Lounsbury, 1898; Newman, 1912; Annecke & Moran, 1982; Barnes, 1991). CM is an important pest to the pome fruit industry not only within South Africa but in temperate regions worldwide (Anonymous, 1906; Barnes, 1980; Phillips & Barnes, 1974; Nel, 1983; Addison, 2005). It has also been recorded as a pest on walnuts and occasionally on almonds, wild almonds, pecan nuts, pomegranates, apricots, peaches, and plums (Table 3) (Phillips & Barnes, 1974; Nel, 1983; Barnes, 1991; Blomefield, 1994). In 1983, CM rated fifth in the 101 most important plant-feeding pests in South Africa (Moran, 1983); in 1996 it was changed to third (Bell & McGeoch 1996). In the 1980's its infestation potential in South Africa was one of the highest in the world (Myburgh, 1980).

Oriental fruit moth, Grapholita molesta

The oriental fruit moth (OFM) is originally from the Orient, Siberia, China, Korea, and Japan, hence the vernacular name (Collins, 1933; Summer, 1966, Rothschild & Vickers, 1991; Alford, 2007). It was positively identified in 1990 in South Africa (Blomefield & Geertsema, 1990; Victor *et al.*, 1991). It has become an important pest on peaches, nectarines, apricots, apples, pears, plums, almonds, and persimmons and some ornamental flowering plants (Collins, 1933; Victor *et al.*, 1991; Alford, 2007). In the season 1990/1991 OFM caused over R1 million rand losses to the peach industry in the Western Cape with some farmers recording up to 70% fruit loss due to damage caused by OFM (Anonymous, 1991; Viljoen, 1992).

Litchi Moth, Cryptophlebia peltastica

The litchi moth (LM) is native to the African continent and has been found in South Africa, Madagascar, Seychelles, and Mauritius (Bradley, 1963; McGeoch, 1993; Krüger, 1998; Timm *et al.*, 2006). Larvae of LM are often found in pods of indigenous trees such as karooboerboon (*Schotia cafra*) and flamboyant

(*Poinciana regia*) (Annecke & Moran, 1982) and also in galls of *Ravenlia macowania* (Uredinales), on *Acacia karoo* and *Uromycladium tepperianum* (Uredinales) on the Port Jackson willow (*Acacia saligna*). In the case of the last host, LM may be considered a biological control agent against this weed pest (McGeoch, 1993; Krüger, 1998; Timm *et al.*, 2006). LM is a pest on litchi and macadamia (Annecke & Moran, 1982; de Villiers, 2001b). In 1996, Bell & McGeoch (1996) ranked it 19th worst pest compared to Moran's ranking in 1983 where it had no pest-status ranking. In Friedenheim, South Africa, an infestation of 8.9% and in other areas up to 15% was recorded on litchi fruit (de Villiers & Stander, 1989; Newton & Crause, 1990).

Macadamia nut borer, Thaumatotibia batrachopa

The macadamia nut borer (MNB) is an Afrotropical moth not yet recorded beyond the African continent (Timm *et al.*, 2006). MNB only has two known host plants in South Africa, macadamia and litchi, but it is the dominant pest on macadamia in South Africa (Timm *et al.*, 2006). The first official record of MNB as a macadamia pest in South Africa was in 1999. However in 1972, an individual of MNB was reared from citrus by E.C.G. Bedford from a larvae found in an unusual infestation in this crop (de Villiers, 2001a). However, since then no further occurrence of MNB on citrus has been reported (Newton, 1998). In Malawi, MNB is a major pest of macadamia and litchi, but it is also found on snot apple (*Azanza garckeana*), Mauritius thorn (*Caesalpinea decapetala*), and feijoa (*Feijoa sellwiana*) (de Villiers, 2001a). A 20% crop loss has been recorded in Malawi on macadamia (de Villiers, 2001a).

Pear leafroller/ Carnation worm, Epichoristodes acerbella

The pear leafroller (PLR), also known as the carnation worm, is also native to South Africa but was accidentally introduced with cuttings to Denmark in the 1960s from where it subsequently spread to various European countries (Thygesen, 1965, quoted in Nuzzaci, 1973; Allen, 1980; Timm *et al.*, 2008). The damage that PLR causes to crops and flowers make it one of the most important pests in South Africa and it is a notorious carnation pest worldwide (Bolton, 1979; Timm *et al.*, 2008). It attacks stone fruit, pome fruit, and grapes, but also feeds on weeds such as false dandelion (*Hypochoeris radiacata*), wild radish (*Raphanus raphinistrum*), sheep sorrel (*Rumex angiocarpous*), and *Arctotheca* sp. (Myburgh & Basson, 1961; Bolton, 1979; Timm *et al.*, 2008). In 1924, 75% of carnation shoots were found to be infested at a local florist in the Eastern Cape (Gunn, 1926).

Apple leafroller, Lozotaenia capensana

The apple leafroller (ALR) is an African insect that is polyphagous; it has been recorded from apples, pears, citrus, peaches, plums, apricots, avocados, granadilla, marula, sometimes garden roses, but also on weeds such as *Arctotheca* sp., false dandelion (*Hypochoeris radiacata*), wild radish (*Raphanus raphanistrum*), *Rumex acetocella*, and *Tacsonia nollissima* (Myburgh & Basson, 1961; Borrow, 1977; Annecke & Moran, 1982; Begemann *et al.*, 1998; van den Berg, 2001). It has also been reared on *Pinus radiata* (Geertsema, pers. comm), and unpublished data from van den Berg showed ALR infestation in macadamia (van den

Berg, 2001). In 1996, Bell & McGeoch (1996) determined the pest status for ALR, ranking it 11th compared to Moran's (1983) ranking where it was placed 22nd. In 1976, in the Zebediela area the total amount of citrus fruit lost due to ALR was 32.4% and of the Valencia's cultivar 43.4% (Begemann *et al.*, 1998).

1.4. Aims and objectives

The aim of the present study was to establish an easy identification system for tortricid moth pests of fruit, including the construction of interactive taxonomic keys of all life stages with associated morphological information.

Objectives:

- To collate and analyse all available literature on seven major species of economic importance and to compile more comprehensive morphological descriptions;
- ii) to develop an interactive key based on LUCID software using morphological data (published and own study) of local and potential invasive tortricid moth species for use by fruit industry stakeholders; and
- iii) to determine the species status of *T. leucotreta*, using male genitalia and shape morphometrics.

The chapters of this thesis are structured as individual papers and therefore some repetition is unavoidable.

1.5. References

- Addison, M.F. (2005). Suppression of codling moth *Cydia pomonella* L. (Lepidoptera: Tortricidae) populations in South African apple and pear orchards using sterile insect release. *Acta Horticulturae* **671**: 555-557.
- Alford, D.V. (2007). Pests of Fruit Crops- A color handbook. Academic Press, Boston, USA.
- Allen, A.A. (1980). *Epichoristodes acerbella* (Lepidoptera: Tortricidae) first capture of the imago at large in Britain, U.K. *Entomologist's Record and Journal of Variation* **92**: 33.
- Amante, V. dR. & Norton, G.A. (2003). Developing interactive diagnostic support tools for tropical root crops. *Third Taro Symposium, Proceedings of an International Scientific Meeting jointly organized by the Secretariat of the Pacific Community and the International Plant Genetic Resources Institute,* Fiji Islands.
- Annecke, D.P. & Moran, V.C. (1982). *Insects and mites of cultivated plants in South Africa*. Butterworths, Durban, 383 pp.
- Anonymous (1906). Appendix IV, Report of the (Cape)Government Entomologist for the year 1906.
- Anonymous (1991). Oosterse vrugtemot nou orals in Wes-Kaap. Agricultural News, 24 June 1991.

- Aschenborn, H. (1978). False Codling moth, *Cryptophlebia leucotreta* Meyr. *Farming in South Africa*, H.36.
- Barnes, B.N. (1980). Codling moth in Apples, Farming in South Africa. G5.
- Barnes M.M. (1991). Codling moth occurrence, host race formation, and damage. In: *World Crop Pests*. *Tortricid Pests*. Van der Geest, L.P.S. & Evenhuis, H.H. (eds). Elsevier, Amsterdam, pp. 23-48.
- Begemann, G.J., Barrow, M.R. & Bedford, E.C.G. (1998). Lepidoptera: Butterflies and moths. Family Tortricidae. Apple leaf roller *Tortrix capensana* Walker, pp. 200-207 In: *Citrus Pests in the Republic of South Africa*. 2nd ed. (revised). E.C.G. Bedford, M.A. van den Berg & E.A. de Villiers (eds.). Institute for Tropical and Subtropical Crops, Nelspruit.
- Bell, J.C. & McGeoch, M.A. (1996). An evaluation of the pest status and research conducted on phytophagous Lepidoptera on cultivated plants in South Africa, *African Entomology* **4**(2): 161-170.
- Bloem, S., Carpenter J.E, & Hofmeyr, J.H. (2003). Radiation biology and inherited sterility in false codling moth (Lepidoptera: Tortricidae). *Journal of Economic Entomology* **96** (6): 1724-31.
- Blomefield, T.L. & Geertsema, H. (1990). First record of the Oriental Fruit moth, *Cydia molesta* (Lepidoptera: Tortricidae: Olethreutinae), a serious pest of peaches, in South Africa. *Phytophylactica* **22**: 355-357.
- Blomefield, T.L. (1994). Codling moth resistance: is it here, and how do we manage it? *Deciduous Fruit Grower* **44**: 130-132.
- Bolton, M.C. (1979). Some effects of temperature, humidity, and larval diet on the cycle of he South African carnation worm *Epichoristodes acerbella* (Lepidoptera, Tortricidae). *Journal of the Entomological Society of Southern Africa* **42**: 129-136.
- Barrow, M. R. (1977). *The Biology, Economic Significance and Control of* Tortrix capensana (*Walker*) (*Lepidoptera: Tortricidae*) on Citrus at Zebediela Estate. University of Natal, Pietermaritzburg (MSc Thesis), Pietermaritzburg.
- Bradley, J.D. (1963). Some important species of the genus *Cryptophlebia* Walsingham, 1899, with descriptions of three new species (Lepidoptera: Olethreutidae). *Bulletin of Entomological Research* **43**: 679-689, pl. XXIV-V.
- Bradley, J.D., Tremewan, W.G. & Smith, A. (1973). *British Tortricoid Moths-Cochylidae and Tortricidae: Tortricinae*, Ray Society, London, pp. 6-20.
- Brown, R. L. (1987). Tortricidae (Tortricoidea), pp. 419–433. In: Stehr, F. W. (ed.), *Immature Insects*, volume 1. Kendall/Hunt, Dubuque, Iowa.
- Brown, J. W. (2005). World Catalogue of Insects Volume 5, Tortricidae (Lepidoptera), Apollo Books, Stenstrup, Denmark, 741 pp.

- Brown, J. W. & S. E. Miller. (1999). A new species of *Coelostathma* (Lepidoptera: Tortricidae) from Cocos Island, Costa Rica, with comments on the phylogenetic significance of abdominal dorsal pits in the Sparganothini. *Proceedings of the Entomological Society of Washington* **101**: 701-707.
- Catling, H.D. & Aschenborn, H. (1978). False codling moth *Cryptophlebia leucotreta* Meyr. *In*: Citrus pests in the Republic of South Africa (ed. E.C.G. Bedford), pp. 165-170. *Science Bulletin*. *Department of Agricultural Technical Services Republic of South Africa*, No. **391**: 165-170.
- Collins, C.W. (1933). The Oriental Moth (*Cnidocampa flavescens* Walker) and its control. *United States Department of Agriculture, Circular nr.* 277, Washington, D.C.
- Common, I.F.B. (1990). *Moths of Australia*, Melbourne University Press, Melbourne, pp. 274-281.
- Daly, H.V. (1985). Insect morphometrics, Annual Review of Entomology 30: 415-438.
- Dampf, A. (1908). Über den Genitalapparat von *Rhopobota naevana* Ha. (Lep., Tortricidae) nebst Bemerkungen zur Systematik der Olethreutinae. *Deutsche Entomologische Zeitschrift Iris* **21**: 304-329.
- de Villiers, E.A. & Stander, G.N. (1989). Proefresultate met triflumuron vir die beheer van die lietsjiemot. South African Litchi Growers' Association Yearbook 2: 20-21.
- de Villiers, E.A. (2001a). Macadamia nut borer. In: *Pests and beneficial arthropods of tropical and noncitrus subtropical crops in South Africa*. Van den Berg, M.A., de Villiers, E.A. & Joubert, P.H. (eds). Institute for Tropical and Subtropical Crops, ARC, Nelspruit, South Africa, pp. 317-320.
- de Villiers, E.A. (2001b). Litchi moth. In: Pests and beneficial arthropods of tropical and non citrus subtropical crops in South Africa. Van den Berg, M.A., de Villiers, E.A. & Joubert, P.H. (eds). Institute for Tropical and Subtropical Crops, ARC, Nelspruit, South Africa, pp. 325-328.
- Dugdale, J.S., Gleeson, D., Clunie, L.H. & Holder, P.W. (2005). A diagnostic guide to Tortricidae encountered in field surveys and quarantine inspections in New Zealand: Morphological and Molecular characters. Ministry of Agriculture and Forestry, Wellington, New Zealand, pp. 24-28.
- Entomological Society of America (ESA) (2003). List of Exotic Insect Species that are of significant economic concern to the United States.
- Gilligan, T.M., Wright, D.J. & Gibson, L.D. (2008). *Olethreutine Moths of the Midwestern United States*. *An Identification Guide*. Ohio Biological Survey, Bulletin New Series, Volume XVI Number 2. Columbus, USA, 334 pp.
- Gunn, D. (1921). The false codling moth (*Argyroploce leucotreta Meyr.*). Scientific Bulletin of Agriculture and Forestry, Union of South Africa **21**: 1-28.
- Gunn, D. (1924). The carnation worm, Union of South Africa, Dept. of Agriculture, Bulletin 18.
- Holloway, J.D., Bradley, J.D. & Carter, D.J. (1987). CIE Guides to insects of importance to man Lepidoptera, C.A.B. International, Wallingford, pp. 129-131.

- Horak, M. (1984). Assessment of taxonomically significant structures in Tortricinae (Lep., Tortricidae), Mitteilungen der Schweizerischen Entomologischen Gesellschaft 57: 3-64.
- Horak, M. (1999). The Tortricoidea in: *Handbuch der Zoologie: Lepidoptera, Moths and Butterflies, Volume 1: Evolution, Systematics, and Biogeography,* Walter de Gruyter, Berlin, pp.199-215.
- Horak, M. (2006). Olethreutine Moths of Australia (Lepidoptera: Tortricidae), CSIRO Publishing, 472 pp.
- Horak, M. & Brown, R.L. (1991). Taxonomy and Phylogeny in: *World Crop Pests: Tortricid Pests*, Editors: van der Geest, L.P.S. & Evenhuis, H.H. Elsevier, Amsterdam, p. 23.
- Karisch, T. (2003). Zur Schmetterlingsfauna von St Helena, 2. Teil: Kleinschmetterlinge (1) (Insecta:Lepidoptera: Tortricidae, Glyphipterigidae, Cosmopterigidae, Plutellidae, Pterophoridae). Linzer biologische Beiträge 35: 1081-1085.
- Kennel, J. (1908). Die Palaearktischen Tortriciden [1. Teil], Zoologica, 54, 727 pp.
- Komai, F. (1999). A taxonomic review of the genus *Grapholita* and allied genera (Lepidoptera: Tortricidae) in the Palaearctic region. *Entomologica Scandinavica Supplement* **55**: 1-226.
- Krüger, M. (1998). Identification of the adults of Lepidoptera inhabiting *Ravenelia macowiana* Pazschke (Uredinales) galls on *Acacia karroo* Hayne (Fabaceae) in southern Africa. *African Entomology* **6**: 55-74.
- Lounsbury, C.P. (1898). Codling moth. Agricultural Journal, 13: 597-616.
- MacKay, M.R. (1959). Larvae of the North American Olethreutidae (Lepidoptera). *The Canadian Entomologist*, supplement 10, Volume XCI.
- McGeogh, M.A. (1993). The Microlepidoptera associated with a fungus gall on *Acacia karroo* Hayne in South Africa. *African Entomology* **1**: 49-56.
- McGeogh, M.A. & Krüger, M. (1994). Identification and diagnoses of Lepidoptera larvae inhabiting galls introduced by *Ravenelia macowaniana* Pazschke on *Acacia karroo* Hayne in South Africa. *African Entomology* **2** (1): 37-43.
- Meyrick, E. (1895). *A Handbook of British Lepidoptera*. Macmillan and Co, London and New York, 743 pp.
- Monsalve, S., Dombroskie, J., Lam, W., Rota, J. & Brown, J. W. (2011). Variation in the female frenulum in Tortricidae (Lepidoptera). Part 3. Tortricinae. *Proceedings of the Entomological Society of Washington* **113**: 335-370.
- Moran, V.C. (1983). The phytophagous insects and mites of cultivated plants in South Africa: pattern and pest status. *Journal of Applied Ecology* **20**: 439-450.

- Mutanen, M. & Pretorius, E. (2007). Subjective visual evaluation vs. traditional and geometric morphometrics in species delimitation: a comparison of moth genitalia. *Systematic Entomology* **32**: 371-386.
- Myburgh, A.C. & Basson, S.G. (1961). *Tortrix capensana* (Wlk.) and *Epichorista ionephela* (Meyr.) as pests new to apples and pears (Lepidopt.: Tortricidae). *Journal of the Entomological Society of Southern Africa* **24**: 348-349.
- Myburgh, A.C. (1980). Infestation potential of codling moth. *Deciduous Fruit Grower* **30**: 368-377.
- Nel, P.J. (1983). Insect pests Codling moth. *Deciduous fruits and vines Pests and diseases and their control*, David Philip, Cape Town, pp. 1-7.
- Newman, L.J. (1912). Descriptive account of Codling Moth, together with notes on its controls, *Bull 30 Department of Agriculture and Industries*, Division of Botany and Plant Pathology, Entomological Section, Western Australia, Government Printers Perth, p11.
- Newton, P.J. (1998). False Codling Moth, *Cryptophlebia leucotreta* (Meyrick). pp. 192-200. In: *Citrus Pests in the Republic of South Africa*. 2nd ed. (revised). E.C.G. Bedford, M.A. van den Berg & E.A. de Villiers (eds.). Institute for Tropical and Subtropical Crops, Nelspruit.
- Newton. P.J. & Crause, C. (1990). Oviposition on *Litchi chinensis* by *Cryptophlebia* species (Lepidoptera: Tortricidae). *Phytophylactica* **22**: 365-367.
- Nuzacci, G. (1973). Epichoristodes acerbella (Lepidoptera, Tortricidae). Entomologica (Bari) 9: 147-148.
- Osborne, D.V. (1963). Some aspects of the theory of dichotomous keys. New Phytologist 62 (2): 144-160.
- Patočka. J. & Turčáni. M. (2005). *Lepidoptera Pupae, Central European Species, Text Volume, Apollo Books, Stenstrup, pp. 187, 238-242.*
- Phillips, P.A. & Barnes, M.M. (1974). Host race formation among sympatric apple, walnut, and plum populations of the codling moth, *Laspeyresia pomonella*. *Annals of the Entomological Society of America* **68** (6): 1053-1059.
- Pinhey, E.C.G. (1975). Moths of Southern Africa, Tafelberg Publishers, Cape Town, pp. 37-38.
- Pierce, F. N. & J. W. Metcalfe. (1922). The genitalia of the group Tortricidae of the Lepidoptera of the British Islands. Oundle, Liverpool, England, 101 pp.
- Powell, J.A. (1964). *Biological and taxonomic studies on tortricine moths, with reference to the species in California*, University of California Press, Berkeley and Los Angeles, pp. 317.
- Powell, J. A. (1976). Oviposition behavior of *Templemania* (Lepidoptera: Tortricidae). *Pan-Pacific Entomologist* **52**: 91-92.

- Powell, J. A. & I. F. B. Common. (1985). Oviposition patterns and egg characteristics of Australian Tortricine moths (Lepidoptera: Tortricidae). *Australian Journal of Zoology* **33**: 179-216.
- Powell, J. A. and J. W. Brown. (2012). Tortricoidea, Tortricidae (part): Tortricinae (part): Sparganothini and Atteriini. In Hodges, R. W. (ed), The Moths of North America, fascicle 8.1. Wedge Entomological Research Foundation, Washington, DC, 230 pp.
- Razowski, J. (2002). *Tortricidae (Lepidoptera) of Europe, Volume 1*, Tortricinae and Chlidanotinae. František Salmka, Bratislava, Slovakia, pp. 9-13.
- Rothschild, G.H.L. & Vickers, R.A. (1991). Biology, Ecology and Control of the Oriental Fruit Moth in: *World Crop Pest: Tortricid Pests*, Editors: van der Geest, L.P.S. & Evenhuis, H.H. Elsevier, Amsterdam, pp. 389-411.
- Schwartz, A. (1981). 'n Bydrae tot die biologie en beheer van die valskodlingmot, *Cryptophlebia leucotreta* (Meyr.)(Lepidoptera: Eucosmidae) op nawels. PhD (Agric) Thesis, University of Stellenbosch.
- Scoble, M.J. (1992). The Lepidoptera: Form, Function and Diversity, Oxford University Press, New York.
- Stofberg, F.J. (1954). False codling moth of citrus. Farming in South Africa 29: 273-276, 294.
- Stotter, R.L. (2009). Spatial and temporal distribution of False Codling Moth across landscapes in the Citrusdal area (Western Cape Province, South Africa). MSc-Thesis, University of Stellenbosch, pp. 101.
- Summer, M.F. (1966). The oriental fruit moth in California. California Agricultural Experimental Station, Circulation 539, 18 pp.
- Thygesen, T. (1965). Jagttagelser over *Epichorista inophela* (Meyr.), en sydafrikansk viklerart på nellike. *Biologi og bekaempelse – Tidsskrift. PlaAvl*, 69.
- Timm, A.E. (2005). Morphological and molecular studies of Tortricid moths of economic importance to the South African fruit industry. Phd (Agric) dissertation, Stellenbosch University.
- Timm, A.E., Geertsema, H. & Warnich, L. (2006). Analysis of population genetic structure of two closely related tortricid species of economic importance on macadamias and litchis in South Africa.

 **Agricultural and Forest Entomology 8: 113-119.
- Timm, A.E. Warnich, L. & Geertsema, H. (2007). Morphological and molecular identification of economically important Tortricidae (Lepidoptera) on tropical and subtropical fruit in South Africa. *African Entomology* **15** (2): 269-286
- Timm, A.E., Warnich, L. & Geertsema, H. (2008). Morphological and molecular identification of economically important Tortricidae (Lepidoptera) on deciduous fruit tree crops in South Africa. *African Entomology* **16**: 209-219.

- Timm, A.E., Geertsema. H. & Warnich. L. (2010). Population genetic structure of economically important Tortricidae (Lepidoptera) in South Africa: a comparative analysis. *Bulletin of Entomological Research* **100**: 421-431.
- Van der Geest, L.P.S., Wearing, C.H. & Dugdale, J.S. (1991). Tortricids in Miscellaneous Crops In: World crop pests: Tortricid pests. Van der Geest, L.P.S. & Evenhuis, H.H. (eds). Elsevier, Amsterdam, pp. 563-571.
- Van den Berg, M.A. (2001). *Tortrix capensana* Walker, Apple leaf roller, In: *Pests and beneficial arthropods of tropical and noncitrus subtropical crops in South Africa*. Van den Berg, M.A., de Villiers, E.A. & Joubert, P.H. (eds). Institute for Tropical and Subtropical Crops, ARC, Nelspruit, South Africa, pp. 317-320.
- Victor, W., Smit, D. & Chambers, K. (1991). Oosterse vrugtemot. Ander peste en plae kan volg. *Deciduous Fruit Grower* **41**: 202-208.
- Viljoen, B. (1992). Oriental fruit moth combatted successfully. Farmers Weekly, 25 September 1992.
- Walters, D. E. & Winterton, S. (2007). Keys and the Crisis in Taxonomy: Extinction or Reinvention? *Annual Review of Entomology* **52**: 193-208.

CHAPTER 2

Morphological study and development of a taxonomic key for the larval stages of economic importance tortricids in South Africa

2.1. Abstract

Cydia pomonella, Thaumatotibia leucotreta, Grapholita molesta, Cryptophlebia peltastica, Thaumatotibia batrachopa, Epichoristodes acerbella and Lozotaenia capensana are the seven most economically important tortricids in South Africa, causing extensive damage and losses to the fruit industry. The correct identification of these species in the larval stage is important as misidentifications could lead to ineffective pest management. In South Africa no comprensive, accuarate or user-friendly key exists to distinguish between these species. Thus the aim was to compile, from an extensive literature study and own morphological analyses of the larvae, descriptions of the larval stages of each species and to develop an interactive LUCID key.

2.2. Introduction

Worldwide, many tortricid species are of economic importance as the family includes some of the most significant agricultural pests (Powell, 1964; MacKay 1959; Holloway et al., 1987; Razowski, 2002; Timm, 2005). In South Africa, seven tortricid species are of economic significance, primarily to the fruit industry (Timm, 2005): Cydia pomonella (Linnaeus, 1758) (Codling moth), Thaumatotibia leucotreta (Meyrick, 1913) (False codling moth), Grapholita molesta (Busck, 1916) (Oriental fruit moth), Cryptophlebia peltastica (Meyrick, 1921) (Litchi moth), Thaumatotibia batrachopa (Meyrick, 1908) (Macadamia nut borer), Epichoristodes acerbella (Walker, 1864) (Pear leafroller/Carnation worm), and Lozotaenia capensana (Walker, 1863) (Apple leafroller) (Brown, 2005). The larvae of all these species feed on a range of cultivated crops causing extensive damage and loss to fruit production (Powell, 1964; Timm, 2005). MacKay (1959) mentioned larvae of North America Olethreutinae are rather similar in apperance and are thus often confused or misidentified. Identification keys for tortricid species in South Africa are currently unsatisfactory because they are incomplete. McGeoch & Krüger (1994) developed a key for identifying moth larvae associated with Ravenelia galls on Acacia karroo, and that study included one economically important tortricid species, C. peltastica. Unfortunately, McGeoch & Krüger (1994) provided no morphological description for C. peltastica. Timm (2005) produced a dichotomous key to distinguish among the larvae of six economically important tortricid species in South Africa, but for some of these species a complete description for all the life stages is lacking. Although dichotomous keys continue to play a major role for the identification of species (Osborne, 1962), they often leave the user without a satisfactory identification. In a dichotomous key each couplet addresses a feature for which there are two possible answers of contrasting characters (Osborne, 1962; Amante & Norton, 2003; Walters & Winterton, 2007). The answer chosen directs the user to either another couplet or to an end point (Amante & Norton, 2003; Walters & Winterton, 2007). A major problem with dichotomous keys is the "unanswerable couplet." An unanswerable couplet is that for which the user is unable to choose among the two options provided and thus cannot continue with the key (Amante & Norton, 2003; Walters & Winterton, 2007). Matrix keys, such as LUCID key, are interactive and allow the user to select more than one character or skip characters and still get an end solution (Amante & Norton, 2003; Walters & Winterton, 2007). The main objective of this study was to develop descriptions of the larval stages of each species so that they can be compared and distinguished, and then to develop an interactive key, using the LUCID Key program, for use by workers in the fruit industry.

2.3. Material and methods

Literature of various authors were studied, summarized (Table 4; Appendix 2.1), and compared for descriptions of the larval stages of *T. leucotreta*, *C. pomonella*, *T. batrachopa*, *G. molesta*, *C. peltastica* and *E. acerbella*. The descriptions were then compared with specimens of larvae in an effort to fill in gaps in the various descriptions based on own morphological studies (see Results). The morphological characteristics described in this chapter may be less than comprehensive for internal or inconspicuous features, the goal, being the development of a Lucid key for the specific use by personnel in the fruit industry.

2.3.1. Insect material

Final instar larvae of *T. leucotreta*, *C. pomonella*, *C. peltastica*, and *G. molesta* were obtained from established laboratory colonies: *T. leucotreta* from XSIT, Citrusdal, South Africa and Rhodes University, Grahamstown, South Africa; *C. pomonella* from Entomon, Stellenbosch, South Africa; *C. peltastica* from Bioriver Science, Addo, South Africa; *G. molesta* from Embrapa Grape and Wine, Bento Gonçalves, Brazil and the Applied Entomology Department in the Institute of Agricultural Research, Eidgenössische Technische Hochschule Zürich, Switzerland; and *T. batrachopa* from Upotn farm, Umhlali, South Africa. Additional larvae were collected locally from *Acacia saligna* and *A. pygnantha* galls. Unfortunately, no larvae of *L. capensana* were obtained. Voucher specimens were deposited in the Entomological Museum of the Stellenbosch University, South Africa.

2.3.2. Preparation of specimens

Larvae were killed in boiling water so as to extend the specimens, injected with Kahle's Fluid as a preservative, and stored as reference material. Kahle's fluid was selected for its better colour retention of specimens.

2.3.3. Preparation of images

Photos of larvae were taken with a Leica MZ 16A automontage microscope for two dimensional image analysis with a Leica DFC 290 fixed digital camera and Leica Application Suite (LAS) v.2.7. software. Photos were edited in Adobe Photoshop Element v.9.0.0 (Adobe System Incorporated).

2.3.4. Nomenclature

Nomenclature for the setal arrangements of the head capsule follows Heinrich (1916 (in Stehr, 1987)) and Swatschek (1958) (Fig. 10), that of the thorax and abdomen follows Hinton (1946; in Stehr, 1987) (Fig. 11), and that of the anal shield follows MacKay (1959) (Fig. 12). Figure 2 is adapted from MacKay (1959) and Stehr (1987). The style and format of the descriptions follows Timm *et al.* (2007, 2008) for uniformity.

Diagnostic characteristics for this study are based on MacKay (1959). However, MacKay did not include the chaetotaxy of the mandibles, which was included by Timm *et al.* (2007, 2008) and in this study.

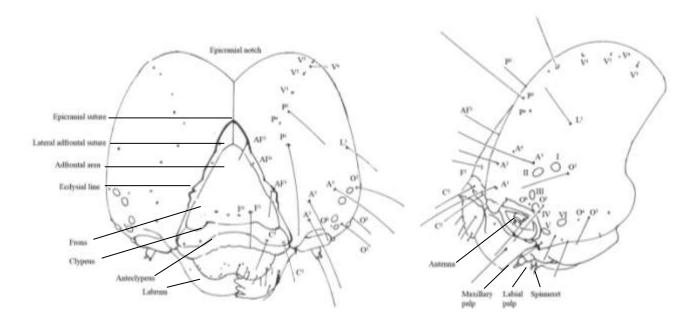


Figure 10: Head capsule, frontal and lateral view (Redrawn from Stehr, 1958; Figs 26.1, 26.2, adjusted with Swatschek, 1958;

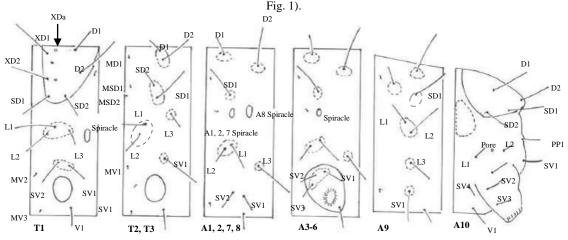


Figure 11: Lepidoptera setal maps (Redrawn from Stehr, 1958; Figs 26.20-26.25, and adapted from MacKay, 1959; Fig. 1).

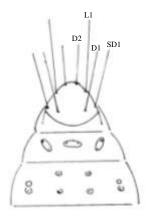


Figure 12: A8-A10 dorsal view (Redrawn from MacKay, 1959; Fig. 1).

2.3.5. Key development

A diagnostic key was developed using LUCID key 3.5.2 (Lucid, The University of Queensland), based on a key developed by Gilligan & Epstein (2012).

2.4. Results

2.4.1. Thaumatotibia leucotreta (Figure 13)

General: Setal bases medium to dark brown. Pinacula convex and distinct.

Head: Chaetotaxy: Stemma II closer to stemma I than III by approximately half the diameter of II. Distance between stemma II and III more than half the diameter of II. Stemma IV much closer to III than to V. O^2 anteroventral to stemma I. Line joining O^1 and A^2 closer to stemma II than to III. Line joining O^1 and A^1 and O^1 and O^1 and O^1 and O^2 and O^2 and O^3 nearly at a right angle.

Thorax: Prothorax: Spiracle rounded, larger than those on A1-8. Prothoracic shield: Pale brown to brown, either unicolorous or with posterior shading. Peritreme of spiraculum large, medium to dark brown. L-group pinaculum darker than other pinacula. Chaetotaxy: XD¹ and XD² similar in length, in line with SD¹. D¹ very short, D² equal or slightly longer than SD¹ and longer than XD¹ and XD². SD² approximately half length of SD¹, situated at an upwards angle posterior to SD¹. L¹ longest, L² shortest, L³ less than half as long as L¹, longer than L². SV¹ long, SV² half length of SV¹. V¹'s tiny; close together, slightly medial to coxa. Distance between coxa and V¹ greater than between V¹s. Meso- and metathorax: Chaetotaxy: D-group pinaculum in line with SD group. D¹ short, less than half length of D². D² as long as SD¹. SD² equal in length to D¹. L¹ long, L² and L³ equal in length. L³ situated on a separate pinaculum, larger than L¹ and L² pinaculum. SV¹ slightly longer than L¹. V¹s further apart than on prothorax. Thoracic legs: Seven coxal setae, one minute, five medium, varying in length, and one long seta. One pair of setae situated distally on mesal part of femur. Six setae arranged around tibia; two pairs of setae, one pair longer, situated dorsally, and shorter pair ventrally on tarsus.

Abdomen: Chaetotaxy: SD^1 on A1-7 situated dorsally to spiracle. On A8, SD^1 anterior to spiracle. A1-7: On A3-6, D^1 less than half length of D^2 , in line with SD^1 , spiracle, and L-group. D^2 longer than SD^1 . SD^1

long, SD^2 minute, found mostly on same pinaculum, but sometimes on separate pinaculum. L^1 long, L^2 short, L^3 similar in length to L^1 ; L^1 - L^2 and L^3 pinacula equal in size. V^1 s on A1-2 closer together than on A3-6, farther apart than on prothorax. Distance between V^1 s on A3-6 equal to distance between V^1 on meso- and metathorax. V^1 s on A7 equal distance as on A1-2. <u>A8:</u> SD^1 and SD^2 situated anterior to spiracle, SD^1 equal in length to D^1 and SD^2 minute. L^1 longer than L^3 but slightly shorter than SD^1 . L^2 shortest. SV^1 long, SV^2 1 / 3 of SV^1 . V^1 s further apart than on prothorax. <u>A9:</u> D^2 s sharing a pinaculum forming a "saddle" across mid-dorsum of segment. D^2 equal in length to SD^1 . D^1 on same pinaculum as SD1, length half of SD^1 . SD^2 absent. L-group trisetose. L^1 approximately equal in length to SD^1 . L^2 shortest, L^3 slightly longer than L^2 . SV^2 absent, SV^1 slightly shorter than L^1 . V^1 s further apart than on A8.

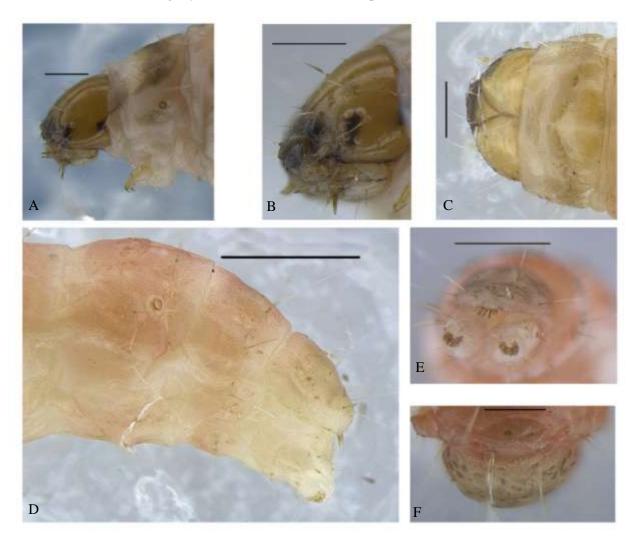


Figure 13: *Thaumatotibia leucotreta* larvae, A) lateral view of head and T1; B) stemmata; C) dorsal view of head and prothoracic shield, D) lateral view of A8 – A10; E) anal fork; F) dorsal view of A9 and anal shield. Scale: D = 1mm, A-C, E-F = 0.5mm.

2.4.2. Cydia pomonella (Figure 14)

General: Larvae slender with a rugose integument. Spiracle, rounded, light brown. Pinacula body colour to slightly dark greyish brown.

Head: Dark pigmented area at the postgenal juncture (posteriorly to the stemmatal area). Chaetotaxy: Stemma VI more oval compared to other stemmata (rounded). Stemma III closer to IV than to II. Stemma V slightly closer to IV than to VI. Stemma IV separated from stemma VI by distance nearly half diameter of IV. Stemma V separated from VI by distance slightly more than half diameter of V. A³ slightly closer to A² than to L¹. Lines joining O¹ and A¹ equidistant to slightly closer to stemma III than to II. Lines joining O¹ and L¹ through median of stemma I. Lines joining O¹ and A² through median of stemma II. Antenna elongate, shorter, less robust than labial palps, with long terminal seta. Five mandibular teeth, outer three large and pointed, second largest, fourth smaller, fifth smallest and bluntly rounded. Mandibular teeth often appear blunt in more mature larvae.

Thorax: Pinacula body coloured to slightly dark greyish brown, setal bases with light brown colouration. Prothorax: **Prothoracic shield:** Body colour to slightly darker greyish brown. **Chaetotaxy:** XD¹ and XD² equal in length. XD^a posterior to XD¹ and distance between XD^a and XD¹ equal to diameter of pinaculum of XD¹. XD^b posteriodorsal to XD¹, separated by four and a half times pinaculum diameter of XD¹. XD¹ and XD² equal in length. SD¹ long, SD² short, XD² longer than SD² but shorter than SD¹. D¹ vertically posteriorly to D^2 above the level of XD^1 , setae equal or slightly longer than SD^1 . D^2 short, equal to SD^2 . XD¹, XD² and SD¹ in line. Spiracle pale brown to brown. L-group pinaculum not extending below spiracle and darker than other pinacula. L¹ longer than SD¹. L² short, L³ very short. SV² more less than half as long as SV¹. V¹s situated between thoracic legs and closer together than on meso- and metathorax. Meso- and metathorax: D¹ and D² dorsal to SD-group, in vertical position, D² longer than D¹. SD¹ longer than SD², but shorter than D^2 . SD^2 and D^1 equal in length. L^1 and L^2 on one pinaculum, anteroventral to SD-group and L^3 . L^3 situated posterioventral to SD-group and posteriodorsal to L^1 and L^2 . L^3 pinaculum larger than L^1 and L^2 . L^2 and L^3 equal in length. SV^1 long, posteriodorsal to thoracic legs and in line with L^3 pinaculum. V^1 s on meso- and metathorax equidistant, not fused to coxae. Thoracic legs: Seven setae, situated on coxa, one very tiny, five medium, varying in length, and one long. One pair setae situated distally on mesal part of femur. Six setae arranged around tibia; two pairs of setae. Claws curved.

Abdomen: Chaetotaxy: SD^1 and SD^2 with common pinaculum on abdominal segments, but SD^2 on A1-8 reduced or absent. A1-7: D^1 shorter than D^2 . SD^1 long, sharing pinaculum with SD^2 , the latter tiny, anteroventral to SD^1 . L^1 shorter than L^2 , sharing a pinaculum, L^2 and SV^1 equal in length. L^3 slightly shorter than L^2 , closer to SV^1 and in line with D^2 . SV^2 and SV^3 equal in length. SV^3 on A7 occasionally absent. SV^2 anterior ventrad to SV^1 . SV^3 anterior-dorsad to SV^1 . SV^1 twice as long as SV^2 or SV^3 . A8: D^1 and D^2 pinaculum in line. D^1 shorter than D^2 . SD^1 slightly shorter than L^2 . SD^2 ventral to SD^1 , very short, SD^1 anterior to spiracle. Spiracle positioned on the anterior two-thirds of segment. L^1 shorter than L^2 , L^3 equal in length to L^2 . L^3 pinaculum equal in size to L^1 and L^2 pinacula. L^3 in line and closer to SV group

than to L^1 and L^2 . SV^1 shorter than SV^2 . V^1 s same distance apart as on A7. <u>A9</u>: D^2 s situated on same pinaculum forming a "saddle". D^2 and SD^1 equal in length. D^1 much shorter than SD^1 and sharing the same pinaculum. SV^1 shorter than SV^2 . V^1 s distance same distance apart or farther than V^1 s on A8. **Crochets** pale brown to brown.

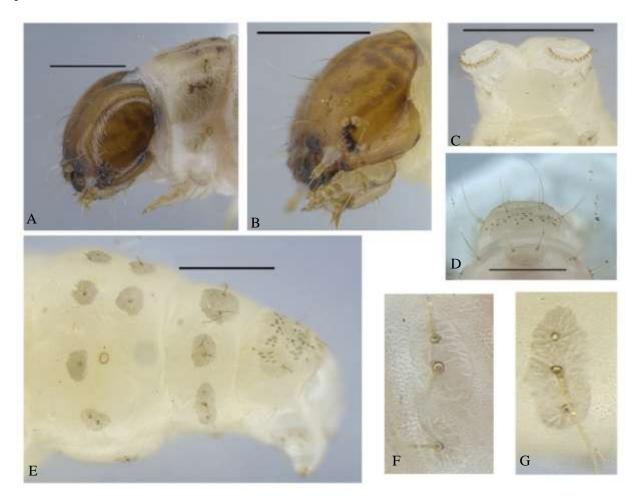


Figure 14: *Cydia pomonella* larvae, A) lateral view of head and T1; B) stemmata; C) Anal comb absent; D) dorsal view of A9 and anal shield; E) lateral view of A8 – A10; F) L-group on A9 trisetose, 2 setae on 1 pinaculum and 1 on a separate pinaculum; G) L-group on A9 trisetose, all 3 setae on 1 pinaculum. Scale: A, D-E = 1mm, B = 0.5mm.

2.4.3. *Grapholita molesta* (Figure 15)

General: Pinacula concolourous with body and setal base dark brown.

Head: Stemmata irregularly rounded. **Chaetotaxy:** Blackened sclerotized area around stemma III–VI. A³ closer to A¹ than to A². Stemma III very close to IV. Stemma IV closer to III than to V. Stemma V slightly posterior to IV and closer to IV than to VI, separated by VI by nearly the diameter of V. Distance between IV and VI half the diameter of IV. Lines joining O¹ and A¹ passing through anterior ventral edge of stemma II. Lines joining O¹ and A¹ passing through median or anterior edge of stemma II. Lines joining O¹ and L¹ through edge anterior of stemma I. Five mandibular teeth, outer three large and pointed, second largest, fourth smaller, fifth smallest and blunt. Spinneret rounded.

Thorax: Prothorax: Spiracle brown to dark brown, larger than on abdominal segments **Prothoracic Shield:** Orange brown, without markings. **Chaetotaxy:** SD² approximately ¹/₃ to ¹/₄ length of SD¹. XD^a posterior ventral to XD¹, separated by one and a half to two times setal base of XD¹. XD^b posterior-dorsad at an angle to XD², separated by four to five times setal base of XD². XD¹ half length of SD¹, XD² twice as long as SD². XD¹ and XD² in line with SD¹. D¹ short, almost as long as SD², and posterior-ventrad to D² at the same level as XD¹. D² almost as long as SD¹. L-group pinaculum darker than other pinacula, not extending horizontally below spiracle. L¹ longest, almost as long as SD¹, L³ short, ¹/₄ length of L². SV¹ two and a half times longer than SV². V¹ tiny, ventral to thoracic legs, close together. Meso and metathorax: **Chaetotaxy:** D¹ short, half as long as D². SD¹ equal in length to D¹. SD¹ longer than D². L¹ and L² on the same pinaculum, equal in size as L³ pinaculum. L¹ three times as long as L² and L³. SV¹ long, but shorter than L¹. V¹ tiny, situated farther apart than on prothorax. **Thoracic legs:** Concolourous with body at coxa, with a small V-shaped sclerotized area at anterior part of leg. Seven seta, situated on coxa, one very tiny, five medium, varying in length, and one long. One pair of setae situated distally on mesal part of femur. Six setae arranged around tibia; two pairs of setae, one pair longer situated dorsally on and the shorter pair ventrally on the tarsus. Claws curved.

Abdomen: Chaetotaxy: $\underline{A1-7}$: D^2 posterioventral to D^1 , D^2 longer than D^1 . SD^1 and SD^2 on same pinaculum, but SD^2 puncture-like, often inconspicuous, sometimes separated from SD^1 . SD^1 long. L^1 and L^2 on single pinaculum positioned anteroventral vertical close to spiracle. L^2 longer seta, L^1 equal in length to L^3 . L^3 on a separate pinaculum, posterioventral to spiracle, closer to SV group than the to L^1 and L^2 . SV^2 and SV^3 equal in length, SV^1 twice as long. SV^2 anterior ventrad to SV^1 . SV^3 anterior-dorsad to SV^1 . V^1 seta tiny, found on the inside of the prolegs. V^1 s on A^2 closer together than those on A^1 -6, equal to V^1 s distance on A^1 -6. Same as A^1 -7 except for the following: D^1 and D^2 closer together and almost in horizontal to each other. Spiracle larger, situated on the posterior two-thirds of the segment. SD^1 anteroventral to the spiracle. SD^1 in line with L-group. SD^2 closer to spiracle than to SD^1 . SV^2 setal length A^1 -7 of A^2 -10 on a shared dorsal "saddle". A^2 -10 longer than A^2 -10 on shared pinaculum. A^2 -10 and A^2 -10 on shared pinaculum. A^2 -11 and A^2 -22 equal in

length. L-group trisetose, all setae on same pinaculum, L^1 longest and in line with L^2 and L^3 , L^2 setae equal in length. SV^1 three times as long as SV^2 . V^1 s tiny, slightly further apart than on A8.

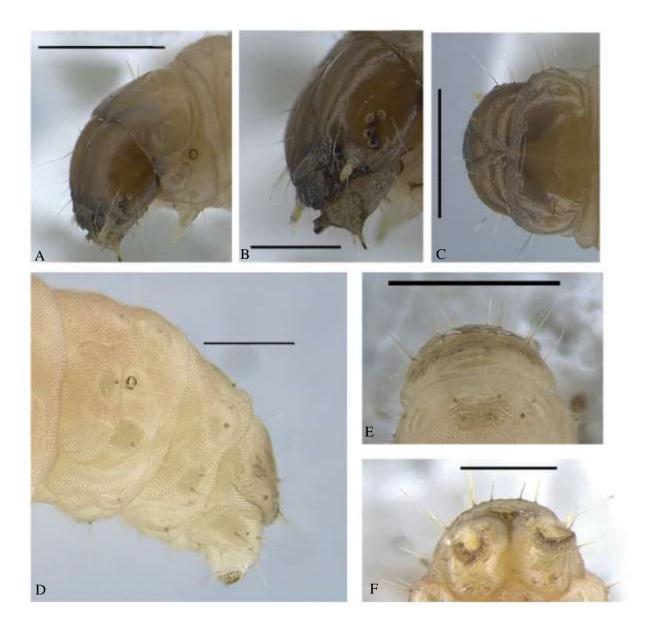


Figure 15: *Grapholita molesta* larvae A) lateral view of head and T1; B) stemmata; C) dorsal view of head and prothoracic shield; D) lateral view of A8 – A10; E) dorsal view of A9 and anal shield; F) anal fork; Scale: A,C, E = 1mm, B, D, F = 0.5mm.

2.4.4. *Cryptophlebia peltastica* (Figure 16)

General: Body pale brown to pinkish white. Setae moderately long, with dark brown bases. Pinacula large, darker than body colour.

Head: Head variable, pale to dark brown. Darker pigmentation at postgenal juncture. Chaetotaxy: Stemmata rounded. Stemma II closer to I than to III by half a diameter, Stemma IV and VI separated by distance nearly equal to diameter of IV. Stemma V closer to IV than to VI by less than half a diameter of V. O^2 posterior and in a horizontal line to stemma I. Line through O^1 and L^1 crossing edge of stemma II. A^2 closer to A^1 than to A^3 . A^3 closer to L^1 than to A^2 .

Thorax: Prothorax: Spiracle dark brown, larger than those on A1-A7. L-group pinaculum extending horizontally below spiracle, larger and darker than other pinacula. Prothoracic Shield: Darker lateral margins and or posterior shading. Chaetotaxy: SD² half (or slightly less than half) as long as SD¹. XD¹ shorter than XD², SD¹ longer than XD². XD¹ and XD² in line with SD¹. XDª postero-dorsad to XD¹. D¹ short, almost equal in length to SD², D² and SD¹ equal in length. D¹ and D² slightly posterior dorsal to SD². L¹ longest and L³ shortest. SV² twice as long as SV¹. V¹ tiny, ventral to thoracic legs. Meso and metathorax: Chaetotaxy: D¹ short, dorsal to SD¹, D² longer than D¹ and about the same length as SD¹. SD² about the same length as D¹. L¹ and L² on same pinaculum, smaller than L3 pinaculum. L¹ longer than L² and L³. SV¹ long, dorsal to thoracic legs. V¹ tiny, same as on prothorax. Thoracic legs: Coxa concolourous with body basally, gradually darkening to brown at femur, tibia and tarsus with a dark brown claw. Small V-shaped sclerotized area at the anterior part of coxa. Seven seta on the coxa: one pair setae situated distally on mesal part of femur. Six setae arranged around tibia; two pairs of setae, one pair longer situated dorsally on and the shorter pair ventrally on the tarsus.

Abdomen: Chaetotaxy: A1-7: D^2 displaced posteroventral of D^1 , D^2 longer than D^1 . SD^1 and SD^2 on same pinaculum, but SD^2 puncture-like, often inconspicuous, usually separated from SD^1 . SD^1 long. L^1 and L^2 on shared pinaculum, positioned anteroventral, vertical, close to spiracle. L^2 longer, L^1 and L^3 equal in length. L^3 on separate pinaculum, posterioventral to spiracle, closer to SV-group than to L^1 and L^2 . SV^2 and SV^3 equal in length, SV^1 twice as long as SV^2 and SV^3 . SV^3 anterior-dorsad to SV^1 . V^1 tiny, medial to prolegs. V^1 s on A7 closer together than those on A1-6, equally distant to those on A8. <u>A8:</u> Same as A1-7 except for: D^1 and D^2 closer together, almost in horizontal line. Spiracle larger, situated on posterior two-thirds of segment. SD^1 anteroventral to spiracle. SD^1 in line with L-group. SD^2 closer to spiracle than SD^1 . L-setae as on A1-7. SV^1 longer than SV^2 , situated on same pinaculum close to V^3 . V^3 absent. V^4 closer together than those on A1-A6 or A9. <u>A9:</u> V^3 0 on a shared dorsal "saddle". V^3 1 longer than V^4 2 on shared pinaculum. V^4 3 and V^4 4 and V^4 5 equal long. L-group trisetose, on same pinaculum. V^4 4 longest, in line with V^4 5 and V^4 6 and V^4 7 equal in length. V^4 8 longer than V^4 9 equal in length. V^4 9 longer than V^4 9 equal in length. V^4 9 longer than V^4 9 longer than V^4 9 equal in length. V^4 9 longer than V^4 9 longer than V^4 9 longer than V^4 9 equal in length. V^4 9 longer than V^4 9 longer langer langer langer lange



Figure 16: Cryptophlebia peltastica larvae A) lateral view of head and T1; B) stemmata; C) dorsal view of head and prothoracic shield; D) lateral view of A8 – A10; E) dorsal view of A9 and anal shield; F) anal fork absent. Scale: A, C - F = 1mm, B = 0.5mm.

2.4.5. Thaumatotibia batrachopa (Figure 17)

General: Pinacula large, greyish brown, easily observed with naked eye, with dark brown bases. Setae moderately long and easily observed.

Head: Dark pigmentation at stemmatal area and at postgenal juncture. Chaetotaxy: Distance between stemma II and III about half the diameter of II, stemma IV closer to III than to V and separated from VI by less than half a diameter of IV. Stemma V closer to IV than to VI. P^1 closer to Adf^2 than to F^1 . Line through O^1 and A^1 extending medially though stemma II. Line through O^1 and O^1 and O^2 extending medially through stemma II.

Thorax: Prothorax: Spiracle dark brown, larger than those on abdominal segments. L-group pinaculum extending horizontally below spiracle, larger and darker than other pinacula. Prothoracic Shield: Darker lateral margins or shaded posteriorly. Chaetotaxy: SD² less than half as long as SD¹ and equal to XD¹. XD¹ less than half as long as XD², SD¹ longer than XD². XD¹ and XD² in line with SD¹. XD^a posterodorsad to XD¹. D¹ short, about equal in length to SD², D² almost equal in length to SD¹. D¹ and D² slightly posterior dorsal to SD². L¹ longest, equivalent to SD¹ and D¹, L³ shortest, somewhat inconspicuous. SV¹ and L¹ equal in length SV² short, but longer than L². V¹ tiny, ventral to thoracic legs; distance to coxae greater than between V1s. Meso and metathorax: Chaetotaxy: D1 short, about as long as SD2, D2 and SD1 equal in length, more than twice as long as D¹. L¹ and L² on same pinaculum, smaller than L³ pinaculum. L¹ longer than L² and L³. SV¹ long, dorsal to thoracic legs. V¹s tiny, further apart than on prothorax. Microsetae present: MD¹ and MD² tiny, on same pinaculum anterior to and in line with SD-group. MV² and MV³ micro setae anterior to coxa and less prominent than MD¹ and MD². V¹s on T2 and T3 not fused with coxae. Thoracic legs: Coxa concolourous with body basally, gradually darkening to brown at femur, tibia and tarsus with a dark brown claw. Small V-shaped sclerotized area at anterior part of coxa. Seven seta located on coxa. One pair setae situated distally on mesal part of femur. Six setae arranged around tibia; two pairs of setae, one pair longer situated dorsally and shorter pair ventrally on tarsus.

Abdomen: SV-group pinacula concolourous with body. Chaetotaxy: $\underline{A1-7}$: D^2 displaced posteroventral of D^1 , D^2 two times as long as D^1 . SD^1 and SD^2 on same pinaculum, but SD^2 puncture-like, with whitish base. SD1 long, equal in length to D^2 . L^1 and L^2 on one pinaculum, anteroventral vertical, close to spiracle. L^1 twice as long as L^2 . L^3 on separate pinaculum, posterioventral to spiracle, closer to SV group than to L^1 and L^2 . SV^1 , SV^2 and SV^3 setae equal in length on A1-A6. On A7, SV^1 longest and SV^3 shortest. SV^2 anterior ventrad to SV^1 . SV^3 anterior-dorsad to SV^1 . V^1 's tiny, on A1-A2 and A7 distance of V^1 pinaculum apart. On A3-6, V^1 s medially to prolegs. $\underline{A8}$: Same as A1-7 except D^1 and D^2 closer together, almost in horizontal line. Spiracle larger, situated on posterior V^2 3 of segment. V^2 4 anteroventral to spiracle. V^2 5 shortest. V^2 6, in line with V^2 7 puncture-like, with whitish base. V^2 8 equal in length to V^2 9 and V^2 9 closer together than twice as long as V^2 9, situated on the same pinaculum close to V^2 9, V^2 9 absent. V^2 9 closer together than those on A7. V^2 9 on a shared dorsal "saddle". D2 longer than V^2 9 and V^2 9 on shared pinaculum. V^2 9 longer than V^2 9 on same pinaculum, V^2 9 longer than V^2 9 on shared pinaculum.

line with L^2 and L^3 . L^2 and L^3 setae equal in length. SV^1 equal in length to L^1 , in line with SD^1 and L-group. V^1 s slightly farther apart than on A8.

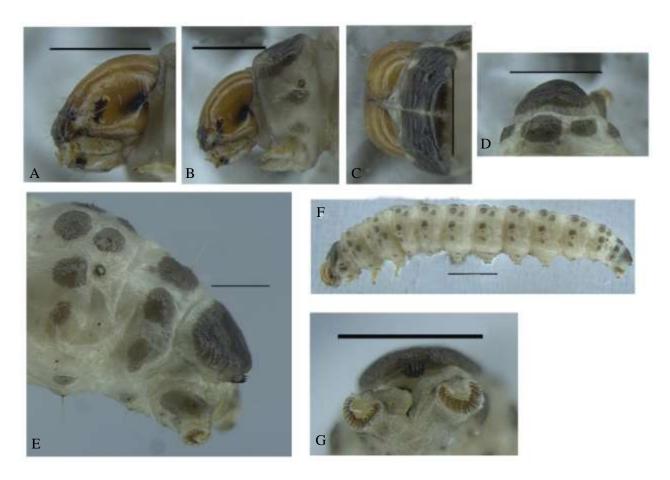


Figure 17: *Thaumatotibia batrachopa* larvae A) lateral view of head and T1; B) stemmata; C) dorsal view of head and prothoracic shield; D) dorsal view of A9 and anal shield E) lateral view of A8 – A10; F) lateral view of final instar larvae indicating large greyish pinacula; G) anal fork. Scale: A–D, G = 1mm, E = 0.5mm, F = 2mm.

2.4.6. Epichoristodes acerbella (Figure 18)

General: Body light green with pinacula concolourous with, or slightly lighter than, body colour. Setae moderately long.

Head: Hypognathous, darker pigmentation at postgenal juncture. **Chaetotaxy:** Stemmata II-IV surrounded by darker pigmentation. Stemma II separated from I by distance greater than its diameter. Stemma III closer to IV than to II. Stemma IV closer to V than to III, almost connecting. V closer to IV than to VI. Stemma V slightly closer to VI, separated by about the diameter of V. Stemma IV separated from VI by almost a diameter of IV. Stemma V separated from VI by more than a diameter. O² posterior and in line to stemma I. A² more or less equidistant to A¹ and A³. A³ equidistant to A² and L¹. Line through O¹ and A¹ closer to II than to III. Line through O¹ and L¹ extending medially through stemma I. Spinneret bluntly rounded and approximately seven times longer than wide.

Thorax: Prothorax: Spiracle larger than those on A1-7, dark brown. L-group pinaculum concolourous with body with a darker dorsal pigmented line, slightly larger than other pinacula, not extending below the spiracle. Meso- and metathorax: Chaetotaxy: D^1 and D^2 on one pinaculum, D^1 dorsal to D^2 , and D^2 longer than D1. L-group ventral to spiracle, L^1 and L^2 on a shared pinaculum, L^3 on a separate pinaculum situated posterior to L^1 and L^2 , L^1 longest. SV^1 long, dorsal to the thoracic leg. V^1 s on T2 and T3 not fused with coxae. Thoracic legs: Light green, concolourous with body.

Abdomen: Chaetotaxy: $\underline{A1-7}$: D^1 and D^2 almost in horizontal line, D^2 setae slightly longer than D^1 s. SD^1 separated from spiracle by approximately half the diameter of spiracle. SD^2 highly reduced or absent. SD^1 very long, dorsal to spiracle. L-group ventral to spiracle, L^1 and L^2 on a shared pinaculum, L^3 on a separate pinaculum situated posterioventral to L^1 and L^2 and its setae longer than L^1 and L^2 . Three SV setae situated on the same pinaculum, anterodorsal to crochets, SV^1 longest. SV^1 ventral to SV^1 . SV^3 anterior-dorsal to SV^1 . V^1 s on A1-A6 farther apart than on those T2-T3. V^1 s on A7 closer together. $\underline{A8}$: Same as A1-7 except SD^1 anteroventral to spiracle by half the diameter of the spiracle. SD^2 sometimes inconspicuous. D^1 and SD^1 on separate pinaculum. L^1 and L^2 anteroventral to the spiracle. SV^3 absent. V^1 closer together than on A7. $\underline{A9}$: Same as A1-7 except D^1 shorter than that seta on A1-8. SD^2 absent. D^1 and SD^1 on separate pinaculum. D^2 s not sharing a pinaculum. L-group trisetose; L^2 closer to L^3 than to L^1 , L^1 longest. Distance between V^1 s equal to or less than distance of V^1 s on A8. Anal fork: Darkening to tip.

Specimens studied differed from that illustrated by Timm *et al.* (2008; Fig. 17) in that the D^2 s were not on a common pinaculum ("saddle") on A9; D^1 did not share a pinaculum with SD^1 ; and the L-group was trisetose, with all setae sharing the same pinaculum. The features we observed agree with those reported by Nuzzaci (1973; Appendix 2.1, Fig. 16).

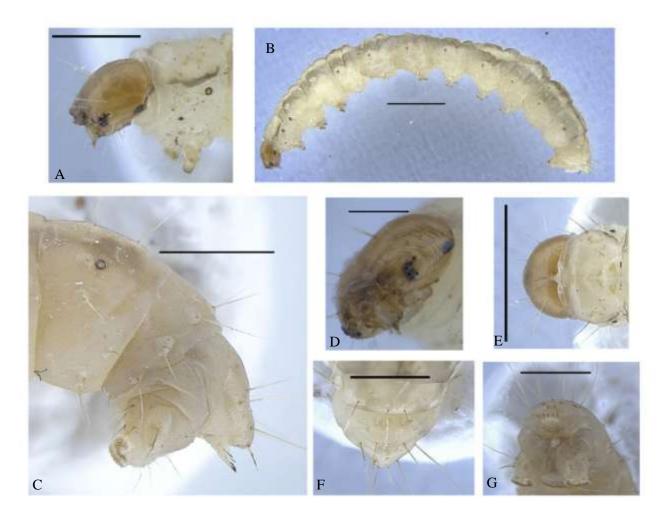


Figure 18: *Epichoristodes acerbella* larvae A) lateral view of head and T1; B) lateral view of final instar larvae indicating green lateral line; C) lateral view of A8 – A10; D) stemmata; E) dorsal view of head and prothoracic shield; F) dorsal view of A9 and anal shield G) anal fork. Scale: A, C, F-G = 1mm, D = 0.5mm, B, E= 2mm.

2.4.7. Lozotaenia capensana

Despite the fact that this species is an important minor pest of apples, no specimens were obtained.

2.5. Summary of literature and morphological study

A complete summary of characters extracted from literature (see Appendix) (white boxes) and own morphological study (green boxes) is compiled in Table 4

Table 4: Important morphological characteristics to identify and distinguish between the six economically important tortricids larvae (White boxes as in literature. Dark grey and highlight boxes are morphological characteristics that were studied and added).

General	T. leucotreta	C. pomonella	G. molesta	C. peltastica	T. batrachopa	E. acerbella	
Size	12-25 mm length	15-19mm length	10-15mm	20mm	13-23 mm length	17-21	
Shape	Slender elongated	Slender elongated	Elongated	Elongated	Elongated	Elongated	
Colour	Cream to light red, orange	Body colour to light brown	Whitish, light brown or more often reddish	Whitish brown to light pink to light red,	Cream to grey green	Light green with stripes: dark green on dorsal- yellow green on lateral midlines	
Integument structure			Rugos	se			
Setal pinacula	Easily observed	Moderate in size	Moderately large	Easily observed, large,.	Moderately large	Conspicuous	
Setal pinacula colour	Darker than body colour	Body colour or darker greyish	Body coloured	Darker than body colour	Medium to dark greyish brown	Lighter than body colour	
Setal length	Moderately long	Moderately long, spine-like appearance	Short, spine-like	Moderately long	Moderately long	Moderately long	
Spinulation of integument	Easily apparent	Easily observed	Slender, darker than body colour	Slender, darker than body colour	Easily observed	Easily observed	
Head	T. leucotreta	C. pomonella	G. molesta	C. peltastica	T. batrachopa	E. acerbella	
Head colour	Yellowish brown to dark brown	Yellow brown	Yellow brown to black	Light to dark brown	Yellow brown	Yellow brown to olive green	
Head pigmentation	Darker pigmentation at ocellar area and postgenal juncture	Dark brown pattern & blackish pigment on ocellar areas and at postgenal juncture.	Often overlaid with darker pattern; dark pigmentation on ocellar area & at postgenal juncture.	Darker pigmentation at ocellar area and postgenal juncture	Darker pigmentation at ocellar area	Darker pigmentation at postgenal juncture	
Head shape	Hypognathous						
Aver. width prior pupation	1.31 mm	1.71 mm	0.92-1.11mm	1.7mm	1.5mm	1.04mm	
Head position	Vertical angle acute	Vertical angle acute	Vertical angle more or less acute	Vertical angle acute	Vertical angle acute	Vertical angle acute	
Adfrontals	Tapering anteriorly, extending to vertical angle	Extending to vertical angle	Extending to vertical angle	Extending to vertical angle, tapering anteriorly	Extending to vertical angle, tapering anterior- & posteriorly	Narrow, extending to vertical angle	
Ocellar shape	Rounded	Rounded	Rounded	Rounded	Rounded	Rounded	
Stemmata size & shape	Equal in size, irregularly rounded	Large distinct, convex, irregularly rounded except for VI, more oval	Irregularly rounded, I larger than others	II-VI rounded equal size, I elongate, approx. 1.5x diameter of III.	Equal in size, irregularly rounded	Inconspicuous except for III, surrounded by dark pigmentation	
Stemma II position	Closer to I than III	Closer to I than III or equidistant	Less than diameter away from stemmata I and III	Closer to I than III, by less than half a diameter of II.	Equidistant from I & III	Closer to III than I.	
Stemma III position	Closer to IV than II	Closer to IV than II	Very close to IV	Closer to IV than II	Closer to IV than II	Closer to IV than II	
Stemma IV	Closer to III than V	Closer to III than V	Equidistant from III and V, sometimes closer to III	IV closer to III than VI	IV closer to III than VI	Closer to V than III, almost connecting	

Stemma V position	Close to IV than VI	Closer to IV than VI	Closer to IV than VI	Closer to IV than VI	Closer to IV than VI	Closer to IV than VI
Stemma V separated from stemma VI	By nearly half diameter of V	By slightly more than half the diameter of V	By nearly diameter of V	By slightly 1.5x diameter of V	By equal diameter of V	By more than a diameter of V
Stemma IV separated from stemma VI	By distance slightly less than diameter of IV	By ½ diameter of IV	By ½ diameter of IV	By distance slightly less than diameter of IV	By distance less than ½ diameter of IV	By distance slightly less than diameter of IV
O¹ distance from stemma II & III	Equidistant	Equidistant, or slightly closer to III	Closer to stemma III	Closer to stemma III	Equidistant	Equidistant
O ² position	Posterior ventrad to I	More ventral than caudal to O ¹	Ventral to I,	Posterior and in line with I	Posterior ventrad to I	Posterior and in line with I
A ² position to A ¹ and A ²	Closer to A ¹ than A ³	Equidistant	Equidistant	Closer to A ¹ than A ³	Closer to A ¹ than A ³	Equidistant
A ³ position	Closer to L ¹ than A ²	Closer to A ² than L ¹	Equidistant to A ² and L ¹	Closer to L ¹ than A ²	Closer to L ¹ than A ²	Equidistant to A ² and L ¹
Lines joining O ¹ & A ¹	Closer to III than II	Equidistant or closer to III than II	Through anterior ventrad edge of II	Closer to III than II	Through median of III	Closer to II than III
Lines joining O ¹ & L ¹	Through median of I	Through median of I	Through median of I	Through median of I.	Through median of I	Through median of I
Lines joining O ¹ & A ²	Closer to II than III	Through median of II	Through median or anterior edge of II	Closer to II than III.	Equidistant from II & III	Closer to II than I
P ¹ position	Closer to Adf ² than F ¹	Closer to Adf ² than F ¹	Closer to Adf ² than F ¹	Closer to Adf ² than F ¹	Closer to Adf ² than F ¹	Closer to Adf ² than F ¹
Mandibles	Five teeth	Five teeth , darker in denticulate region	Five teeth	Five teeth	Five teeth	Five teeth
Mandibles 1 - 3 teeth	Larger, acuminate, often 2 &3 flattened	Large and pointed, often 2 & 3 flattened	Large and pointed, second largest	Larger, acuminate, often 2 and 3 flattened	Larger, acuminate, often 2 and 3 flattened	2 and 3 large and pointed
Mandibles teeth 4	Smaller and flattened	Smaller, often flattened	Smaller	Smaller and flattened	Smaller and flattened	Smaller and flattened
Mandibles teeth 5	Straight-edged	Bluntly rounded	Smallest and bluntly rounded	Straight-edged	Straight-edged	Straight-edged
Distal end of spinneret	Rounded,	Tapered,	Rounded,	Rounded,	Rounded,	Rounded
Spinneret length	8x longer than wide	6-6.5x longer than wide	7-8.5x longer than wide	7x longer than wide	7x longer than wide	7x longer than wide
Thorax	T. leucotreta	C. pomonella	G. molesta	C. peltastica	T. batrachopa	E. acerbella
Prothoracic shield colour	Yellowish brown to dark brown	Body coloured to slightly greyish brown	Yellowish brown	Yellow brown	Yellow brown	Yellow or body coloured
Prothoracic shield pattern	Darker patches Fully coloured or with posterior shading	Brownish pattern- speckled appearance	Sometimes with green or brown pigment present	Some medium brown pigmentation	Lightly sclerotized with small patches of darker pigmentation.	No pattern
SD ¹ position on prothoracic shield	Equidistant to SD ² than XD ²	SD ¹ closer to SD ² than XD ²	Equidistant to SD ² than XD ²	SD ¹ slightly closer to SD ² than XD ²	SD ¹ equidistant from XD ² and SD ²	Equidistant to SD ² than XD ²
D ¹ position on prothoracic shield	Slightly posterior to D ² , slightly below level of XD ¹	Slightly posterior to D^2 ,, above level of XD^1	Slightly posterior to D ² , in line with XD ¹	Posterior to XD ¹ and anterior to D ²	Slightly posterior to D ² , slightly below level of XD ¹	Slightly posterior to D ² , above the level of XD ¹
Spiracle shape	Circular, large	More oval than circular	Circular	Oval/Circular	Very prominent, circular	Circular,
Spiracle colour	Medium to dark brown	Light to medium brown	Dark brown -black	Dark brown	Dark brown	Dark brown

L group pinaculum on T1	Enlarged, extending below spiracle	Not extending below spiracle	Not extending below spiracle	Extending beyond spiracle	Extending beyond spiracle	Not extending below spiracle		
L ¹ distance & angle from L ² & L ³	Equidistant, straight line	Equidistant, straight line	Equidistant or closer to L^2 , below a straight line joining L^2 and L^3 ,	Equidistant & in straight- line	Straight line	Equidistant, straight line		
L-group length	L^1 longest, L^2 shortest, $L^{31}/_3$ to a $^1/_4$ x shorter than L^1	L ² twice as long as L ³ , L ¹ longer	L ¹ longest,	L ¹ longest, L ³ shortest	L ¹ longest, L ³ shortest	L ¹ longest, L ³ shortest		
D ¹ position to D ² on T2-3		Dorsal						
V¹s position on T2 & T3	Separated from coxae	Separated from coxae	Fused to coxae	Separated from coxae	Separated from coxae	Separated from coxae		
Thoracic claws	Small, curved, medium brown	Short, light brown	Pale, curved, slender	Medium brown, curved	Medium brown, curved	Curved		
<u>Abdomen</u>	T. leucotreta	C. pomonella	G. molesta	C. peltastica	T. batrachopa	E. acerbella		
Spiracle shape & size	Oval, small, seldom larger than setal bases	Small, elliptical, larger than setal base.	Moderate	Small, circular, seldom larger than setal base	Small, circular, seldom larger than setal base	Circular, smaller than setal base		
SD ¹ distance from spiracle	1.5x spiracle diameter	2-3x its diameter	1.5-3x SD ¹ diameter except on A8	2.5x its diameter from spiracle	1.5x spiracle diameter	0.5x spiracle diameter		
Position of spiracle on A8	Posterior third of segment	Anterior two-thirds	Posterior third of segment	Posterior third of segment	Posterior third of segment	Anterior two-thirds		
SD ¹ and SD ² position	On same pinaculum	On same pinaculum	On same pinaculum, sometimes SD ² separated	On A1 same pinaculum, On A2-A8 separate pinacula.	On same pinaculum	On same pinaculum		
SD ² A1-A8	Highly reduced, appearing absent	Highly reduced, appearing absent	Highly reduced, appearing absent	Highly reduced, appearing absent	Highly reduced, white setal base	Highly reduced, appearing absent		
SD ¹ position to spiracle on A8	Anteroventral, 3x spiracle diameter	Anterior or slightly anterior ventral, 1.5-2x its diameter	Approx. anterior, 1- 1.5x its diameter	Anteroventrally, 1.5x spiracle diameter	Anteroventrally, 2x spiracle diameter	Anteroventrally, by half spiracle diameter.		
L group on A9	Trisetose	Trisetose, or bisetose with L ³ separated on own pinacula	Trisetose	Trisetose	Trisetose	Trisetose		
SV group	A7 Bisetose, A9 Unisetose	A1-A2 trisetose, A1-A2, A7-A8 bisetose, A9 unisetose	A9 Unisetose	A9 Bisetose	A8 Bisetose, A9 Unisetose	Bisetose on A8 and A9		
SV ² position to SV ¹ on A1-A2	Ventrad and slightly cephalad	Anterior-ventrad	Anterior-ventrad	Ventrad and slightly cephalad from SV1	Anterior-ventrad	Ventral		
SV ³ position to SV ¹ on A1-A2	Antero-dorsad	Antero-dorsad	Antero-dorsad, or absent	Dorsal	Antero-dorsad	Antero-dorsad		
SV group on A1, 2, 7,8, and 9	3:3:2:2:1	3:3:2:2:1 or 3:3:2,1:1:1 or 2:3:2:2:1 or other combinations	2:3,2:2:2:1; 3,2:3:2:2:1; 2:3,2:2:2:2;	3:3:3:2:2	3:3:2:2:1	3:3:3:2:2		
On A9 D ¹ & SD ¹		On separate pinaculum						
On A9, D ² s position		Not sharing pinaculum						
On A9 L ¹ distance to L ² and L ³	Equidistant	Equidistant or slightly further apart	Equidistant	Equidistant	Equidistant	Equidistant or slightly closer to L ³		
V ¹ on A9 compared to A8	Slightly further apart	Equidistant	Equidistant or further apart	Slightly further apart	Slightly further apart	Equidistant or closer together		
Anal comb	Present	Absent	Present	Absent	Present	Present		
Anal comb colour	Darkly pigmented	Absent	Yellow to dark brown,	Absent	Darkly pigmented	Transparent		

Abdomen	T. leucotreta	C. pomonella	G. molesta	C. peltastica	T. batrachopa	E. acerbella
Prong shape	Basal part of prong strongly tapered dorsally, width of base $^{1}/_{4}$ length of tooth, upper levels of larger prongs medially at same level	Absent	Equal in length and parallel, two mesal spines sometimes blunt	Absent	Basal part of prong strongly tapered dorsally, width of base nearly ¹ / ₄ length of tooth, prongs merging into distinct medial structure	Width of base approx equal to or less than length of prongs. Basal part of each prong tapered dorsally
Number of prongs	2 –10 bluntly dentate prongs	Absent	4-5 prongs	Absent	5-8 prongs	6-9 bluntly dentate prongs
Anal shield colour	Medium brown	Darker than body colour	Brown	Yellow brown	Yellow brown	Yellow or body coloured
Anal shield pattern	Sometimes small brown pigmentation	Speckled or patchy appearance (larger patches)	Sometimes small brown pigmentation	Sometimes small brown pigmentation	Sometimes small brown pigmentation	No pigmentation
Anal shield shape	Tapering posteriorly, posterior margin evenly rounded.	Posteriorly rounded	Posteriorly rounded	Posteriorly rounded	Posteriorly rounded	Rounded and strongly tapered posteriorly
L^1s distance compared to D^1s	Further apart	Further apart, sometimes equidistant	Further apart	Further apart	Equidistant	Closer together
D ² s & L ¹ s setal length	D ² s half as long as L ¹ s	L^1 long or longer than anal segment, D^2 s half of L^1 s	D ² s half as long as L ¹ s	L1 slightly further lateral than D1s	D2 more than half as long as L1	D2s shorter than L1s
D¹s distance	Closer to SD ¹ than each other.	Closer to SD ¹ than each other	Equidistant from SD ¹ and each other	Closer to SD ¹ than each other almost in straight line.	Closer to SD ¹ than each other.	Closer to SD ¹ than each other.
D¹s & SD¹s setal length	D ¹ s slightly shorter than SD ¹	D ¹ s shorter than SD ¹	D ¹ s slightly shorter than SD ¹	D ¹ s shorter than SD ¹	Equally long or D ¹ slightly longer	Equally long
Proleg crochets arrangement	Irregularly triordinal	Unevenly uniordinal	Uniordinal	Biordinal	Triordinal	Unevenly uniordinal, almost biordinal
Crochets number	36-42	28-35	30-40	50-58	34-44	32-54
Anal proleg crochets arrangement	Irregularly triordinal, absent in medial half	Unevenly uniordinal , "in situ" more or less ovoid	Uniordinal, "in situ" sometimes oval or almost circular	Biordinal, absent in medial half.	Triordinal, absent in medial half	Unevenly uniordinal, almost biordinal
Anal crochet numbers	24-32	15-25	19-25	46-54	26-32	28-36

2.6. Discussion and conclusion

A few larval morphological characteristics are distinct and diagnostic for specific species, and are summarized below.

<u>General</u>: Setal pinacula are usually concolourous with or darker than the body, however, in *E. acerbella* the pinacula are lighter than the body colour. In *T. batrachopa* the pinacula are very distinct and visible with the naked eye, giving the larvae a spotted appearance. The prothoracic and anal shields of *C. pomonella* have characteristic patterns of spots, and those of *E. acerbella* lack markings. Crochets are triordinal in *T. leucotreta* and *T. batrachopa*, biordinal in *C. peltastica*, and uniordinal in *C. pomonella*, *G. molesta* and *E. acerbella* (in the latter, weakly biordinal).

Head: The head in the studied specimens are yellow brown to dark brown, except for that of E. acerbella, which is yellow to olive green. C. pomonella has a distinctive pattern on the head, giving it a more mottled appearance than in the other species. T. batrachopa has no darker pigmentation at the stemmatal region. E. acerbella has pigmentation around stemmata II-IV, and the other stemmata are inconspicuous. Stemmata are equal in size except in G. molesta and C. peltastica, where stemma I is larger. Stemma II is closer to I than to III, except for C. pomonella where the three are sometimes equidistant. In T. batrachopa they are equidistant, and in E. acerbella stemma II is closer to III than to I. Stemma VI is closer to III than to V except in G. molesta where it is also sometimes equidistant, and in E. acerbella where it is closer to V than III, almost immediately adjacent. O¹ is equidistant to II and III, except in C. pomonella, where it is sometimes closer to III. In G. molesta and C. peltastica O1 is always closer to III than to II. A2 is closer to A¹ and A³ in T. leucotreta, C. peltastica, and T. batrachopa. A² is equidistant to A¹ and A³ in C. pomonella, G. molesta, and E. acerbella. The spinneret is usually rounded distally except in C. pomonella where it is tapered. The spinneret at least $8\times$ as long as wide in T. leucotreta and G. molesta, but less than $7\times$ as long as wide in C. pomonella. It is usually about 7× as long as wide in C. peltastica, T. batrachopa, E. acerbella. Thorax: The pre-spiracular group (L-group) pinaculum is unmodified in C. pomonella, G. molesta and E. acerbella, but in Thaumatotibia and Cryptophlebia it extends below the spiracle. Distinct micro setae are easily observed on T2-T3 only in T. batrachopa. V¹s are fused to T2-T3 coxae only in G. molesta.

Abdomen: The spiracle on A8 is situated on the posterior two-thirds of the segment except on *C. pomonella* and *E. acerbella* where it is situated on the anterior third. SD² usually shares a pinacula with SD¹, but it is sometimes on a separate pinacula in *G. molesta*. In *T. batrachopa* SD² shares a pinacula with SD¹ only on A1, and they are on separate pinacula on A2-A8. D¹ and SD¹ share a common pinaculum on A9 except in *E. acerbella* where they are on separate pinacula. The D² setae share a pinaculum, forming a dorsal "saddle" on A9 in all but *E. acerbella* where they are on separate pinacula. L-group on A9 is trisetose and on same pinaculum in all but *C. pomonella* where L¹ and L² share a pinaculum and L³ is on a separated pinaculum. Distance between V¹s A9 compared to A8 is usually farther apart, except in *G. molesta* where they sometimes are equidistant, always equidistant in *C. pomonella*, and closer together in *E. acerbella*. The anal comb is present in all but *C. pomonella* and *C. peltastica*.

From the above it is clear that distinct and unique suites of characters exist that can be applied in the development of a diagnostic key (Appendix 2.1).

2.7. References

- Amante, V. dR. & Norton, G.A. (2003). Developing interactive diagnostic support tools for tropical root crops. Third Taro Symposium, Proceedings of an International Scientific Meeting jointly organized by the Secretariat of the Pacific Community and the International Plant Genetic Resources Institute, Fiji Islands.
- Brown, R.L. (1987). Tortricidae (Tortricoidea). In: Stehr, F.W. (Ed.) *Immature Insects*. Kendall/Hunt, Iowa, pp. 419-427.
- Dugdale, J.S., Gleeson, D., Clunie, L.H. & Holder, P.W. (2005). A diagnostic guide to Tortricidae encountered in field surveys and quarantine inspections in New Zealand: Morphological and Molecular characters. Ministry of Agriculture and Forestry, Wellington, New Zealand, pp. 24-28.
- Garman, P. (1917). The Oriental Peach pest (*Laspeyresia molesta*, Busck), a dangerous new fruit insect of Maryland. *The Maryland State College of Agriculture, Agricultural Experiment Station*. Bulletin No. **209**: 1-16.
- Gilligan, T.M., Epstein, M.E. & Hoffman, K.M. (2011). Discovery of False Codling Moth, *Thaumatotibia leucotreta* (Meyrick), in California (Lepidoptera: Tortricidae) *Proceedings of the Entomological Society of Washington* **113** (4): 426-435
- Gilligan, T. M. & Epstein, M. E. (2012). TortAI, Tortricids of Agricultural Importance to the United States (Lepidoptera: Tortricidae). Identification Technology Program (ITP), USDA/APHIS/PPQ/CPHST, Fort Collins, CO. [accessed at http://idtools.org/id/leps/tortai/].
- Heinrich, C. (1916). On the taxonomic value of some larval characters in the Lepidoptera. *Proceedings of the Entomological Society of Washington* **18**: 154-164.
- Hinton, H. E. (1946). On the homology and nomenclature of setae of lepidopterous larvae, with some notes on phylogeny of Lepidoptera. *Transactions of the Entomological Society of London* **97**: 1-37.
- Holloway, J.D., Bradley, J.D. & Carter, D.J. (1987). *CIE Guides to insects of importance to man-Lepidoptera*, C.A.B. International, Wallingford, pp. 129-131.
- Horak, M. (1999). The Tortricoidea in: *Handbuch der Zoologie: Lepidoptera, Moths and Butterflies, Volume 1: Evolution, Systematics, and Biogeography*, Walter de Gruyter, Berlin, pp. 199-215.
- Komai, F. (1999). A taxonomic review of the genus *Grapholita* and allied genera (Lepidoptera: Tortricidae) in the Palaearctic region. *Entomologica Scandinavica Supplement* **55**: 1-226.
- Lopez, A.W. (1929). Morphological studies of the head and mouthparts of the mature codling moth larva, *Carpocapsa pomonella (Linn). University of California Publications in Entomology* **6** (3): 19-36.

- MacKay, M.R. (1959). Larvae of the North American Olethreutidae (Lepidoptera). *The Canadian Entomologist*, supplement 10, Volume XCI.
- McGeogh, M.A. & Krüger, M. (1994). Identification and diagnoses of Lepidoptera larvae inhabiting galls induced by *Ravenelia macowiana* Pazschke on *Acacia karroo* Hayne in South Africa. *African Entomology* **2**: 37-43.
- Nuzacci, G. (1973). Epichoristodes acerbella (Lepidoptera, Tortricidae). Entomologica (Bari) 9: 147-148.
- Osborne, D.V. (1962). Some aspects of the theory of dichotomous keys, New Phytologist 62 (2): 144-160.
- Powell, J.A. (1964). *Biological and taxonomic studies on tortricine moths, with reference to the species in California*, University of California Press, Berkeley and Los Angeles, p. 317.
- Razowski, J. (2002). *Tortricidae (Lepidoptera) of Europe, Volume 1*, Tortricinae and Chlidanotinae. František Salmka, Bratislava, Slovakia, pp. 9-13.
- Stehr, F.W. (1987). Immature insects. Kendall Hunt, Iowa, 754 pp.
- Swatschek, B. (1958) Abhandlungen zur Larvalsystematik der Insekten Die Larvalsystematik der Wickler (Tortricidae und Carposinidae). Deutsche Akademie der Wissenschaften zu Berlin, Deutsche Akademie der Landwirtschaftwissenschaften zu Berlin. Nr 3. 269 pp.
- Timm, A.E. (2005). Morphological and molecular studies of tortricid moths of economic importance to the South African fruit industry, Phd (Agric) dissertation, Stellenbosch University.
- Timm, A.E. Warnich, L. & Geertsema, H. (2007). Morphological and molecular identification of economically important Tortricidae (Lepidoptera) on tropical and subtropical fruit in South Africa. *African Entomology* **15** (2): 269-286.
- Timm, A.E. Warnich, L. & Geertsema, H. (2008). Morphological and molecular identification of economically important Tortricidae (Lepidoptera) on deciduous fruit tree crops in South Africa. *African Entomology* **16** (2): 209-219.
- Williams, J.R. (1953). The larvae and pupae of some important Lepidoptera. *Bulletin of Entomological Research* **43** (4): 691-701.
- Walters, D. E. & Winterton, S. (2007). Keys and the Crisis in Taxonomy: Extinction or Reinvention? *Annual Review of Entomology* **52**: 193-208.
- Wood, W.B & Selkregg, E.R. (1918). Further Notes on *Laspeyresia molesta*, *Journal of Agricultural Research* **XIII**, No. 4. Pp. 59-70.

2.8. Appendix 2.1

Published descriptions of the larvae of the tortricids presently studied.

2.8.1 Thaumatotibia leucotreta

Williams (1953), Dugdale *et al.* (2005), Timm *et al.* (2005) and Gilligan *et al.* (2011) all provided descriptions of *T. leucotreta* larvae.

Stofberg (1948) described the larva of *T. leucotreta*, but not used in this study for reasons discussed by Timm (2005).

Williams (1953) described *T. leucotreta* as:

When mature, length about 15 mm, breadth about 2.5 mm. Colour: head rather light brown; body pale with reddish hue, tubercles without, or with faint, pigmentation. Head wider than long. Ocelli 3 and 4 very close together. Spiracles round. Body with spines as in figure 2. Anal comb present. Chaetotaxy: head with puncture V^a between and equidistant from V^2 and V^3 . Puncture P^b nearer P^2 than P^1 . Seta P^2 nearer P^2 than P^3 . Seta P^2 nearer P^3 than P^3 in Seta P^3 nearer P^3 and slightly below the level of P^3 in Seta P^3 and P^3 with common tubercle on abdominal segments, P^3 on 8 antero-ventrad from spiracle. Setae P^3 and P^3 subequal on abdomen, on 8 P^3 ventrad and slightly cephalad from P^3 as on 1-7. Subventral group bisetose on 7, unisetose on 9. On 1 and 2 P^3 ventrad and slightly cephalad from P^3 antero-dorsad P^3 . Tubercle of ventral seta not in contact with coxa on II & III.

Komai (1999) described T. leucotreta as:

Body length of mature larvae 15 mm. Head yellow brown. Body orange or pink in final instar. pinacula large, darker than body colour. Spiracle on A8 near the posterior margin. Prolegs with 31-40 crochets arranged in a biordinal circle. Anal fork present. Chaetotaxy: SD¹ and SD² on same pinaculum on A1-A7; D¹ and SD¹ on same pinaculum on A9; SV group on A1-A6 trisetose, on A7 and A8 bisetose, A9 unisetose; L group trisetose on A9.

Dugdale *et al.* (2005) established a key for tortricids encountered in the field in New Zealand and the following characteristics where described in the key:

Setal group SV on abdominal segments A7-9 not with 3,2 and 2 setae respectively, but usually 3,2,1 or 2,2,2, or 2,2,1; Head capsule in most species with axes of setal series P and MD forming an obtuse angle between 100° and 140° . Setal group SV on A7 with 2 setae. A9 setae D1, D2, SD1 either arranged on 3 separate pinacula (D1+SD1; D2+D2; D1 + SD1) or these can be narrowly joined; setal group SV on A1, A2 bisetose or trisetose; A7-9 setae V1 usually equally far apart. T1 prespiracular setal group A1, A2 setal group SV, and A9 setal group L all trisetose. Head capsule seta P1 close to adfrontal suture (separated by \pm 3× P1 socket diameter); A9 setal group SV unisetose. A9 setae D1 on a line anterior to setae D2, Sd1 in dorsal view. Anal shield longer than wide; anal comb with 5-6 teeth, narrower than the distance between the D2; A9 setal pinacula D2 + D2, SD1+ D1 separate or narrowly joined.

Timm et al. (2007) described T. leucotreta as (Fig. 19):

General. Larva slender, elongate, cream to light red. Integument rugose. Setal pinacula easily observed, darker than body colour; spinulation of integument easily apparent, setae moderately long. Head yellowish brown with dark pigmentation at ocellar area and postgenal juncture. Prothoracic and anal shields distinct, medium brown with darker patches due to moderate and extensive sclerotization. Thoracic legs medium brown.

Head (Figs 19a-e). Hypognathous, dorsal surface flattish and broad. Average width prior to pupation 1.31 mm (n = 29). Vertical angle acute. Adfrontals tapering anteriorly and extending to vertical angle. P^1 closer to Adf^2 than F^1 . Ocellar areas rounded. Stemmata approximately equal in size, irregularly rounded. Stemma II closer to stemma I than III. Stemmata III and IV situated close together. Stemma V closer to stemma IV than VI. Stemma V separated from VI by distance nearly equal to half diameter of stemma V. O^1 equidistant from

stemma II and III. A^2 closer to A^1 than A^3 . A^3 closer to L^1 than A^2 . Line joining O^1 and A^1 closer to stemma III than stemma II. Line joining O^1 and L^1 through median of stemma I. Mandible with five teeth, the outer three large, usually acuminate although second or third often flattened, fourth smaller and flattened and fifth straightedged. Antenna elongate, shorter, less robust than labial palps. with long terminal seta. Distal end of spinneret rounded, about eight times longer than wide.

Thorax (Figs 19f, j). Prothoracic shield with anterior lateral margin obtuse, slightly concave, curved at about one-third of its length and curved convexly towards the mid-line, lateral margin fairly straight and posterior margin evenly rounded towards mid-line. On prothorax, spiracle circular; L^1 equidistant from L^2 and L^3 , L^1 more or less in a straight line with L^2 and L^3 , SD^1 equidistant from XD^2 and SD^2 . On meso- and metathorax D^1 dorsal to D^2 . Thoracic claws small, curved.

Abdomen (Figs 19g-j). Spiracles oval, small, seldom larger than setal bases. SD¹ separated from spiracle by approximately 1.5 times diameter of spiracle. On segment 8 (A8) spiracle slightly anterior to midvertical line through segment; SD¹ anteroventral to spiracle at about three times spiracle diameter. SV group on A1, 2, 7, 8 and 9 usually 3:3:2:2:1. On A9, D¹ and SD¹ on same pinaculum; L¹ equidistant to L² and L³. Anal fork well developed, darkly pigmented, with 2–10 bluntly dentate prongs. Basal part of each prong strongly tapered dorsally, with width of the base nearly one-quarter length of tooth, upper levels of larger prongs medially at about same level. Anal shield tapering posteriorly, evenly rounded along posterior margin, lateral margin acute, angled anteriorly, anterior margin broadly curved. L¹s further apart than D¹s. D²s half as long as L¹s. D1s slightly closer to SD¹s than to each other, D¹s slightly shorter than SD¹s. Crochets of abdominal prolegs irregularly triordinal, 36–42 and 24–32 on anal prolegs. Anal prolegs with crochets absent in medial half.

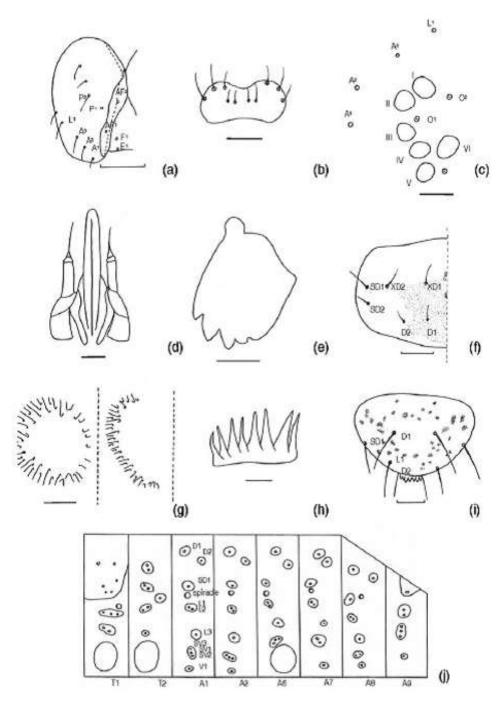


Figure 19 : *Thaumatotibia leucotreta* final instar larva. a, Head (frontal aspect); b, labrum; c, stemmata; d, spinneret; e, mandible; f, prothoracic shield; g, crochets on ventral and anal prolegs; h, anal comb; i, anal shield; j, setal map. Scale bars: a, f, i = 1 mm, b–e, g, h = 0.1 mm. (Taken from Timm *et al.*, 2007; Fig. 2)

Gilligan et al. (2011) described T. leucotreta as follows:

First instar larvae are ca. 1mm in length and are pale with dark pinacula. Mature larvae are ca. 12-18mm long with yellowish brown to dark brown head and prothoracic shield. The abdomen is orange to pink with large pinacula that are darker than the body colour. *Thaumatotibia leucotreta* larvae can be distinguished from many tortricids in California by the following combinations of characters: L pinaculum on T1 enlarged, extending beneath and beyond (posterad of) spiracle; D¹ and SD¹ on A9 on same pinaculum, separate from D²; L group on A9 trisetose; anal comb present with 2-10 teeth. Other larval characters of *T. leucotreta* include: SD 2 on A1-8 highly reduced or appearing absent; SV groups on A1,2,7,8,9 with 3:3:2:2:1 setae; spiracle on A8 displaced posterad of SD pinaculum; V setae on A9 slightly farther apart than those on A8. Enlarged L-pinaculum on A9.

2.8.2 Cydia pomonella

Swatschek (1958), MacKay (1959), Brown, (1987) and Dugdale *et al.* (2005) all provided descriptions of *C. pomonella* larvae.

Lopez (1929) did an extensive study on the head and mouth parts of *C. pomonella* larvae. However, for this study only the labrum and mandible sections where used.

Mandibles. The mandibles articulate ventrally with the cranial margin of the head by means of the hypocondyles, and dorsally with the cranial margin through the epicondyles. The mandibles are about 0.4mm long, about 0.4mm, wide and are five-toothed. They are much darker in the denticulate region. From the dorsal aspect concave. Two setae, one long and one short, arise from the dorsal surface of the mandibles.

Swatschek (1958) described *C. pomonella* in German, part of his elaborate description has been abridged and text freely translated: bracketed nomenclature is that of Hinton (1946) (Figs. 20 and 21).

Larvae whitish with reddish hue. Head and prothoracic shield light to dark brown. Prothoracic and anal shield with darker spots. Body either faintly or not at all granulated. Ocellus 2 closer to 1 than 3. On the prothoracic shield, IIIa (SD^1) is closer to III (SD^2) than IX (XD^2) . IV (L^1) ventral from V (L^2) and VI (L^3) equidistant to both. V (L^2) twice as long as VI (L^3) , IV (L^1) even longer. On A1 and A2, group VII (SV) has 3 setae, on A7 and A8, 2 setae and on A9, one setae. On all abdominal segments, IIIa (SD^2) is on the same pinaculum as III (SD^1) . IV (L^2) and V (L^1) positioned vertically on A1 and horizontally on the other abdominal segments. On A2, spiracles elliptical, and larger than the setal base of III (SD^1) . On A8, II (D^2) and I (D^1) equidistant. On A9, I (D^1) , II (D^2) and III (SD^1) situated on same pinaculum. VI (L^3) is separate from IV (L^2) and V (L^1) . A microsetae is positioned anteriorly to the pinacula of I (D^1) and III (SD^1) . VIII (V^1) on A9 equally apart as on A8. Abdominal prolegs crochets 28-35 and anal proleg crochets, 23.

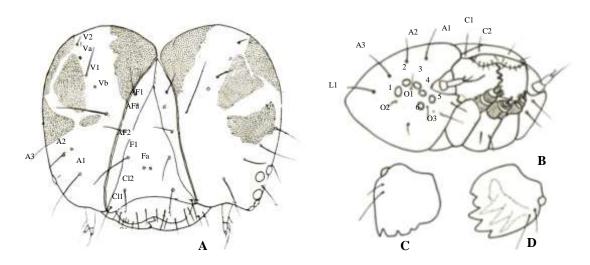


Figure 20: A) Dorsal head chaetotaxy of *Cydia pomonella*, **B)** Lateral head chaetotaxy; **C)** Mandible right; **D)** Mandibles left (Collated and modified from Swatschek, 1958; Figs 1, 3, 5, 6)



Figure 21: A) Prothoracic shield; B) Anal shield of Cydia pomonella (Collated and modified from Swatschek, 1958; Figs, 89, 90).

MacKay (1959) described *C. pomonella* as follows (Fig. 22):

General: Mature length between 15-19 mm; larvae moderately stout with light brown body colour, setal pinacula moderate in size, of body colour or darker. Spinulation of integument easily observed the spinules slender with bases of the body colour. Setae moderately long, often spine-like in appearance. Head yellow brown, often overlaid with a darker brown pattern and blackish pigment on ocellar areas. Prothoracic and anal shields usually somewhat darker than body colour with a brownish pattern on the former and a speckled appearance on the latter. Thoracic legs light brown and short. Anal fork absent.

Head: "Hypognathous, head capsule as an average length of 1.42 mm and 1.71 mm wide. Vertical angle acute. Adfrontals extending to vertical angle. The distance between E^2 and F^1 about two thirds of that between E^2 s and F^1 s. P^1 much closer to Adf^2 than F^1 . Ocellar areas rounded. Stemma II usually much less than its diameter from stemma I and III. Stemma IV usually slightly closer to stemma III than to V. O^1 equidistant from stemma I and III or slightly closer to III. O^2 more ventral than caudal to stemma I. A^2 more or less equidistant from A^1 and A^3 . Spinneret about six to six and half times as long as wide and tapered to distal end.

Thorax: On prothorax, spiracle more oval than circular; L^1 more or less equidistant from L^2 and L^3 , and distinctly below a straight line joining L^2 and L^3 but sometimes in line with both. SD^1 usually closer to SD^2 than to XD^2 but occasionally equidistant from both. On mesothorax and metathorax D^1 more or less dorsal to D^2 . Coxae of metathoracic legs their own diameter or more apart. Claws moderately slender, dorsal setae shorter than claws.

Abdomen: Spiracle small. SD^1 on A1-7 usually two or three times its diameter from spiracle. Spiracle on A8 tending to be on posterior half of segment and SD1 anterior or slightly anterior ventral to it and one and a half or two times its diameter from it. L^1 and L^2 more or less ventral to spiracle. On A9, D^1 & SD^1 on the same pinaculum; L^1 , L^2 and L^3 on same pinaculum, or apparently L^3 more often distant from L^1 and L^2 and on own pinaculum. SV group on A1, 2,7,8,9 usually 3:3:2:2:1 or 3:3:2, 1:1:1 or 2:3:2:2:1 or other combinations; V^1 s setae on A9 the same distance or slightly father apart than these on A8.

Anal fork absent. Anal shield rounded posteriorly; L^1 usually farther apart than D^1 's but occasionally about same distance, and as long or longer than the anal segment. D^2 's less than half as long as L^1 's; D^1 's usually somewhat closer to corresponding SD^1 than to each other, sometimes distinctly so, and shorter than SD^1 s. Crotchets unevenly uniordinal, 30-35 on ventral prolegs and 15-25 on anal prolegs. Crotchets "in situ" more or less ovoid.

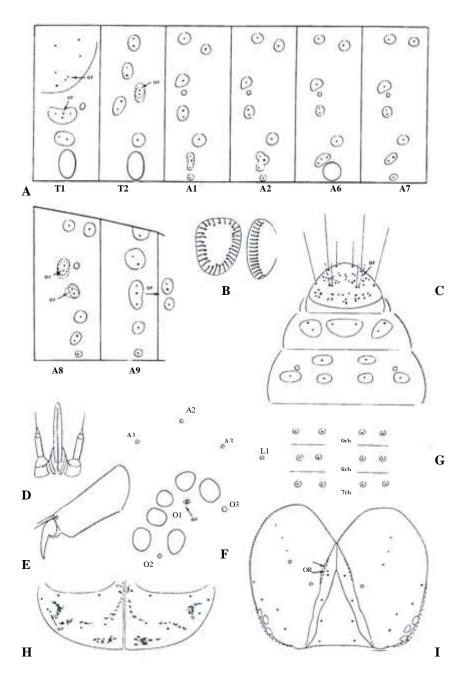


Figure 22: *Cydia pomonella* larvae, **A)** Setal map; **B)** Ventral and anal prolegs; **C)** Dorsal view of A8-A10; **D)** Spinneret; **E)** Thoracic legs; **F)** Ocellar area; **G)** V¹s position on A7-A9; **H)** Prothoracic shield; **I)** Dorsal head. (Taken and modified from MacKay, 1959; Fig. 50).

Brown (1987) established a key for some common tortricid larvae on apple, peaches and generically related fruit and gave a short description and illustration (Fig. 23):

14-18 mm. Head yellow-brown, often overlaid with darker pattern, prothoracic and anal shields with dark speckling, spinules of integument distinct. Anal comb absent.

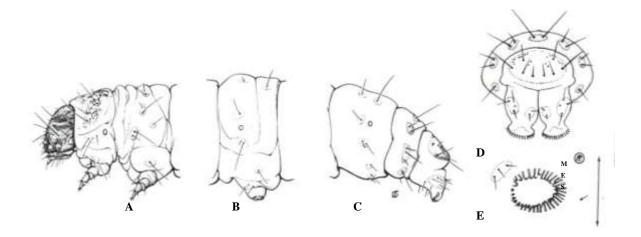


Figure 23: Cydia pomonella, A) Head, T1, T2; B) A3; C) A8-A10 D) A9-A10 caudal view E) A6 crochets (Taken from Brown, 1987; Fig 26.126).

Dugdale *et al.* (2005) established a key for tortricids encountered in the field in New Zealand and the following characteristics where described in the key:

Setal group SV on abdominal segments A7-9 not with 3,2 and 2 setae respectively, but usually 3,2,1 or 2,2,2, or 2,2,1. Head capsule in most species with axes of setal series P and MD forming an obtuse angle between 100° and 140° (However, *Cydia pomonella* has these setae series in line). Setal group SV on A7 with 2 setae. A9 setae D^1 , D^2 , SD^1 either arranged on 3 separate pinacula $(D^1+SD^1; D^2+D^2; D1+SD^1)$ or these can be narrowly joined; setal group SV on A1, A2 bisetose or trisetose; A7-9 setae V1 usually equally far apart. T1 prespiracular setal group A1, A2 setal group SV, and A9 setal group L all trisetose. Head capsule seta P^1 close to adfrontal suture (separated by $\pm 3 \times P^1$ socket diameter); A9 setal group SV unisetose. A9 setae D^1 on a line anterior to setae D^2 , SD^1 in dorsal view. Anal shield wider than long; anal comb absent, A9 setal pinacula $D^2 + D^2$ and D^1+SD^1 separate. T2, T3 seta V^1 pinacula separate from coxal sclerite; prolegs with outer crotchets more widely spaced than the rest of the series; Anal shield speckled in most instars; A1-6 setal pinaculum SD with an anteroventral lobe bearing seta SD^2 in early instars; head capsule setal series P and MD axes forming an angle closer to 180 °.

2.8.3 Grapholita molesta

Garman (1917), Wood & Selkregg (1918), MacKay (1959) Brown, (1987) and Dugdale *et al.* (2005) all described *G. molesta* as follows:

Garman (1917) (Fig. 24):

*For uniformity, Hinton's (1946) setal nomenclature is also given.

General: Head capsule black and shining, sometimes provided with pale markings; body white to pinkish, the prothoracic and anal shields usually brown; length of full-grown larva 12-15mm. Length of labium one and one-half times its width, cardo as long as the stripes; mentum expanded at the caudal extremity and with two heavy setae near the middle; maxillary palpi three- segmented, the basal segment with a long seta on the inner surface; galea-lacinia with four distinct teeth, two of which are chitinized, the tips of the chitinized, projections with blunt tubercles; labial palpi slender, composed of two segments, a very short, globular, distal segment tipped with a long seta and a long proximal segment with a short seta at the apex; tip of the labium heavily chitinized; antennae four-segmented, the third segment with a long seta near the distal end, the distal segment about one-half the diameter of the preceding segment and provided with a blunt projection and a long seta; third segments with two short setae, one long setae and a blunt projection similar to that of the distal segment; the anterior setae being nearly equidistant and in almost a straight line; anterior puncture directly mesad of the second anterior seta; first posterior seta near the adfrontal suture and about midway between the vertical

triangle and the articulation of the mandible; arms of the epicranial suture concave in the dorsal third; ultraposterior tubercles four in number, at least three of which are provided with short setae.

Thorax and abdomen: Segment nine of the abdomen with alpha sometimes represented by a tubercle or short seta near the dorso-meson delta (II) (D2)located on the same pinaculum with rho (III) (SD1); kappa (IV) (L1) and mu (V) (L2) on the same pinaculum and about equidistant; kappa (L) (pre-spiracular) group of the prothorax with the middle seta longest and equidistant from the remaining two; kappa (L) group (V, VI) bisetose on segments one to eight; pi (VII) (SV) group unisetose on segment seven, eight and nine. Crochets uniordinal, densely placed, the lateral hooks sometimes short; number of crochets on prolegs 3 to 6 varying from about 25 to 35; hooks of irregular length infrequent, at least nothing present which approaches the biordinal type; crochets of the anal prolegs in full grown specimens 19 to 20, the cuticle immediately cephalad without thickest pad of spines; anal fork with four to five spines usually of equal length and parallel, the two mesal spines sometimes blunt.

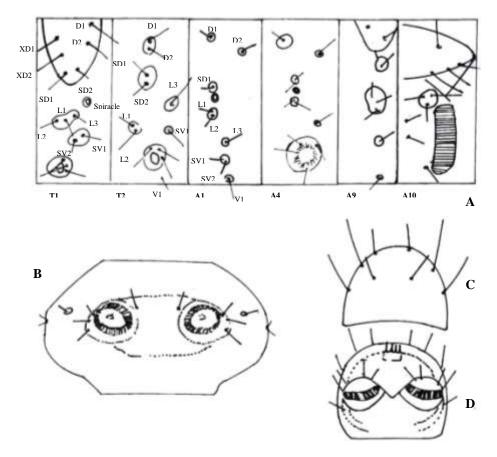


Figure 24: *Grapholoita. molesta* **A)** Setal maps of the larva; **B)** Ventral view of forth abdominal segment; **C)** Dorsal view of anal shield; **D)** Ventral view tenth abdominal segment of the larvae (Redrawn from Garmen, 1917; Fig. 3, 4, 5).

Wood & Selkregg (1918) (Fig. 25):

* For uniformity, Hinton's (1946) setal nomenclature is also provided.

The larva is cylindrical; without secondary hair; colour varying from white deep pink, usually more strongly suffused with pink on dorsal side. Legs and prolegs normal. Crochets (31 o 46) uniordinal, in a complete circle. Anal fork developed, yellow to black in colour, three to six pointed, prominent. Setal areas broadly chitinized, grayish brown. Thoracic shield light yellow edged with yellowish brown, narrowly divided, moderately broad. Spiracles dark brown or black, small circular, slightly produced; spiracle on prothorax and that on abdominal segments 1 to 7. Entire body, except chitinized areas, evenly and finely scobinate; what appears to be a coarse pubescence under low magnification proves, under high magnification, to be a mass of short aculei.

Body setae yellow shading to deep brown, moderately long. Prothorax with Ia (XD^1) and Ib (XD^2) on, and Ic (SD^1) behind the anterior margin of the shield; IIa (D^1) and puncture y (XD^b) caudad of Ia (XD^1) , IIb (D^2)

directly laterad of IIa (D^1); puncture x (XD^c) dorsad of and approximate to Ib (XD^2), lower than the level of IIb (D^2); Ib (XD^2), Ic (SD^1) and IIc (SD^2) equidistant; prespiracular shield oval, situated ventro-cephalad of the spiracle; IV (L^1) and V (L^2) on the same chitinization, under the spiracle approximate. Abdominal segment 8 with II (D^2) only slightly below the level of I (D^1); III (SD^1) and IIIa (SD^2) cephalad of the spiracle. Abdominal segment 9 with all setae in a line I (D^1) and III (SD^1) closely approximate; V (L^1), IV (L^2), and VI (L^3) on the same chitinization, approximate; VII (SV group) unisetose.

Head light brown, with darker brown mottling; hind margin, ocellar area, and tips of trophy black.

Head capsule nearly spherical, slightly flattened, broadly oval in outline viewed from above, a little wider than long; greatest width well behind the middle; incision of dorsal hind margin about one-fourth the width of the head; distance between dorsal extremities of hind margin less than one-half the width of the head. Frons (Fr) only slightly longer than wide, reaching to middle of head; adfrontal ridges (AdfR) sinuate; longitudinal ridge half the length of the frons; adfrontal suture (AdfS) reaching to dorsal incision of hind margin. Projection of dorsal margin over ventral slightly less than one-third the diameter of the head.

Ocelli six, in normal tortricid arrangement; III, IV and V in a straight line; I larger than the others.

Epistoma with the normal setae (E_1, E_2) .

Frontal punctures (F^a) lying rather closely together, anterior to the setae (F_1) ; distance between punctures less than from puncture (F^a) to seta (F_1) ; adfrontal seta (Adf_1) nearer to F_1 than to Adf; adfrontal puncture (Adf^a) approximate to Adf_2 .

Epicranium with the normal number of primary setae and six punctures, and with three small ultra posterior puncture. Anterior and lateral setae (A_1 , A_2 , A_3 , and L_1) in line, with distance between A_1 and A_2 , A_2 and A_3 and L_1 about equal; puncture (A^a) postero-dorsad of A_2 ; A_1 , A_2 and A_3 on a level respectively with F^a , F_1 and Adf_1 . Posterior setae (P_1 and P_2) and punctures (P^a and P^b) at middle of head; P_1 on a level with adfrontal puncture (Adf^a) P_2 and puncture (P^b) on a level with beginning of longitudinal ridge (LR); P_2 , P_1 , and adfrontal seta (Adf_1) in a line; puncture (P^a) approximate to and equidistant from A_3 and A_4 . Lateral seta (A_4) on a line with A_4 and adfrontal puncture (Adf^a); lateral puncture (Adf^a) directly posterior to the seta. Ocellar setae (A_4) well separated. A_4 0 approximate to and equidistant from ocelli II and III, within the area bounded by the ocelli; A_4 1 closely approximate to and postero-ventrad of ocellus I; A_4 2 and A_4 3 and A_5 4 and A_7 5 and A_8 6 and remote from A_8 6 and remote from A_8 7 and A_9 8 and A_9 9 and remote from A_9 9 and A_9 9 and remote from A_9 9

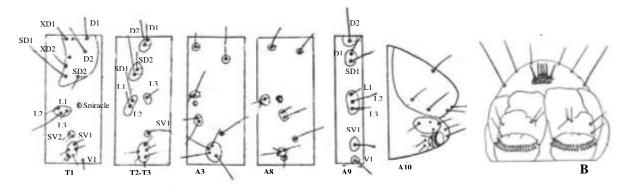


Figure 25: Grapholita molesta A) Setal map, B) Ventral view of anal prolegs and caudal end of abdomen. (Taken and modified from Wood & Selkregg, 1918; Fig. 8)

MacKay (1959) (Fig. 26):

General: Length 10 or 12 mm. Length and width, respectively, of head on six specimens averaging 0.92mm and 1.11mm. Position of P^1 in relation to anterior and posterior margins of head ranging from 33:42 to 31:46. Head yellow-brown, often overlaid with a darker pattern; dark pigmentation on ocellar area and at postgenal juncture. Thoracic shield yellowish, occasionally with some green or brown pigment present. Thoracic legs

pale. Anal shield usually with some brown pigmentation. Setal pinacula moderately large, easily observed usually pale except possibly on eight and nine segments; setae short, sometimes spinelike in appearance. Spinulation of integument easily observed, the spinules slender and darker than body colour. The integument in preserved specimens variable in colour, whitish, light brown or more often reddish. Anal fork present. Larvae moderately slender, hypognathous.

Head: In dorsal view variable in outline, but ocellar area rounded and vertical angle more or less acute. Adfrontals extending to vertical angle. The distance between E^2 and F^1 about two thirds of that between E^2 s or F^1 s. P^1 closer to Adf^2 than to F^1 . A^2 more or less equidistant from A^1 and A^3 . O^2 ventral to ocellus I. O^1 closer to ocellus III than to I. Ocellus II less than its diameter from I and usually slightly less than its diameter from III. Ocellus IV sometimes equidistant from III and V, but usually closer to III than to V. All ocelli more or less equal in size. Spinneret about seven to eight and a half times as long as wide.

Thorax: On prothorax, spiracle circular; SD^1 much closer to SD^2 than to XD^2 , SD^2 very short and delicate. D^1 s closer to corresponding D^2 s than to each other and in a straight line with D^2 s or slightly posterior to that line. L^1 usually slightly closer to L^2 than to L^3 and below a straight line joining L^2 and L^3 . On mesothorax and metathorax D^1 more or less dorsad of D^2 .

Abdomen: Spiracle moderate in size, and SD^1 about one and a half to three times its diameter from it, except on segment 8. Spiracle on segment 8 tending to be on posterior half of segment, and SD^1 more or less anterior to it and one or one and a half times its diameter from it. L^1 and L^2 usually directly below spiracle even on segment 8, but tending to be slightly posterior to a vertical line through spiracle on some specimens, and L^2 usually dorsal to L^1 . SV group on segments 1,2,7,8 and 9 usually 3:3:2:2:1 but unstable and occasionally 2:3,2:2:2:1 or 3, 2:3:2:2:1 and even 2:3,2:2:2:2 on one specimen. On segment 9, D^1 and SD^1 on the same pinaculum; L^1 usually equidistant from L^2 and L^3 . V1s the same distance apart or more often farther apart on A9 than those on segment 8.

Anal shield: Rounded posteriorly. L^1s farther apart than D^1s and about length of anal segment. D^2s about half as long as L^1s . D^1s about equidistant from corresponding SD^1s and each other and usually equidistant from corresponding SD^1s and each other and usually shorter than SD^1s

Legs: Coxae of metathoracic legs their diameter or less apart; pinacula of V¹s fused with coxae. Claws curved but slender; dorsal setae as long as or much longer than claws. Crotchets uniordinal and comparatively long, 30 or 40 on ventral prolegs and about 25 on anal prolegs; crotchets 'in situ' sometimes oval, sometimes almost circular. Anal fork moderately developed, dark brown, easily seen.

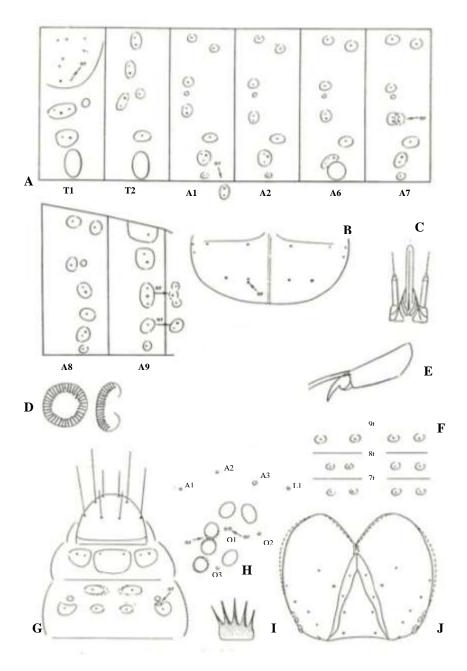


Figure 26: *Grapholita molesta* **A)** Setal map; **B)** Prothoracic shield; **C)** Spinneret; **D)** Ventral and anal prolegs; **E)** Thoracic legs; **F)** V¹s position on A7-A9; **G)** Dorsal view of A8-A10; **H)** Ocellar area; **I)** Anal Comb; **J)** Dorsal head. (Taken and modified from MacKay, 1959: Fig 54).

Brown (1987) established a key on some common tortricid larvae on apple, peaches and generically related fruit and gave a short description and illustration (Fig. 27):

9-13 mm. Pinkish to near white with light brown head; length and width of head more than 9 and 9.5mm respectively thoracic and anal shields lightly sclerotized, pinacula large and pale; spinules of integument distinct, slender and darker than body color; anal comb present.

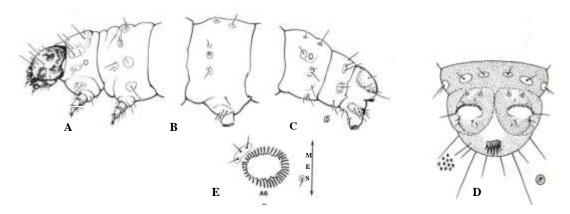


Figure 27: *Grapholita molesta* A) Head, T1, T2; B) A3; C) A8-A10 D) A9-A10 caudal view E) A6 crochets (Taken from Brown, 1987; Fig 26.124).

Dugdale *et al.* (2005) established a key on tortricids encountered in the field in New Zealand and the following characteristics where described in the Key:

Setal group SV on abdominal segments A7-9 not with 3,2 and 2 setae respectively, but usually 3,2,1 or 2,2,2, or 2,2,1; Head capsule in most species with axes of setal series P and MD forming an obtuse angle between 100° and 140° (However, *Cydia pomonella* has these setae series in line). Setal group SV on A7 with 2 setae. A9 setae D1, D2, SD1 either arranged on 3 separate pinacula (D1+SD1; D2+D2; D1 + SD1) or these can be narrowly joined; setal group SV on A1, A2 bisetose or trisetose; A7-9 setae V1 usually equally far apart. T1 prespiracular setal group A1, A2 setal group SV, and A9 setal group L all trisetose. Head capsule seta P1 close to adfrontal suture (separated by \pm 3× P1 socket diameter); A9 setal group SV unisetose. A9 setae D1 on a line anterior to setae D2, SD1 in dorsal view. Anal shield wider than long; anal comb with 4 teeth, A9 setal pinacula D2 + D2 and D1+SD1 separate. T2, T3 setae V1 fused to coxal sclerite; prolegs with crotchets evenly arranged around the planta ("sole" of the proleg); Anal shield \pm immaculate except in the last instar; A1-6 setal pinaculum SD \pm circular in early instars; head capsule setal series P and MD axes forming an angle closer to 90° .

2.8.4 Cryptophlebia peltastica

Williams (1953) and Timm et al. (2005) both described C. peltastica.

Williams (1953)

Larva when mature, length about 20 mm, breadth about 3 mm. Colour: head dark brown; body with reddish hue which is often pronounced, body with tubercles slightly smaller, and lightly pigmented so spotting is not pronounced. Head wider than long. Ocelli 3 and 4 widely separated, but closer together than the others. Spiracles broadly oval. Chaetotaxy: head with puncture V^a between V2 and V3 usually slightly nearer the former. Pb near P2, P1 closer to Adf2 than F1. AF^a much nearer to AF2 than AF1. Body with puncture XD^a postero-dorsad from XD¹. Seta D1on I directly above D2, and slightly above the level of XD1. Setae SD¹ and SD² with common tubercle on 1, on 2-8 SD² with its own small tubercle which is occasionally confluent (especially on 8) with the SD1 tubercle. Seta L1 on 8 postero-ventrad from L2 as on 1-7 Subventral group bisetose on 9. On 1 and 2 SV2 ventrad and slightly cephalad from SV1, SV3 antero-dorsad from SV1. Tubercle of ventral seta quite separate from coxa on II and III.

Timm *et al.* (2007) (Fig. 28):

General. Larva elongate, light pink to light red. Integument rugose. Setal pinacula easily observed, with brown pigmentation. Spinules slender, darker than body colour. Head brown with darker pigmentation at ocellar areas. Prothoracic shield dark brown. Anal shield yellow brown with some medium brown pigmentation. Thoracic legs medium brown.

Head (Figs 28 a-e). Hypognathous, dorsoventrally flattened. Average width prior to pupation 1.7 mm (n = 11). Adfrontals extending to vertical angle, tapering anteriorly. Vertical angle acute. P^1 closer to Adf^2 than F^1 . Stemmata II – VI rounded, approximately equal in size. Stemma I elongate, length nearly 1.5 times diameter of III. Stemma IV closer to III than VI. Stemmata IV and VI separated by distance slightly less than diameter of stemma IV. Stemma V and VI separated by distance roughly 1.5 times diameter of stemma V.O¹ closer to stemma III than stemma II. Line through O^1 and A^1 closer to stemma III than III, through O^1 and O^1 and

Thorax (Figs 28f, j). Prothoracic shield with anterior lateral margin obtuse, lateral margin fairly straight and posterior margin evenly rounded towards mid-line. On prothorax, spiracle circular, L^1 equidistant from and in straight line with L^2 and L^3 , SD^1 slightly closer to SD^2 than XD^2 . On meso- and metathorax D^1 dorsal to D^2 . Thoracic claws curved.

Abdomen (Figs 28g-j). Spiracles small, circular, seldom larger than setal bases, SD^1 approximately 2.5 times its diameter from spiracle except on A8. On A8 spiracle slightly posterior to mid-vertical line through segment; SD^1 situated anteroventrally to spiracle and about 1.5 times of spiracle diameter. L^1 and L^2 usually ventral to spiracle and on A8 L^2 slightly anterodorsal to L^2 . SV group on A1, 2, 7, 8, 9 usually 3:3:3:2:2. On A9, D^1 and SD^1 on same pinaculum, L^1 usually equidistant to L^2 and L^3 . Anal fork absent. Anal shield rounded posteriorly. L^1 s slightly further lateral than D^1 s. D^1 s closer to corresponding SD^1 s than to each other and almost in a straight line. Prolegs with 50–58, and anal prolegs with 46–54 mostly biordinal crochets. Anal prolegs with crochets absent in medial half.

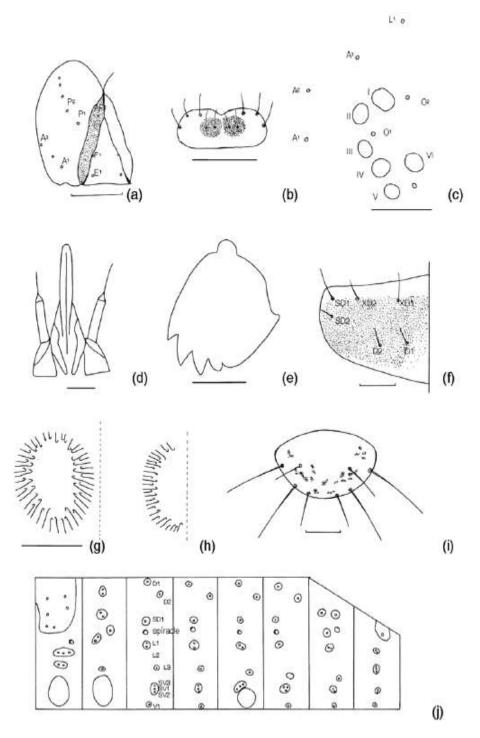


Figure 28: *Cryptophlebia peltastica* final instar larva. a, Head (frontal aspect); b, labrum; c, stemmata; d, spinneret; e, mandible; f, prothoracic shield; g, crochets on ventral prolegs; h, crochets on ventral prolegs; i, anal shield; j, setal map. Scale bars: a, f, i = 1 mm, b—e, g, h = 0.1 mm. (Taken from Timm *et al.*, 2007; Fig 6)

2.8.5 Thaumatotibia batrachopa

Timm et al. (2007) described T. batrachopa as follows (Fig. 29):

General: Larva elongate, cream to grey green. Setal pinacula moderately large, easily observed. Integument rugose and spinulation of integument conspicuous, spinules slender and darker than body colour. Head yellow brown with darker pigmentation at ocellar area. Prothoracic and anal shields yellow brown, lightly sclerotized with small patches of darker pigmentation. Thoracic legs medium brown.

Head (Figs 29 a-e). Hypognathous, dorsoventrally flattened. Average width prior to pupation 1.5 mm (n = 10). Vertical angle acute. Adfrontals extending to vertical angle, tapering anteriorly and posteriorly. P¹ closer to Adf² than F¹. Ocellar areas rounded. Stemmata approximately equal in size, irregularly rounded. Stemma II equidistant from I and III. Stemmata III and IV close together, IV closer to III than VI. Stemmata V and VI separated by distance nearly equal to diameter of V. O¹ equidistant from II and III. A² closer to A¹ than A³. A³ closer to L¹ than A². Line through O¹ and A² equidistant from stemma II and III. Lines joining O¹ and A¹ and O¹ and A³ nearly right-angled. A¹, A², A³ and L¹ on regular rounded arc. Mandible with five teeth, outer three pointed, second largest, fourth smaller and fifth smallest and straight-edged. Antenna elongate with long terminal seta, shorter, less robust than labial palpus. Distal end of spinneret rounded, about seven times longer than wide.

Thorax (Figs 29 f, j). Prothoracic shield with anterior lateral margin obtuse, slightly concave and curved about one-third its length, curved convexly towards mid-line, lateral margin fairly straight, posterior margin evenly rounded towards mid-line. On prothorax, spiracle very prominent, circular L^1 , L^2 and L^3 in straight line. SD^1 equidistant from XD^2 and SD2. On meso- and metathorax D^1 dorsal to D2. Thoracic claws curved.

Abdomen (Figs 29 g-j). Spiracles circular, small, seldom larger than setal bases, SD¹ usually separated by distance 1.5 times diameter of spiracle except on A8. On A8 spiracle on mid-vertical line through segment; SD¹ situated anteroventrally of spiracle at about twice its diameter. L² anterodorsal to L¹ on same pinaculum and L¹ and L² anterodorsal to spiracle. On A9, D¹ and SD¹ on same pinaculum; L¹ equidistant from L² and L³. SV group on A 1, 2, 7, 8, and 9 usually 3:3:2:2:1. Anal fork well developed, darkly pigmented, with 5–8 prongs. Basal part of each prong strongly tapered dorsally, width of the base nearly one-quarter length of tooth, prongs merging into distinct medial structure. Anal shield posteriorly rounded. L¹s approximately same distance apart from each other as D¹s. D²s slightly more than half as long as L¹s. D¹s somewhat closer to corresponding SD¹s than to each other. Prolegs with 34–44, and anal prolegs with 26–32 unevenly arranged triordinal crochets with crochets absent in medial half.

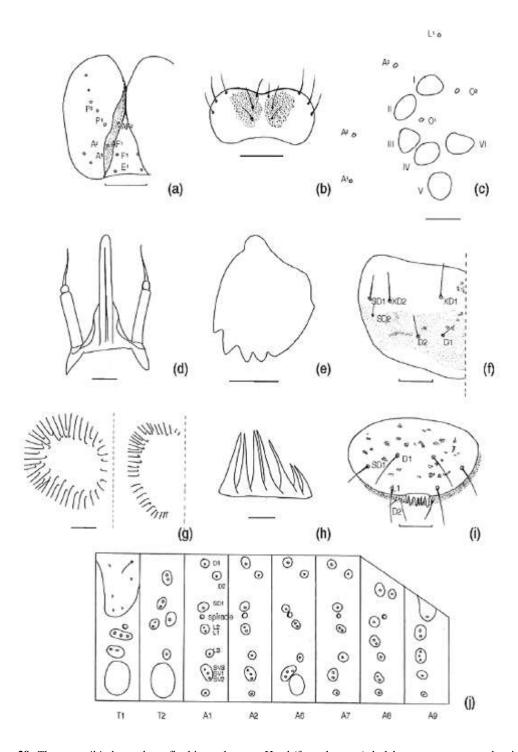


Figure 29: Thaumatotibia batrachopa final instar larva. a, Head (frontal aspect); b, labrum; c, stemmata; d, spinneret; e, mandible; f, prothoracic shield; g, crochets on ventral and anal prolegs; h, anal comb; i, anal shield; j, setal map. Scale bars: a, f, i = 1 mm, b-e, g, h = 0.1 mm. (Taken from Timm et al., 2007; Fig 4)

2.8.6 Epichoristodes acerbella

Nuzzaci (1973) described *E. acerbella* in Italian; part of his elaborate descriptions has been abridged and text freely translated:

General appearance and colouration: The mature larvae reaches a length between 17-21 mm and 1.2 mm in diameter, from the head backwards, the body reaches a maximum of 2 mm across the major segments to eventually reduces to 1.4 mm at the caudal region. The colour of the head is brownish to olive-green with distinct colour of the pronotum; the remainder of the body is yellowish –green. The abdomen of the larva has a medially dorsal line with two lateral bands.

Morphology and taxonomy:

Head (Figs 30-31). The cranium is prognathous, longer than broad, with the epicranial suture about a fifth of the heads length. The frontal sutures are slightly sinus between the anterior angle of the clypeus and the posterior cranium. The epistomal sutures strongly developed, bracing the dorsa; part of the tentorium. The head has following chaetotaxy: Four clypeal setae (Cⁿ), two on each side, with C² longer than C¹, two frontal setae (F¹), one on each side, in line with C², of similar length; four frontal sensilla (F^a), one on each side, the longitudinal axis of the cranium; four adfrontal setae (Afn), two on each side, the anterior AF1 a third the length of AF² in line with the longitudinal axis of the head; two adfrontal sensilla (Af^a), one on each side, situated in a line equidistant from those and between Af¹ and Af², six anterior setae (An), three on each side, diagonally to the cranial longitudinal axis and in line with A² the shortest, A¹ longer than A², and A³ longer than A²; two anterior sensilla (Aa), one on each side, closer to A2. Slightly before connecting lone seta A2 and P1. Six Ocellar setae (Oⁿ), three on each side ventral to the ocelli with O¹ a little longer than O² and O² as long as A³, four Ocellar sensilla, two on each side, one (Oa) situated ventral to SO2 and SO3, the other (Ob) between the third and fourth ocelli; six subocullar setae (SOn), three on each side, situated anterior ventral to the Ocellar region with SO¹, anterior and longer than SO² dorsal to SO³ which is larger than the preceding setae; four subocullar setae, two on each side, anterior ventral margin of the mouth opening (peristioma) and small; two lateral setae (L¹), one on each side situated on a position ventral to A³, its length similar to O¹; two lateral sensilla (L^a), one on each side situated close to L¹; four posterior setae (Pⁿ), two on each side, placed dorsally with P¹ (the longest cranial seta) slightly before Af², P² posterior to a line for A² to the cranial longitudinal axis; four posterior sensilla; two on each side, situated (Pa) on a line between Pa and La and the other (Pb) on a line between Pa and P²; six vertical setae (Vn) three on each side, angled on the anterior-posterior line to the cranial longitudinal axis; two vertical sensilla (Va), one on each side situated between V^2 and V^3 ; two genal setae (G^1), one on each side, close to the foramen occipital; two genal sensilla (G^a), one on each side, situated anterior to G¹ in a line between that seta (G¹) and O³. Six ocelli, five arranged in an arc and 1 placed ventrally to the height of II. Antenna (figure 15). The antenna made up in 4 parts. First segments broader than wide, connected to the skull by a membranous base which can be invaginated. Antennal base can be hollowed out. Second segment much longer than the first, ventral side a placoid sensilla (1) towards distal part. Robust short seta (2) on pinaculum (shorter than the maximum width of this segment). On the tip (apical) part of second segment on the ventral margin there is a prominent seta (3), larger than the entire length of the antenna as well as a subconical sensillium (4) which is a shorter version of the one on the dorsal margin (placoid sensilla). On the same apical part where (long bristle seta is) there is another sensilla half of the smallest sensilla on the subconical (rounded). Third antennal segmented a third of the width of the second segment and has on the distally part towards the margin a subconical sensillium (6). Slightly longer (2) and on the edge. In addition another subconical sensillium (7) slightly shorter than (4). Fourth antennal segment adjoining third segment has two subconical (cone-like) structures of almost equal and slightly longer than third antennal segment. Another conical sensilla adjoining seta (6). Clypeus (Frons) boarders anteriorly to the post labial membrane and posteriorly to the epistomal suture to form a sub triangular shape. Anteclypeus: sub rectangular margin and on its anterior margins concave median incession with the margins rounded and its extremities two sclerifications. Dorsally provided with twelve seta, six on each side of which two are on the anterior margin (front edge), and two in the proximity of the lateral margin (shorter). Inner pair more robust and layer than the clypeus. Another pair almost as long as sub marginal ones behind the anterior median marginal bristles. Ventral face membranous and provided anterior laterally with six sub conical sensilla, rounded tips and compressed. Rear (posterior) formations of placoid sensilla placed behind the sensory medial region. Third pair medial. Placoid sensilla (small/tiny) starts from the front and extends backwards a terminating in an irregular shaped post labral margin. Mandibles (Figure 31): The robust mandibles are sub-pyramidical in shape and provided with five teeth, rising from the inner margin with the third and fourth teeth more developed and pointed. Laterally there are two sensilla on each mandible; ventrally four grooves, diverging medially from the teeth. Maxilla: Laminar cardines hinge in part to the indented posterior of the head capsule and is part to the submental sclerites. The stipites are in part membranous in part sclerotized; in the antero-lateral region two sensilla, the longer one median posteriorly, situated on a longer placoid sensillium. The palpifer with sensillium about fourfifth of the shorter sensillium of the stipite. The maxillary palpus articulates ventrally and dorsally on the palpifer with a sensillium on a placoid sensillium, maxillary palpus has on the first segment on the distal ventral margin, one sensillium with second sensilla probably on a placoid sensillium. On the second segment a distally placed placoid sensillium on the second segment there is a placoid sensillium. The distal part of the third segment subconical sensilla of different lengths on a cylindrical base and distally articulating subconical of various lengths and shapes. A lobe on the ventral side situated on a placoid sensillium with the dorso-lateral side one sensillium. Part of the distal area has two biarticulate sensilla on the cylindrical base, with apical subconical structure on top. Prementum of the labium (Figure 15) sclerotized anteriorrally and at its base two minute sensilla, an the elongated postmentum bi-articulate a basal segment longer than wide and the second segment much smaller than distal base each with a single sensillium on the first distal and apical base of second sensillium, the former about half the length of that of the second segment.

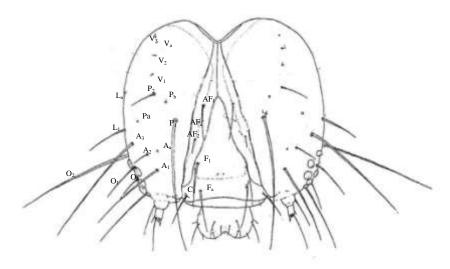


Figure 30: Epichoristodes acerbella, dorsal head chaetotaxy (Taken and adjusted from Nuzzaci, 1973; Fig.12).

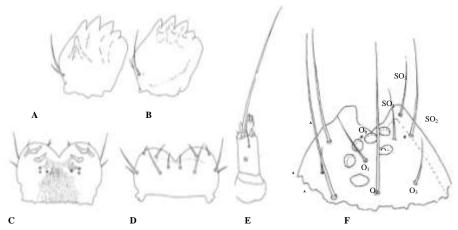


Figure 31: Epichoristodes acerbella, A-B) Ventral and dorsal aspect of left mandible. C-D) Dorsal and ventral labrum; E) Antenna (Taken and adjusted from Nuzzaci, 1973; Fig.13, Fig.14).

Thorax (Fig. 32): **Prothorax** dorsally presents a plaque clarification. <u>Chaetotaxy:</u> Two MXD¹: Far apart on the posterior margin of the dorsal plate. Two MVN on each side anterodorsal to the legs MV² more dorsally. Two

^{*}Bracketed nomenclature is that of Hinton (1946)

XD on each side: towards the antero-lateral aspect of the dorsal plate with XD^2 (SD¹) longer than XD^1 (XD^2). Three punctures (XDa-c). Two D-setae on each side D^1 (XD^1) anterior and longest D^2 (D^1). Two SD setae, SD^2 (D^2) longer and posterior to SD^1 (SD^2). Three L-group setae on one pinaculum anteroventral to pinaculum not reaching below. L^1 (middle setae) longest of all three, Two SV setae SV^1 longer than SV^2 . V^1 very small located behind/below legs. **Meso and metathorax:** Chaetotaxy: One MXD^1 micro bristle ridges. Two MSD^n with MSD^1 anterior to MSD^2 , in one line. Two D setae, D^1 and D^2 on one pinaculum with D^2 (D^1) anterior to and longer than D^1 (D^2). Two SD setae single pinaculum. SD1 ventral to SD^2 and very long compared to SD^2 . L-setae L^1 and L^2 on 1 pinaculum, L^3 on a separate pinaculum and posterior dorso to L^1 and L^2 . L^1 longest of all three. V^1 setae same size and position as on V^1 on prothorax. Three MV microbristle anterior to leg. MV^1 and MV^3 anterior to MV^2 . **Thoracic legs:** Coxa provided with eight bristles, varying length and positions, and three bristles very small.

Abdomen (Fig. 32): Ten segments, eight with spiracles and five with a pair of crotchets. Chaetotaxy 1-7: One MXD1 (microbristle ridges) on each side near anterior margin of segment. One MV³ on each side before SV group. Two D setae, D¹ and D² in line but D² setae slightly longer than D¹. Two SD setae, one pinaculum dorsally to spiracle. SD² tiny, anterior to SD¹. Three L-setae, L¹ and L² on one pinaculum below spiracle, L³ more longer and posterior ventral to L¹ and L². Three SV setae on one pinaculum, SV1 longest of the three. V1 as on the thoracic segments. A8 chaetotaxy: Same as above only difference is that it has no SV3. A9 chaetotaxy: Same as above with the following differences: D¹ shorter than D¹s on other segments. D² longer than D²s on other segments. SD² disappears. L-group united on a single plate. L¹ longest of all three.

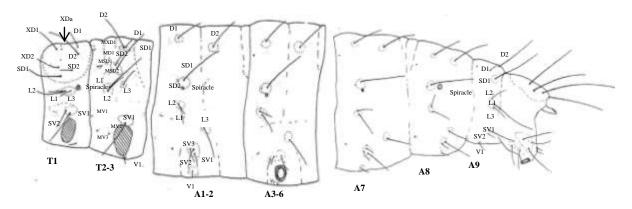


Figure 32: Epichoristodes acerbella setal map (Taken and adjusted from Nuzzaci, 1973; Fig.15).

Timm et al. (2008) described E. acerbella as follows (Fig.33):

General Larva elongate, light green with darker green stripe on dorsal midline and often yellow green stripes at lateral midlines. Setal pinacula conspicuous, lighter than body colour Integument rugose. Spinules long, slender, lighter than body colour, easily observed. Head yellow brown. Prothoracic and anal shields yellow or body coloured, easily observed.

Head (Figs 33 a-e). Average width prior to pupation 1.04 mm (n = 16). Vertical angle acute. Adfrontals narrow, extending to vertical angle. P^1 closer to Adf^2 than F^1 . Ocellar areas rounded. Stemmata inconspicuous with exception of stemma III, surrounded by dark pigmentation. Stemma II closer to stemma III than stemma I. Stemma II separated from stemma I by distance greater than its diameter. Stemmata III, IV and V in straight line, stemma VI at right angles to this line. O^1 equidistant from stemmata II and III. Line drawn through O^1 and O^1 and O^2 closer to stemma II than I. Mandible with five teeth, second and third large and pointed, fourth smaller and slightly flattened, fifth straight-edged.

Thorax (Figs 33 f, j). On prothorax, spiracle small, circular, L^1 equidistant from and in straight line with L^2 and L^3 , SD^1 equidistant from XD^2 and SD^2 . On meso- and metathorax D^1 dorsal to D^2 . Thoracic claws curved.

Abdomen (Figs 33 i-j). Spiracles circular, smaller than setal bases. SD^1 separated from spiracle by approximately half the diameter of spiracle. Spiracle on A8 on midventral line drawn through segment. L^2 usually posteroventral to L^1 . SV group on A1, 2, 7, 8 and 9 usually 3:3:3:2:2. Anal shield: rounded and strongly

tapered posteriorly. D^1s further apart than L^1s . D^2s slightly shorter than L^1s . D^1s closer to corresponding SD^1s than to each other. D^1s anterior to SD^1s .

Anal fork (Fig 33h). well developed, transparent, with 6–9 bluntly dentate prongs of approximately similar length. Width of base approximately equal to or less than length of prongs. Basal part of each prong tapered dorsally.

Proleg (Fig 33j). prolegs with 32–54 and anal prolegs with 28–36 unevenly uniordinal, almost biordinal, crochets.

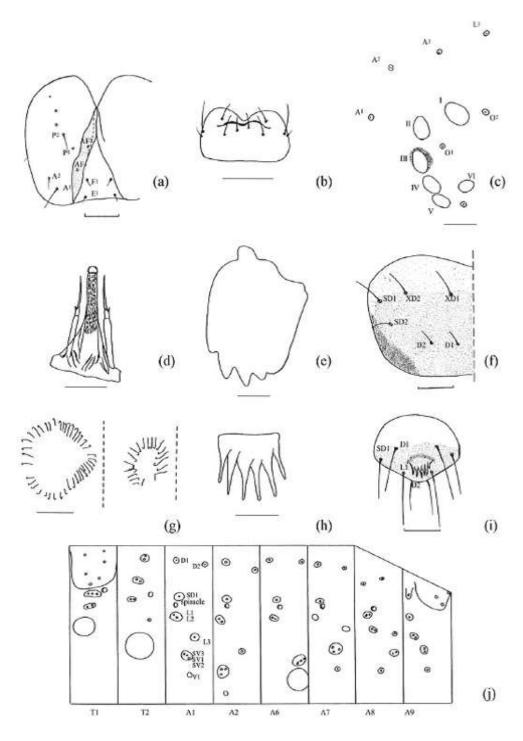


Figure 33: *Epichoristodes acerbella* final instar larva. a, Head (frontal aspect); b, labrum; c, stemmata; d, spinneret; e, mandible; f, prothoracic shield; g, crochets on ventral and anal prolegs; h, anal comb; i, anal shield; j, setal map. Scale bars: a, f, i = 1 mm, b—e, g, h = 0.1 mm. (Taken figure from Timm *et al.*, 2008; Fig. 2).

CHAPTER 3

Morphological study for the pupal stages of economic importance tortricids in South Africa

3.1 Abstract

Seven economic important tortricids causing extensive damage and losses to the fruit industry are present in South Africa, The correct identification of these species in the immature stages is important as misidentifications could lead to ineffective pest management. In South Africa no proper identification guide exists to distinguish between the pupae of these species. Thus the aim was to use the literature available and carry out a morphological study of the pupae to provide descriptions of the pupal stages of each species and to develop an identification guide for this stage.

3.2 Introduction

Seven tortricid species have a major impact on the fruit industry in South Africa, their larvae causing extensive damage and crop losses yearly (Powell, 1964; Timm, 2005). Five of the species belong to Olethreutinae and two to Tortricinae (See Chapter 1, Table 1) (Pinhey, 1975; Karisch, 2001; Brown, 2005): Cydia pomonella (Linnaeus, 1758) (Codling moth), Thaumatotibia leucotreta (Meyrick, 1913) (False codling moth), Grapholita molesta (Busck, 1916) (Oriental fruit moth), Cryptophlebia peltastica (Meyrick, 1921) (Litchi moth), Thaumatotibia batrachopa (Meyrick, 1908) (Macadamia nut borer), Epichoristodes acerbella (Walker, 1864) (Pear leafroller/Carnation worm), and Lozotaenia capensana (Walker, 1863) (Apple leafroller) (Brown, 2005). Misidentifications of these economically important species, especially the immature stages, may lead to ineffective pest management (Timm, 2005) or result in export restrictions. The main objective of this study was to provide a complete, or nearly complete, description of each species that could subsequently be used to distinguish between them, leading to the development of an identification guide. The over-arching goal was to provide a reliable identification tool for workers in the South African fruit industry.

3.3 Material and methods

Literature of various authors was studied, summarized (see Appendices 3.1), and compared for descriptions of the pupal stages of *T. leucotreta*, *C. pomonella*, *G. molesta*, *C. peltastica*, *T. batrachopa*, *E. acerbella*, and *L. capensana*. The descriptions were then compared with specimens at hand, and gaps in the descriptions were filled by additional morphological studies.

For *L. capensana* no published morphological description of the pupa could be found. The morphological characteristics described in this chapter are not always comprehensive for every taxon, but they fulfil the main objective of providing sufficient character information for identification purposes.

3.3.1 <u>Insect material</u>

Pupal specimens of *T. leucotreta*, *C. pomonella*, *C. peltastica*, and *G. molesta* were generously donated from established South African laboratory colonies: *T. leucotreta* from XSIT, Citrusdal, and Rhodes

University, Grahamstown; *C. pomonella* from Entomon, Stellenbosch; *C. peltastica* from Bioriver Science, Addo; *G. molesta* from Embrapa Grape and Wine, Bento Gonçalves, Brazil and the Applied Entomology Department in the Institute of Agricultural Research, Eidgenössische Technische Hochschule, Zürich, Switzerland. Additional pupae of *C. peltastica* were collected from *Acacia saligna* and *A. pygnantha* galls. *T. batrachopa* pupae were received from Upotn farm, Umhlali. Larval specimens of *E. acerbella* were collected in the field and reared to adulthood for confirmation of pupal characters. One specimen of *L. capensana* was collected in an apple orchard at Oak Valley farm, Grabouw; other specimens were from the Entomological Museum of the Stellenbosch University (USEC), South Africa, which is also the depository of the voucher specimens of this study.

3.3.2 Preparations of specimens

For reference material pupae were killed in alcohol and stored in Kahle's Fluid as preservative. Kahle's fluid was selected for its better colour retention of specimens.

3.3.3 Preparation of images

Photos of pupae with a Leica MZ 16A automontage microscope for two dimensional image analyses with a Leica DFC 290 fixed digital camera and Leica Application Suite (LAS) v.2.7. software. Photos were edited in Adobe Photoshop Element v.9.0.0 (Adobe System Incorporated).

3.3.4 Nomenclature

Nomenclature for pupal morphology follows that of Patočka & Turčáni (2005) (Fig. 34) and Timm *et al.* (2007, 2008). For uniformity, the style and format of the descriptions follow that of Timm *et al.* (2007, 2008). Setae are described for the left half of the pupae being a mirror image of the right.

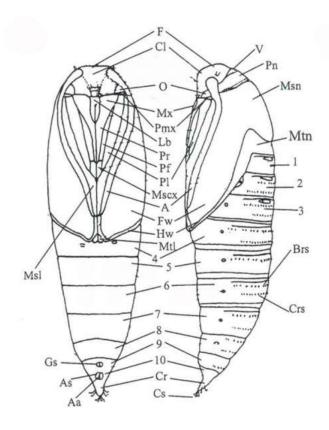


Figure 34: General pupae; A. antennae; Aa. anal area; As. anal opening Brs. basal row of spines; Cl. clypeus; Cr. cremaster; Crs. caudal row of spines; F. frons; Fw. forewings; Gs. genital opening; Hw. hindwings; Lb. labium; Mscx. mesocoxa; Msn. mesonotum; Mtl. metatarsus; Mtn. metanotum; Mx. maxillae; O. eyes. Pf. prothoracic fremor (not always visible); Pl. protarsus. Pmx. maxillary palpi. Pn. pronotum; Pr. maxillae; 1-10. 1st -10th abdominal segments (Taken and edited from Patočka & Turčáni, 2005; Fig B1).

3.3.5 Key development

Morphological details of pupae have not been used in the development of diagnostic keys due to minute difference in these structures not easily discernible to workers in the fruit industry. A summary was compiled using existing literature and own morphological assessments, in order to assist stakeholders in identification of pupae.

3.4 Results

3.4.1 Thaumatotibia leucotreta

Head (Figs 35 A-C). Eyes darker and prominent in mature pupae. Two medial pairs of setae on clypeus, outer pair longer.

Thorax (Figs 35 A-B). Metatarsus extending beyond hindwing, in line with lower margin of forewing. Two pairs of setae on pro- and mesonotum: on pronotum, one lateral pair near forewing and medial pair close to mid-dorsal line. On mesonotum, one pair midway on alar furrow and one pair near mid-dorsal line. On metanotum, one pair of setae situated in anterior corners of the "M"-shaped hindwing.

Abdomen (Figs 35 A-B, D-E). Setae on lateral margin of ventral aspect: four setae on A4, one pair close to spiracle, and two single setae, one medio-anterior and one anterior, close to the mid-ventral line, single seta

sometimes concealed by hindwing. Seven setae on A5-A6, lateral group paired; one group of three, arranged in a triangular pattern, and two single setae; three outer setae longest. Six setae on A7, as for A5-A6, but group of three setae reduced to one pair. Four setae on A8, as for A5-A6, except group of three setae absent and all setae of similar length. Three setae spaced evenly on medio-lateral line on A9.

Dorsal aspect: A1 smooth without spines, single setae close to medial line. Double row of spines on A2-7, three single setae, one on the lateral margin and one close to medial line on anterior ridge, and single seta situated half way between lateral margin and medial line on posterior ridge. A2 anterior row with fewer spines than on other segments. Spines increasing in size on posterior abdominal segments. A8 with double row of dorsal spines in males, anterior row with 8-11 larger irregularly-sized spines, but smaller than on A9. One pair of setae on outer lateral margin and single seta close to mid-dorsal line. Posterior row with 6-8 minute, irregularly-sized spines. Single setae on lateral margins on posterior row of spines. A8 in females with single row of 9-10 larger, irregularly-sized spines, similar to spines on A9. A9 with 5-6 large irregularly shaped spines medially, occasionally with a few extra minute spines. A9 spines larger than those on A10. Distinct single seta laterally-caudal to row of spines. A10 with 2-3 medial small spines. Two larger spines on the lateral margins with a single seta posterior to each large spine.

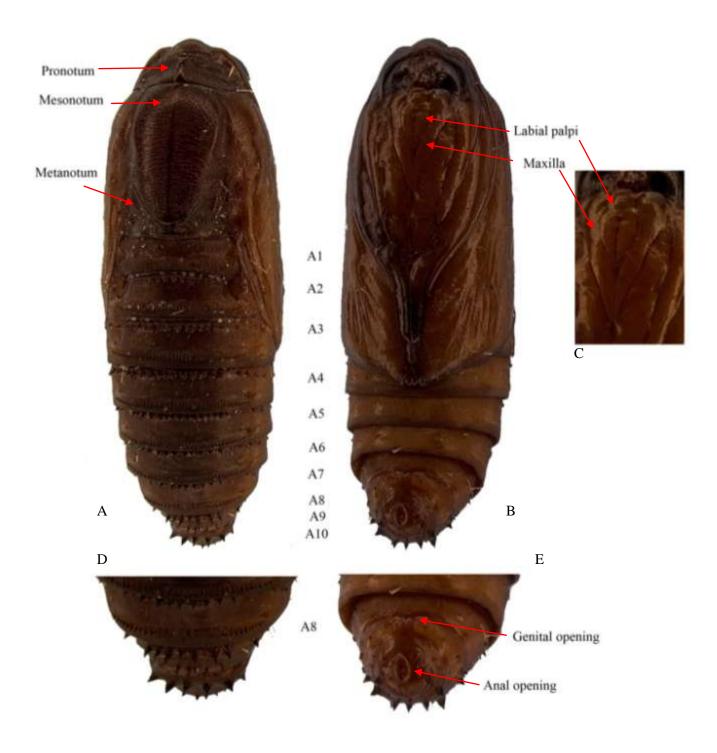


Figure 35: *Thaumatotibia leucotreta.* A. Dorsal view, B. Ventral view, C. Close up of maxilla and labial palpi, D. Dorsal view of A8-10, E. Female ventral view of A8-A10.

3.4.2 Cydia pomonella

General. Frontal and caudal area dark brown, dorsal side darker than ventral. Cremaster absent. Pupation takes place under the bark on trees or in plant litter, usually in a cocoon formed from soil particles and debris (Higbee *et al.*, 2001).

Head (Figs 36 A-C). From smoothly rounded, with one pair of setae. Eyes darker and prominent in mature pupae. Two medial pairs of setae on clypeus, inner pair slightly shorter. Maxilla and proboscis along

midline about 1.5 times length of labial palpus. Structure of antenna sexual dimorphic: in females extending beyond the mesocoxa by a distance longer than the mesocoxa along the midline; in males thickened and prominent, extending past the mesocoxa to the tip of the mesotarsus.

Thorax (Figs 36 A-B). Segments dorsally well delimited. Hindwing concealed almost entirely by forewing, visible only at ventral posterior edge of forewing. Protarsus extending slightly beyond procoxa. Mesotarsus well developed, extending beyond forewing. Metacoxa slightly visible. Two pairs of setae on pronotum and mesonotum. Pronotum 4.0-4.5 times as long as vertex along midline. One lateral pair near forewing and one medial pair close to the mid-dorsal line on pronotum. On mesonotum, one pair of setae midway on alar furrow and one pair near mid-dorsal line. On metanotum, one pair of setae situated in anterior corners of the "M"-shape denoting the hindwing.

Abdomen (Figs 36 A-B, D-E). Spiracles oval, prominent. Setae positioned on lateral margin of ventral aspect: three to four setae on A4, one pair close to spiracle, single setae on medio-anterior to medial line, single seta sometimes concealed by hindwing. Seven setae on A5-A6, lateral group paired; one group of three, arranged in a triangular pattern, and two single setae; two outer setae longest. Six setae on A7, as for A5-A6, but group of three setae reduced to a pair. Lateral pair longest and two single setae shortest. Four setae on A8, as for A5-A6, except group of three setae absent; all setae of equal length. Three setae spaced evenly on medio-lateral line on A9. A10 with four pairs of thickened and distinct perianal and caudal setae. Anterior pair of setae, two on ventral side and two on dorsal side. Genital openings slit-like, ventromedially in females on A8, in males situated ventromedially on A9 with dome-like structures adjoining genital opening.

Dorsal aspect: A1 smooth without spines, single seta close to medial line. Double row of dorsal spines on A2-7, three single setae, one on the lateral margin and one close to medial line on anterior ridge, and single seta situated half way between lateral margin and medial line on posterior ridge. On A2 anterior row with fewer spines than other segments. On A4-A7 six to eight dorsal spines between anteromesad setae. A7 posterior row shorter than those on A2-A6. Spines increasing in size towards posterior abdominal segments. A8–A10 with single row of dorsal spines. Two pairs of setae on A8. A8 with 11-18 and A10 with 7-10 larger irregularly sized spines. A9 with three linear setae. One pair of perianal and one pair of caudal thickened curled setae.

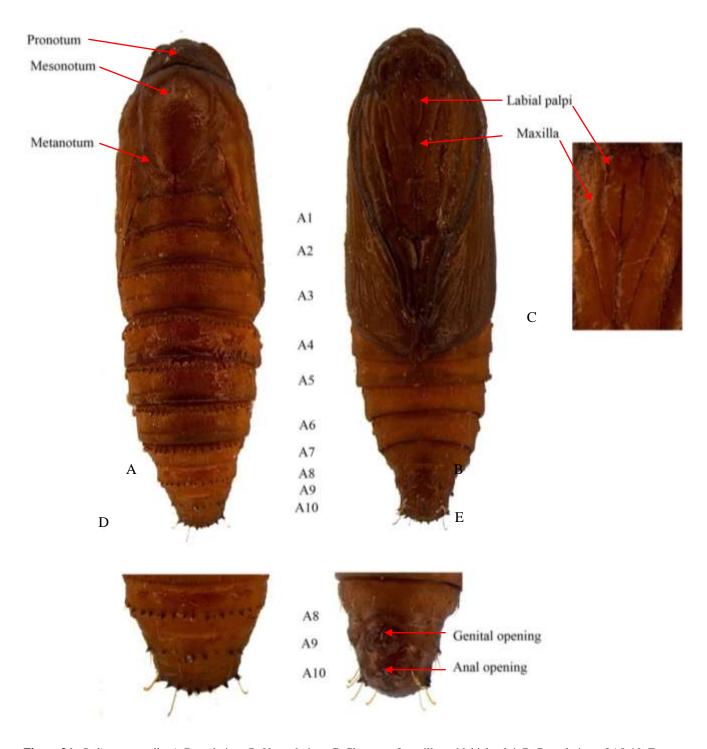


Figure 36: *Cydia pomonella.* A. Dorsal view, B. Ventral view, C. Close up of maxilla and labial palpi, D. Dorsal view of A8-10, E. Male ventral view of A8-A10.

3.4.3 Grapholita molesta

Head (Figs 37 A-C). Frontal region dark brown, from with slightly broadened mesal ridge, bearing one pair of setae. Eyes darker and prominent in mature pupae. Two medial pairs of setae on clypeus, inner pair larger. Antenna without sexual dimorphism, thickened and extending beneath metatarsus.

Thorax (Figs 37 A-B). Hindwing concealed almost completely by forewing, only slightly visible at ventral posterior edge of forewing. Protarsus extending slightly beyond procoxa. Metacoxa slightly discernable. Two pairs of setae on pronotum and mesonotum: on pronotum, one lateral pair near forewing and medial pair close to mid-dorsal line. On mesonotum, one pair midway on alar furrow and one pair near mid-dorsal line. On metanotum, one pair of setae situated in anterior corners of the "M"-shaped hindwing.

Abdomen (Figs 37 A-B, D-E). Segments conspicuously segmented dorsally. Minute setae situated on venter of abdomen. Setae positioned on the lateral margin of ventral aspect: four setae on A4, one pair close to spiracle, and two single setae, one medio-anterior and one anterior. Seven setae on A5-A6, lateral group paired; one group of three, arranged in a triangular pattern and two single setae. Six setae on A7, as for A5-A6, but group of three setae reduced to a pair. Four setae on A8, as for A5-A6, except group of three setae absent and all setae of similar length. Three setae spaced evenly on medio-lateral line on A9. Genital openings slit-like, in males situated ventromedially on A9 with anal rise; in females on A8.

Dorsal aspect: A1 smooth, without spines, single setae close to medial line. Double row of dorsal spines on A2-7, three single setae, one on lateral margin and one close to medial line on anterior ridge, and single seta situated half way between lateral margin and medial line on posterior ridge. On A4-A7 six to nine dorsal spines between anteromesad setae. A2 anterior row with fewer spines than other segments. Spines increasing in size on posterior abdominal segments. A8–A10 with single row of dorsal spines. A8 with four to eight larger spines, with none to three small spines. A9 with five to nine spines and single seta caudal to anterior ridge close to medial line.

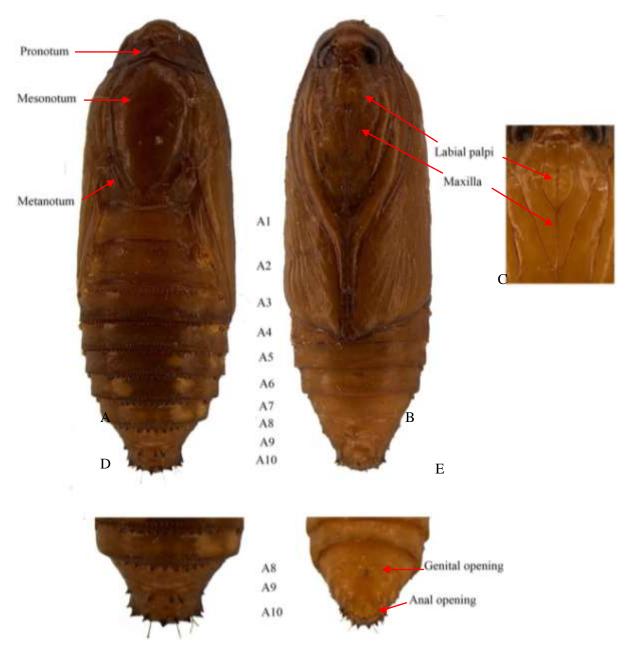


Figure 37: *Grapholita molesta.* A. Dorsal view, B. Ventral view, C. Close up of maxilla and labial palpi, D. Dorsal view of A8-10, E. Female ventral view of A8-A10.

3.4.4 Cryptophlebia peltastica

Head (Figs 38 A-C). Eyes darker and prominent in mature pupae. One medial pair of setae on clypeus.

Thorax (Figs 38 A-B). Metatarsus in line with caudal margin of forewing. Hindwing concealed almost completely by forewing, visible only at the posterior margin. Two pairs of setae on pro- and mesonotum. On pronotum, one lateral pair near forewing and medial pair close to the mid-dorsal line. On mesonotum, one pair midway on alar furrow and one pair near mid-dorsal line. On metanotum, one pair of setae situated in anterior corners of the "M"-shaped hindwing.

Abdomen (Figs 38 A-B, D-E). Setae positioned on lateral margin of ventral aspect: three setae on A4, one pair close to spiracle, and single setae medio-anterior close to medial line. Seven setae on A5-A7 lateral group paired; one group of three, arranged in a triangular pattern, and two single setae; three outer setae longest. Six setae on A8, as for A5-A7, but group of three setae reduced to a pair. Five setae on A9, as on A8 except single medial seta absent.

Dorsal aspect: A1 smooth, without spines, single setae close to medial line. Double row of dorsal spines on A2-7, three single setae, one on the lateral margin and one close to medial line on anterior ridge, and single seta situated half way between lateral margin and medial line on posterior ridge. A2 anterior row with fewer spines than on other segments. Spines generally increasing in size on posterior abdominal segments up to A9. A8 with a single row of dorsal spines, with 10-13 minute spines (smaller than on other segments) irregularly sized with five setae, two pairs and single seta. A9 with six to nine large irregularly shaped spines, larger than spines of A8, with single seta antero-medial to spines. A10 with two-five spines.

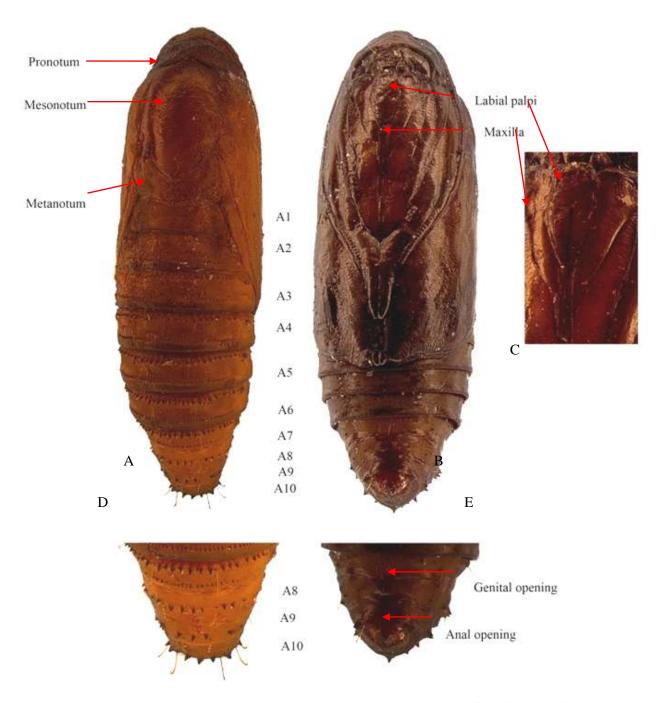


Figure 38: *Cryptophlebia peltastica.* A. Dorsal view, B. Ventral view, C. Close up of maxilla and labial palpi, D. Dorsal view of A8-10, E. Female ventral view of A8-A10.

3.4.5 Thaumatotibia batrachopa

Head (Figs 39 A-C). Frontal region dark brown. Eyes darker and prominent in mature pupae. Two equally-sized medial pairs of setae on clypeus.

Thorax (Figs 39 A-B). Metatarsus extending beyond forewing. Hindwing concealed almost completely by forewing, extending only slightly at posterior edge. Two pairs of setae on pro-and mesonotum. On pronotum, one lateral pair near forewing and medial pair close to the mid-dorsal line. On mesonotum, one pair midway on alar furrow and one pair near mid-dorsal line. On metanotum, one pair of setae situated in anterior corners of the "M"-shaped hindwing.

Abdomen (Figs 39 A-B, D-E). Setae positioned as follows on lateral margin of ventral aspect: four setae on A4, one pair close to spiracle, and two single setae, one medio-anterior and one anterior. Seven setae on A5-A7, lateral group paired; one group of three, arranged in a triangular pattern, and two single setae. Four setae on A8, as for A5-A6, except group of three setae absent and all setae of similar length. Five setae on A8 and A9, two pairs, one lateral pair close to spiracle and one close to mid-ventral line, single setae between pairs. On A10, four pairs of spines, one pair lateral to anal pore, anterior spine larger. Posterior to anal pore: one medio-posterior pair and adjoining laterally two pairs of equal length.

Dorsal aspect, setae described as before (mirror image): A1 smooth, without spines, single setae close to medial line. Double row of dorsal spines on A2-7, three single setae, one on the lateral margin and one close to medial line on anterior ridge, and single seta situated half way between lateral margin and medial line on posterior ridge. A2 anterior row with spines reduced in number and size spines. Spines generally increasing in size on posterior abdominal segments except for A9, number and size reduced. A8 with a single row of dorsal spines, with approximately 10 spines irregularly sized with five setae, two pairs and single seta. A9 with about eight large irregularly shaped spines, with single seta anteromedial to spines. A10 with about four spines.

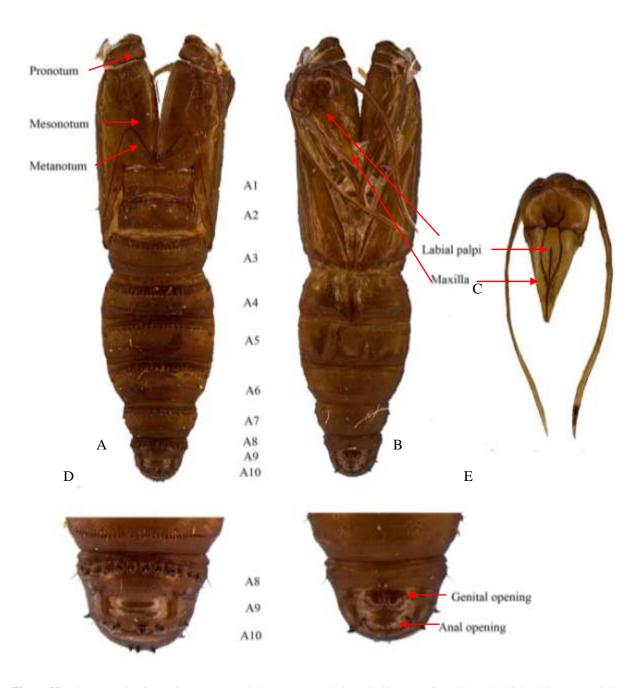


Figure 39: *Thaumatotibia batrachopa* A. Dorsal view, B. Ventral view, C. Close up of maxilla and labial palpi, D. Dorsal view of A8-10, E. Ventral view of A8-A10.

3.4.6 Epichoristodes acerbella

Head (Figs 40 A-C). Two medial pairs of setae on clypeus, inner pair slightly shorter than outer pair. Labial palpus short with a distinct concave bulge.

Thorax (Figs 40 A-B, F). Metatarsus in line with caudal margin of forewing. Hindwing concealed almost entirely by forewing, extending only slightly beyond it at posterior edge. Two pairs of setae on pro- and mesonotum. On pronotum, one lateral pair near forewing and medial pair close to the mid-dorsal line. On mesonotum, one pair midway on alar furrow and one pair near mid-dorsal line. On metanotum, one pair of setae situated in anterior corners of the "M"-shaped hindwing.

Abdomen (Figs 40 A-B, D-E). Distinct darker lateral line along spiracles. Setae positioned as follows on lateral margin of ventral aspect: two single setae on A4, one close to spiracle, the other medio-anterior to medial line. Seven setae on A5-A7, lateral group paired, one group of three, arranged in a triangular pattern, and two single setae. Five setae on A8 and A9, two pairs, one lateral pair close to spiracle and one close to mid-ventral line, single setae between pairs. Anal rise smooth. Genital openings slit-like, in males situated ventromedially on A9; in females on A8. Dorsal aspect, setae described as before (mirror image): A1 smooth, without spines, single setae close to medial line at border of the metanotum. Double row of dorsal spines on A2-A8, spines on anterior ridges facing posteriorly, posterior row of spines smaller than anterior row. On A2-A8, three single setae, one on the lateral margin and one close to medial line on anterior ridge, and single seta half way between lateral margin and medial line on posterior ridge. A2 anterior row with fewer spines than on other segments. Spines on A2 small, on A3-A7 large and equally sized, and on A8 very small, reduced in numbers. Anterior row A8 with 10-16 smaller spines and posterior row with zero to nine minute spines, both rows irregularly sized. A9-A10 smooth, without spines. A9 with three setae, one pair and a single seta close to medial line.

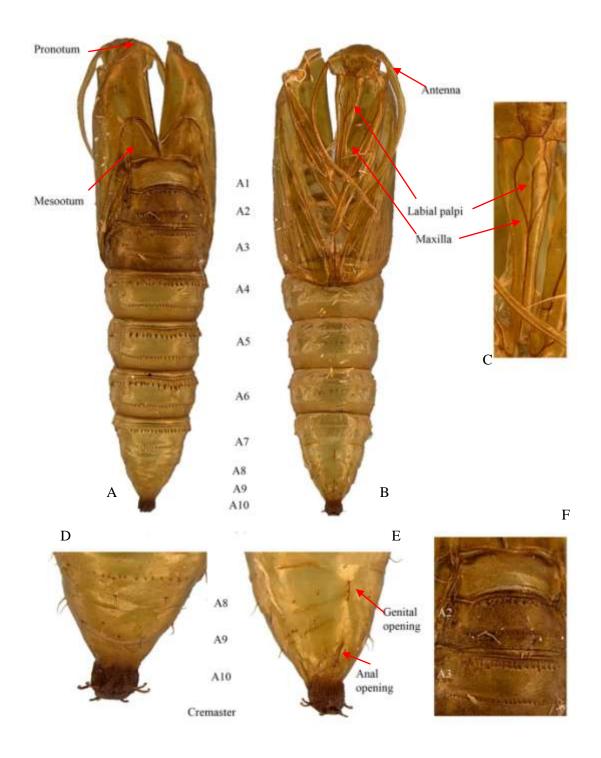


Figure 40: *Epichoristodes acerbella* A. Dorsal view, B. Ventral view, C. Close up of maxilla and labial palpi, D. Dorsal view of A8-10, E. Female ventral view of A8-A10. F. Dorsal view of A2-A3.

3.4.7 Lozotaenia capensana

General. (n=7*) Yellow to light brown. Cremaster well developed, elongate, longer than wide, flattened and rounded at tip, with four pairs of thickened, distinctly curled setae, two situated caudal and one pair on each lateral side.

* Six specimen's retrived from museum collection, and one field-collected.

Head (Figs 41 A-C). Frons smoothly rounded, broad mesal ridge, with one pair of long setae. Two pairs of medial setae on clypeus, outer pair slightly longer. Maxilla along midline approximately 2.0–2.5 times length of labial palpus. Labial palpus slightly concave, but not as bulged as in *E. acerbella* and slightly longer. Antenna extending along mesotarsus to tip of mesotarsus.

Thorax (Figs 41 A-B, F). Segments well delimited dorsally. Pronotum not visible in depicted specimen. Forewing almost completely obscuring hindwing. Protarsus extending beyond procoxa by length of mesocoxa. Metatarsus well developed, extending slightly beyond forewing. Metacoxa faintly exposed. Two pairs of setae on pro- and mesonotum. On pronotum, one lateral pair of setae near forewing and medial pair close to the mid dorsal line. On mesonotum, one pair midway on alar furrow and one pair near mid-dorsal line. On metanotum, one pair of setae situated in anterior corners of the "M" shaped hindwing and another pair close to mid dorsal line.

Abdomen (Figs 41 A-B, D-E). Spiracles prominent, oval. No darker lateral line along the spiracles as in *E. acerbella*. A2-A3 without dorsal cavities and crossfolds. Setae positioned as follows on lateral margin of ventral aspect: three setae, outer one pair and a single seta close to medial line. Seven setae on A5-A6, lateral group paired, one group of three, arranged in a triangular pattern, and two single setae. Six setae on A7, two pairs, one lateral pair close to spiracle and one close to mid-ventral line, two single setae between pairs. Five setae on A8, two pairs, one lateral pair close to spiracle and one close to mid-ventral line, single setae between pairs. Three single setae at posterior margin of A9. Anal rise smooth. Genital openings slit-like, in males ventromedially on A9; in females on A8.

Dorsal aspect: A1 smooth, posterior margin developed slightly with shallow teeth directed posteriorly. Anterior margin of A2 with row of teeth directed anteriorly and posteriorly. Anteriorly directed teeth in posterior ridge of A2. Posteriorly directed spines small. Smaller caudal spines on A2. A3-A7 with anterior row of distinct dorsal spines, posterior row larger than anterior row. On A4-7 six to eight dorsal spines between anteromesad setae. On A2-A8, three single setae, one on the lateral margin and one close to medial line on anterior ridge, and single seta half way between lateral margin and medial line on posterior ridge. A2-A3 anterior row with fewer spines than on other segments. A4-A8 large, increasing in size across segments, A8 anterior row with 12-18 smaller spines, and posterior row with 7-9 minute spines, both rows irregularly sized. A9 with a couple of very small irregular spines on midventral line. Medial spine strong. Three setae, on A9, middle setae at an upright acute angle to other two setae. A10 smooth, without spines.

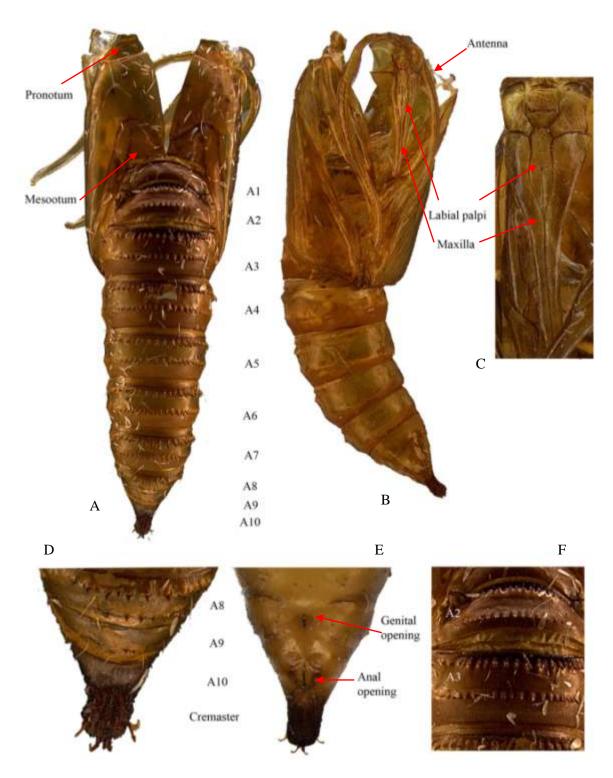


Figure 41: *Lozotaenia capensana* A. Dorsal view, B. Ventral view, C. Close up of maxilla and labial palpi, D. Dorsal view of A8-10, E. Female ventral view of A8-A10. F. Dorsal view of A2-A3.

3.5 Summary of literature and morphological study

A summary of characters abstracted from literature (see Appendix) (white boxes) and own morphological study (green boxes) is given in Table 5.

Table 5: Important and distinct morphological characteristics for identifying and distinguishing between the six economically important tortricids pupae (White boxes as in literatures. Dark grey boxes are morphological characteristics that were studied and added).

<u>General</u>	T. leucotreta	C. pomonella	G. molesta	C. peltastica	T. batrachopa	E. acerbella	L. capensana	
Length & width	8-10 mm long, 2-2.5 mm wide	9-13 mm long, 2-2.5 mm wide	4.5-5.5 mm long, 1.8 mm on average wide	8.5-10.5 mm long, 3-3.5 mm wide.	6-8.5 mm long	8-12 mm long, 2-2.5 mm wide		
Colour	Brown	Brown	Yellow brown	Brown	Brown	Yellow brown	Yellow brown	
Cremaster	Absent	Absent	Absent	Absent	Absent	Well developed, elongate, longer than wide, tip broadly flattened	Well developed, elongate, longer than wide, tip flattened and rounded	
<u>Head</u>	T. leucotreta	C. pomonella	G. molesta	C. peltastica	T. batrachopa	E. acerbella	L. capensana	
Frontal region	Dark brown	Dark brown	Dark brown	Dark brown	Dark brown	Yellow to medium brown	Yellow to medium brown	
Front	Slight broad mesal ridge	Smoothly rounded	Slight broad mesal ridge	Slight broad mesal ridge	Slight broad mesal ridge	Smoothly rounded or slight broad mesal ridge	Smoothly rounded broad mesal ridge	
Frontal setae				One pair of setae	T			
Eyes	Large and prominent	Darker and prominent	Darker and prominent	Large and prominent	Large and prominent	Large and prominent	Large and prominent	
Clypeal setae	2 pairs, outer pair longer	2 pairs, outer pair longer.	2 pairs, inner pair longer	2 pairs, inner pair longer	2 pairs equal in size	2 pairs, outer pair slightly longer	2 pairs, outer pair slightly longer	
Maxilla length	1.5x length of labial palpus	1.5x length of labial palpus.	2x length of labial palpus	1.5x length of labial palpus	2x length of labial palpus	2-2.5x length of labial palps	2-2.5x length of labial palps	
Structure of Antenna	Sexual dimorphism <i>Males</i> : Thickened, prominent, extending beneath the mesocoxa, almost to tip of mesotarsus. Females: Extending beneath the mesocoxa, by the length of mesocoxa.	Sexual dimorphism. Males: Thickened and prominent, extending to the tip of mesotarsus. Females: Extending beyond mesocoxa by length longer than that of mesocoxa.	Expanded at the cephalic extremity and gradually tapering caudad	Prominent, extending beyond mesocoxa, by length less than half of mesocoxa	Prominent, extending beyond mesocoxa, by length of mesocoxa	Extending beyond mesocoxa, by length greater than mesocoxa. Males: Thickened	Prominent, extending to tip of mesotarsus	
<u>Thorax</u>	T. leucotreta	C. pomonella	G. molesta	C. peltastica	T. batrachopa	E. acerbella	L. capensana	
Segments		Well delimited dorsally						
Forewings			Forewings a	lmost obscuring hindwings con	npletely			
Protarsus	Extending past procoxa by 1/4 length of mesocoxa	Extending slightly beyond procoxa.	Extending to tip of procoxa	Extending past procoxa by 1/5 length of mesocoxa	Extending past procoxa by 1/3 length of mesocoxa	Extending past procoxa by 4/5length of mesocoxa	Extending past procoxa by length of mesocoxa	
Mesotarsus	Well developed, clearly delimited. Extending beyond hindwing in line with forewing	Well developed, extending past forewings	Well developed, extending to wing tips	Well developed, clearly delimited	Well developed, Extending past forewings	Well developed, clearly delimited	Well developed, extending slightly past wing tips.	
Metacoxa	Slightly visible in females, in males extending almost to tip of mesotarsus	Slightly visible						
Pronotum	5x as long as vertex along midline.	4-4.5x as long as vertex along midline	4x as long as vertex along midline	4x as long as vertex along midline	4x as long as vertex along midline	4x as long as vertex along midline	Not in specimen	
Pronotum setae		2 pairs, 1 lateral & 1 medial close to mid-dorsal line						
Mesonotalsetae		2 pairs, 1 midway on alar furrow, 1 pair near mid-dorsal line						
Metanotal setae	1 pair, in anterior corner of the "M" shaped hindwings							

<u>Abdomen</u>	T. leucotreta	C. pomonella	G. molesta	C. peltastica	T. batrachopa	E. acerbella	L. capensana
Spiracle	Oblong, prominent	Oval, prominent	Circular	Oblong, indistinct	Oblong, indistinct	Oblong, prominent with a clear darker lateral line crossing along ssegments	Oval, prominent.
Ventral lateral half of abdomen, setae on A4	4 setae, 1 pair close to spiracle, 2 single setae - 1 medio-anterior and 1 anterior	3 - 4 setae on A4, 1 pair close to spiracle, single setae one medio-anterior to medial line, 1 setae sometimes concealed by wings	4 setae, 1 pair close to spiracle, 2 single setae - 1 medio-anterior and 1 anterior	3 setae, 1 pair close to spiracle and a single setae medio-anterior.	4 setae, 1 pair close to spiracle, 2 single setae, 1 medio-anterior & 1 anterior.	3 setae, pair close to spiracl line.	le, single seta close to medial
Ventral lateral half abdomen setae on A5-A7	7 setae on A5-A6, lateral group paired, 1 group of 3, arranged triangular shaped, and 2 single setae; 6 setae on A7, as for A5-A6, but group of 3 setae reduced to a pair			7 setae, lateral group paired, 1 group of 3, arranged triangular shaped, and 2 single setae			7 setae, lateral group paired, 1 group of 3, arranged triangular shaped, and 2 single setae; 6 setae, 2 pairs, 1 lateral close to spiracle, 1 close to mid-ventral line. 2 single setae between pairs
Ventral setae on A8	4 setae as for A5-A6, except group of three setae absent			5 pair, 2 pairs, one lateral close to spiracle, 1 close to mid-ventral line. Single seta between pairs	4 setae as for A5-A6, except group of three setae absent	5 pair, 2 pairs, one lateral close to spiracle, 1 close to mid-ventral line. Single seta between pairs	5 pair, 2 pairs, one lateral close to spiracle, 1 close to mid-ventral line. Single seta between pairs
Ventral setae on A9	Three setae spaced evenly on medio-lateral line			3 setae, 1 lateral pair and 1 single setae close to medial line.	5 pair, 2 pairs, 1 lateral, 1 close to mid-ventral line. Single seta between pairs		3 single setae at the posterior margin
Genital opening: females	Ventromedially on anterior margin on A9, extending to anterior margin of A10	Slit-like, ventro-medial on A8	Slit-like, ventro-medial on A8	Ventromedially on anterior margin on A9 and extending to anterior margin of A10	Ventromedially on anterior margin on A9 and extending to anterior margin of A10	Slit-like, ventro-medial on A8	Slit-like, ventro-medial on A8
Genital opening: males	Ventromedially on A9 and extending to anterior margin of A10	Slit-like, ventro-medial on A9 with 2 dome-like structure next to genital opening	Slit-like, ventro-medial on A9 with 2 dome-like structure next to genital opening	Ventromedially on A9 and extending to anterior margin of A10	Ventromedially on A9 and extending to anterior margin of A10	Slit-like, ventro-medial on A9	Slit-like, ventro-medial on A9
Anal rise	Thickened, distinctly curled setae situated anteriorly to the outermost ventral spine on A9	4 pairs of thickened, distinctly curled setae. 2 on ventral side, 2 pairs on dorsal side	2 hooked setae on either side of anal rise, third hooked seta latero-caudad	2 pairs of thickened, distinctly curled setae medially adjacent to ventral spines on A10	2 pairs of thickened, distinctly curled setae. Lateral to anal pore 1 pair of spines, anterior spine larger. Posterior to anal pore – 1 medioposterior pair and adjoining laterally 2 pairs of equal length	smooth	
Anal rise spines in males	Anterior pair of setae on anal rise anterior to anal pore	Anterior pair of setae on anal rise anterior to anal pore	Anterior pair of setae on anal rise anterior to anal pore	Anterior to pore	Adjacent to anal pore	Smooth	
Anal rise spines in females	Anterior pair of setae on anal rise usually adjacent to anal opening	Anterior pair of setae on anal rise usually adjacent to anal opening	Anterior pair of setae on anal rise usually adjacent to anal opening	Anterior pair setae situated anterior to pore	Anterior pair setae situated anterior to pore	Smooth	
Dorsal spines on A1	Smooth without spines, single setan close to medial line. developed with posterior						

<u>Abdomen</u>	T. leucotreta	C. pomonella	G. molesta	C. peltastica	T. batrachopa	E. acerbella	L. capensana
Dorsal spines on A2	Anterior row extending less that	n halfway across segment, cau	dal row well developed and exter	nding almost across segment	Anterior row reduced, extending less than halfway across segment posterior row prominent.	Anterior and posterior row of spines slight	Anterior margin with anterior and posteriorly directed teeth. Anterior directed teeth in posterior ridge. Posterior directed teeth small
Number of dorsal spines on A3-A7	Posteriorly directed dorsal spines, anterior row distinct, posterior row indistinct	Posteriorly directed dorsal spines, anterior row distinct, posterior row indistinct. A7 posterior row shorter than A2-A6	Double row; Cephalic spine row 2x large and half as numerous than on caudal row	Posteriorly directed dorsal spines, anterior row prominent, posterior row indistinct	Posteriorly directed dorsal spines, anterior row prominent, posterior row indistinct	Anterior row of dorsal spines readily apparent and posterior row slight	Posteriorly directed dorsal spines, anterior row smaller than posterior row
Dorsal setae A2- A7	3 single setae, 1 on the lateral margin and 1 close to medial line on anterior ridge. 1 seta half way between lateral margin and medial line on posterior ridge.						
Number of dorsal spines between antero- mesad setae	3-7 spines	6-8 spines	6-9 spines	4-12 spines	4-8 spines	5-12 spines	6-8 spines
Dorsal spines on A8	Single row of prominent spines, 9-10 larger irregularly-sized spines in females. Double row of irregularly-sized spines in males, cephalic row with 8-11 larger spines, Posterior row with 6-8 minute spines	11-18 single row of spines. directed posteriorly	One row of spines, 4-8 larger spines and 0-3 smaller additional spines.	Single row of prominent posteriorly directed dorsal spines, 10-13 spines	Single row of prominent posteriorly directed dorsal spines. Approximate 10 large and 3 small spines	Spines reduced, irregular sized. Anterior row with 10-16 spines. Posterior row with 0-9 spines	12-18 anterior spines and 7-9 posterior minute spines
Dorsal setae on A8	One pair of setae on outer lateral margin and single seta close to mid-dorsal line. 1 setae on lateral margins on posterior row of spines					3 single setae, 1 on the lateral margin and 1 close to medial line on anterior ridge. 1 seta half way between lateral margin and medial line on posterior row of spines	
Dorsal spines on A9	5-6 larger irregular medial spines, some with smaller supplementary spines	7-9 larger spines	One row of spines, 5-9 spines	Single row of prominent posteriorly directed dorsal spines. 6-9 spines	Single row of prominent posteriorly directed dorsal spines. Approximately 8 spines	No Spines	Couple of spines small spines, strong
Dorsal spines on A10	2-3 medial small spines. Two larger spines on the lateral margin with a single seta below each spine	7-12 spines, irregular in size	6 spines, one row	Single row of prominent posteriorly directed dorsal spines	Single row of prominent posteriorly directed dorsal spines	No Spines	
Spine sizes	Increasing to A10	Increasing to A10	Increasing to A10	Increasing to A10	Increasing to A10	Increasing to A7 reduced in A8	Increasing to A8
Cremaster setae	Absent	Absent	Absent	Absent	Absent	4 pairs of thickened, distinct curled cremastral setae	4 pairs of thickened, distinct curled cremastral setae

3.6 Discussion and Conclusion

A clear distinction exists between species that belong to Olethreutinae (*T. leucotreta*, *C. pomonella*, *G. molesta*, *C. peltastica*, and *T. batrachopa*) and those that belong to Tortricinae (*E. acerbella* and *L. capensana*), and these features are discussed below.

E. acerbella and L. capensana both have an elongated cremaster, whereas the other five species lack a distinct cremaster. L. capensana and E. acerbella can be distinguished from each other by the following: the cremaster of L. capensana is elongate and longer than wide (as in E. acerbella) but narrower and more rounded. The antenna of L. capensana extends beyond the mesocoxa to the tip of the mesotarsus compared to E. acerbella were they extend beyond the mesocoxa by a length greater than that of the mesocoxa. The spiracles in E. acerbella are oblong, arranged along a distinct darker lateral line from A1 to A7, darker lateral line absent in L. capensana. L. capensana has seven setae on A5-A6, six setae on A7, five setae on A8, and three on A9, whereas E. acerbella has seven setae on A5-A7 and five setae on A8 and A9. The posterior row of dorsal spines on A3-A7 is smaller than on the anterior row in E. acerbella, and larger than anterior row in L. capensana. Spines on A8 in E. acerbella are smaller than on A7 but larger in L. capensana. L. capensana also has some small irregular spines on A9; E. acerbella spines on A9 absent.

Although the five species of Olethreutinae do not have diagnostic pupal characteristics that easily distinguish them from each other, certain small differences are present. The males in *T. leucotreta* and *C. pomonella* show signs of sexual dimorphism, the antennae are thickened and more prominent, extending beneath the mesocoxa reaching almost the mesotarsus, while in females the antennae extend beyond the mesocoxa by the length of the mesocoxa (*T. leucotreta*) or slightly longer (*C. pomonella*). Males of *T. leucotreta* also have a double row of spines on A8, whereas in all the other species and females of *T. leucotreta* posses a single row of spines on A8. The outer clypeal setae of *T. leucotreta* and *C. pomonella* is also longer than the inner setae when compared to *G. molesta*, and *C. peltastica*, where the inner pair is longer and in *T. batrachopa* where they are almost of equal length. The maxilla of *G. molesta* and *T. batrachopa* is about 2 times the length of the labial palpus, whereas in the other three species the maxilla is 1.5 times the length of the labial palpus. *T. leucotreta*, *C. pomonella* and *G. molesta*, each have seven setae on A5-A6, 6 setae on A7, four setae on A8 and three setae on A9. *C. peltastica* has seven setae on A5-A7, five setae on A8 and three setae on A9. *S. peltastica* is increases for all species from A2-A10.

3.7 References

- Brown, J. W. (2005). World Catalogue of Insects Volume 5, Tortricidae (Lepidoptera), Apollo Books, Stenstrup, Denmark, 741 pp.
- Fenili, G. A. (1976). Ricerche sul comportamento biologico della *Epichoristodes* (= *Tubula*) acerbella (Walker) Diakonoff vivente in ambienti diversi *Annali dell'Istituto Sperimentale per la Zoologia Agraria* 5: pp. 387-424

- Garman, P. (1917). The Oriental Peach pest (*Laspeyresia molesta*, Busck), a dangerous new fruit insect of Maryland. *The Maryland State Collage of Agriculture, Agricultural Experiment Station*. Bulletin No. **209**: 6.
- Gilligan, T.M., Epstein, M.E. & Hoffman, K.M. (2011). Discovery of False Codling Moth, *Thaumatotibia leucotreta* (Meyrick), in California (Lepidoptera: Tortricidae). *Proceedings of the Entomological Society of Washington* **113** (4): 426-435.
- Karisch, T. (2003). Zur Schmetterlingsfauna von St Helena, 2. Teil: Kleinschmetterlinge (1) (Insecta: Lepidoptera: Tortricidae, Glyphipterigidae, Cosmopterigidae, Plutellidae, Pterophoridae). Linzer biologische Beiträge 35: 1081-1085
- Komai, F. 1999. A taxonomic review of the genus *Grapholita* and allied genera (Lepidoptera: Tortricidae) in the Palaearctic region. *Entomologica Scandinavica Supplement* **55**: 1–226.
- Nuzacci, G. (1973). Epichoristodes acerbella (Lepidoptera, Tortricidae). Entomologica (Bari) 9: 147-148.
- Pinhey, E.C.G. (1975). Moths of Southern Africa, Tafelberg Publishers, Cape Town, pp. 37-38.
- Patočka. J. & Turčáni. M. (2005). *Lepidoptera Pupae, Central European Species, Text Volume*, Apollo Books, Stenstrup, pp.187, 238-242.
- Powell, J.A. (1964). *Biological and taxonomic studies on tortricine moths, with reference to the species in California*, University of California Press, Berkeley and Los Angeles, 317 pp.
- Timm, A.E. (2005). Morphological and molecular studies of tortricid moths of economic importance to the South African fruit industry, Phd (Agric)-dissertation, Stellenbosch University.
- Timm, A.E. Warnich, L. & Geertsema, H. (2007). Morphological and molecular identification of economically important Tortricidae (Lepidoptera) on tropical and subtropical fruit in South Africa. *African Entomology* **15** (2): 269-286.
- Timm, A.E. Warnich, L. & Geertsema, H. (2008). Morphological and molecular identification of economically important Tortricidae (Lepidoptera) on deciduous fruit tree crops in South Africa. *African Entomology* **16** (2): 209-219.
- Williams, J.R. (1953). The larvae and pupae of some important Lepidoptera. *Bulletin of Entomological Research* **43** (4): 691-701.
- Wood, W.B & Selkregg, E.R. (1918). Further Notes on *Laspeyresia molesta*, *Journal of Agricultural Research* XIII, No. 4, pp. 59-70.

3.8 Appendix 3.1

Published descriptions of the pupae of the tortricids studied here as found in the literature.

3.8.1 Thaumatotibia leucotreta

Williams (1953), Timm et al. (2005), and Gilligan et al., (2011) all described T. leucotreta pupae.

Williams (1953) described *T. leucotreta* as follows:

8-10 mm; long, 2-2.5mm wide. Front raised, without depression on its ventral face. Dorsum of II heavily punctured. Legs of III extending to, but not beyond wingtips. Two rows of dorsal spines on 2. Spines on 8 and 9 very well developed. Segment 10 (A10) with spines and with two pairs of hooked setae.

Komai (1999) described *Thaumatotibia* pupae (based on *T. batrachopa, T. encarpa* and *T. leucotreta*) as follows:

Body length 6-10 mm. Body pale yellowish brown. Spiracles transversely ovate. A2-A7 with two rows of dorsal spines; A8-A10 with one row of strong spines (in male of *T. leucotreta*, A8 with two rows of dorsal spines); A10 with a pair of strong spines along anal rise, without hooked setae except two pairs along anal rise

Timm et al. (2007) described *T. leucotreta* pupae as follows (Fig. 42):

General. Pupa 7.94–9.80 mm in length (n = 16), brown, frontal region dark brown. Pupa usually formed in soil, occasionally in fruit; usually enclosed in a cocoon formed from soil particles and debris. Distinct cremaster absent.

Head. Front with slight, broad mesal ridge, bearing one pair of setae. Eyes large, prominent. Maxilla length along midline about 1.5 times length of labial palpus. Structure of antenna with signs of sexual dimorphism: thickened, prominent in males relative to females; extending beneath the mesocoxa by a length approximately equal to the mesocoxa along the midline in females, in males by a length greater than that of the mesocoxa, almost to the tip of the mesotarsus.

Thorax. Segments well delimited dorsally. Forewing almost completely obscuring hindwing ventrally. Protarsus extending beyond procoxa by approximately 1/4 length of mesocoxa. Metatarsus well developed. Coxa of metathorax slightly visible in female, more exposed in male. Pronotum almost five times as long as vertex along midline.

Abdomen. Spiracles oblong, prominent. A2–3 lacking dorsal cavities and crossfolds. A4–7 with posteriorly directed dorsal spines, anterior row distinct, posterior row indistinct, with 3–7 dorsal spines between anteromesad setae. A8–10 each with a single row of prominent posteriorly directed dorsal spines. Anal rise with thickened, distinctly curled setae situated anteriorly to the outermost ventral spine on A9. Females with genital opening ventromedially on anterior margins on A9 and extending to anterior margin of A10; anterior pair of setae on anal rise usually adjacent to anal opening. Males with genital aperture ventromedially on A9, anterior pair of setae on anal rise anterior to anal pore. Ventral spine consisting of paired lateral projections.

Gilligan et al. (2011) described T. leucotreta as follows:

The pupa is pale yellowish brown, ca. 8-10 mm long, and is contained in a silken cocoon constructed with organic debris and soil particles. Important morphological features of the pupa include: segments A2-A7 with two rows of dorsal spines; segments A8-A10 with one row of dorsal spines except in males, where A8 has two rows of spines; A10 with two pairs of hooked setae and one pair of strong spines along anal rise.

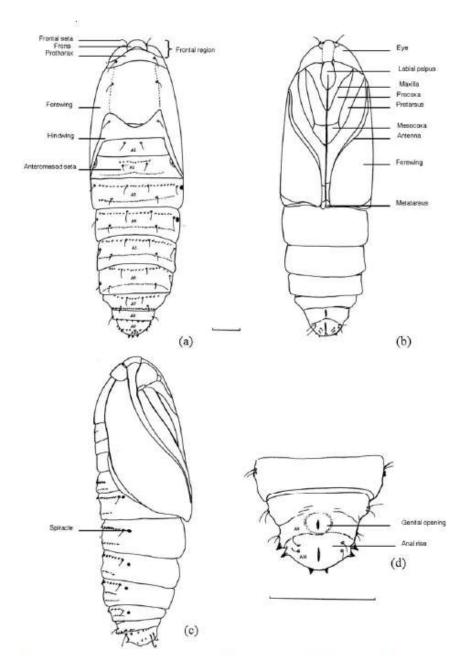


Figure 42: *Thaumatotibia leucotreta* pupa. a) Dorsal aspect (female); b) ventral aspect (female); c) lateral aspect (female); d) terminal abdominal segments (male). Only prominent setae are drawn. Scale bars = 1mm (Taken from Timm *et al.* 2007; Fig. 8)

3.8.2 Cydia pomonella

Garman (1917) mentioned that C. pomonella possesses 10-12 spines on A10.

Patočka & Turčáni (2005) described C. pomonella as follows (Fig. 43):

Pupa 9-11 mm in length. Pupa 9.0-13.0x 2.5-3.5mm in size, red brown slightly glossy sculpture fine.

Head obtusely rounded and lacks projection in lateral view. Proboscis lobes adjacent to each other for at most 2x longer, sometimes also shorter length than prothoracic femora. From has rather thin and long lateral rims dorsally or they are entirely weak. Clypeus lacks transverse ridge in centre.

Mesothoracic legs adjacent to mesothoracic coxae for about 3x greater length than prothoracic legs. Mesothoracic legs adjacent distinctly to maxillary palpi. Metanotum lacks strong transverse wrinkles.

The 4th and 5th abdominal segments have 1-rarely 2-seriate basal rows of spines in centre. The 4th and 5th abdominal segments bear 6-10 (rarely 5) spines (main spines if rows are 2-seriate) in basal row and 18, rarely 14-18 spines in caudal row between dorsal setae. The 9th abdominal segment with numerous spines. Row of spines monoseriate on the 9th abdominal segment. Spines on the 9th abdominal segment moderately large. Spines on the 10th abdominal segment moderate in size to large. Pupa bears 2 pairs of perianal and 2 pairs of caudal setae.

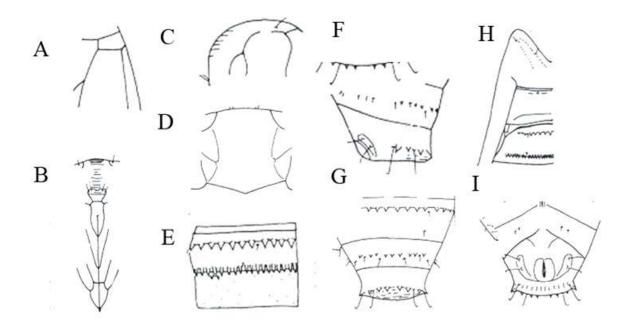


Figure 43: *Cydia pomonella* A) Maxillary palpus and vicinity, B) Head laterally, C) Labrum and vicinity, D) Frons and vicinity dorsally, E) 5th abdominal segment, left half dorsally, F) Abdominal end laterally G) Abdominal end dorsally H) Metanotum, abdomen base left half, I) Abdominal end ventrally (Taken from Patočka & Turčáni, 2005; Plate 135 Figs 44, 49-56)

3.8.3 Grapholita molesta

Garman (1917), Wood & Selkregg (1918) and Patočka & Turčáni (2005) all described G. molesta:

Garman (1917) described G. molesta as follows (Fig. 44):

Uniform brown; length 4.5mm to 5.5.mm. Eye-piece nearly triangular, smooth, the glazed portion indistinct; labial palpi one-half the length of the maxillae, the latter with a length exposed on the meson equal to that of the first coxae; first femora extending from the eye-piece to the tips of the procoxae; second pair of coxae forming a heart-shaped figure, the portion exposed on the meson being about the same as that of the procoxae; mesofemora extending from the level of the tip of the labial palpus to the middle of the mesal margin of the wings, being acute at the cephalic end and constricted caudad; third pair of legs (metathoracic legs) extending beyond the second pair (mesothoracic legs) to the tips of the wings; prothorax measured on the dorso-meson about one-fifth the length of the mesothorax, the caudal margins of which together with the margins of the wings form a decided M; antennae suddenly expanded at the cephalic extremity and gradually tapering caudad.

Abdomen composed of ten segments of which the last three are apparently ankulosed and form a truncated cone; cephalic margins of segments 2 to 9 with a row of heavy setae, the caudal margins of 2 to 7 with rows of much finer setae; caudal margin of ten with six heavy spines between which are somewhat longer hooked setae; two short setae are present on each side of the anal rise.

According to Garman (1917) *G. molesta* can be distinguished from *C. pomonella* by the number of spines on the posterior margins of A10. *C. pomonella* possessing 10 -12 spines.

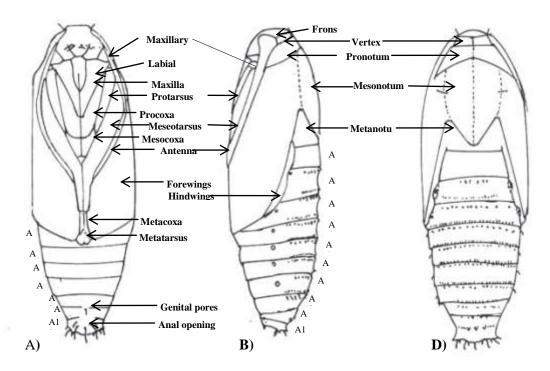


Figure 44: Grapholita molesta A) Ventral B) Lateral, C) Dorsal (Redrawn and modified from Garmen, 1917; Fig. 6)

Wood & Selkregg (1918) described G. molesta as follows (Fig. 45):

The pupa is yellow-brown in colour; without pubescence; average measurement 6.26 mm long by 1.8 mm wide. Frontoclypeal suture indistinct; eyes and glazed discernible; mandibles and clypeus distinctly indicated; 2 pairs of clypeal setae, inner pair slightly longer than outer; clypeo-labral suture not visible; slightly more than half the length of the maxillae; maxillary palpi extending to the proximo-lateral angles of the maxillae; maxillae reaching one-third of the way to the tips of the wings. Metathoracic legs and tips of hind wings reaching just beyond the cephalic edge of the fourth abdominal segment; antennae extending about two-thirds of the wing length, reaching beyond second coxae. A double row of dorsal spines on abdominal segment 2 to 7; abdominal segment 2 with spines of cephalic row uneven in size and arrangement, the row extending usually less than half-way across the segment, the caudal row well developed and extending almost across the segment, the caudal row well developed and extending almost across the segment, the caudal row well developed and half as numerous as those of the caudal row; segments 8 to 10 with one row of spines, the spines gradually increasing in size from segment 8 to segment 10.

No cremaster. Two hooked setae on either side of the anal rise, with a third hooked seta- latero-caudad; margin of the abdomen. Spiracles circular and produced. Anal and genital openings slitlike, the latter single in both sexes.

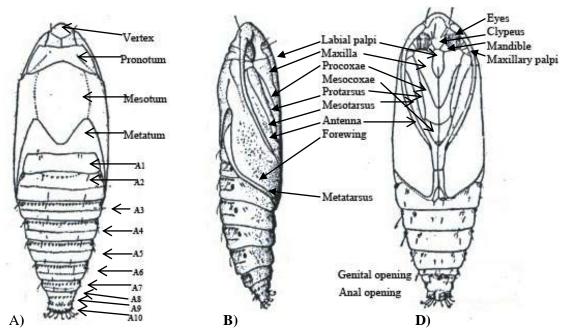


Figure 45: Grapholita molesta A) Dorsal, B) Lateral, C) ventral views (Taken and modified from Wood &Selkregg, 1918; Fig. 9 C-E)

Patočka & Turčáni (2005) described G. molesta as the following (Fig. 46):

Pupa 7-8.5 mm in length. Pupa 7.0-8.5 x 1.8-2.5 mm in size, red brown, sculpture moderately fine, wrinkles locally distinct.

Head obtusely rounded and lacks projection in lateral view. Proboscis lobes adjacent to each other for at most 2x longer, sometimes also shorter length than prothoracic femora (prothoracic legs). From has rather thin and long lateral rims dorsally or they are entirely weak. Clypeus lacks transverse ridge in centre.

Mesothoracic legs adjacent at a point to maxillary palpi. Mesothoracic legs adjacent to mesothoracic coxae (mesothoracic legs) for about 3x greater length than prothoracic legs. Metanotum lacks strong transverse wrinkles.

The 4th and 5th abdominal segments have 1,-rarely 2,-seriate basal rows of spines in centre. The 4th and 5th abdominal segments bear 6-10 (rarely 5) spines (main spines if rows are 2-seriate) in basal row and 18, rarely 14-18 spines in caudal row between dorsal setae. The 9th abdominal segment with numerous spines. Row of spines monoseriate on the 9th abdominal segment. Spines on the 9th abdominal segment moderately small, on the 10the segment large. Pupa bears 2 pairs of perianal and 2 pairs of caudal setae.

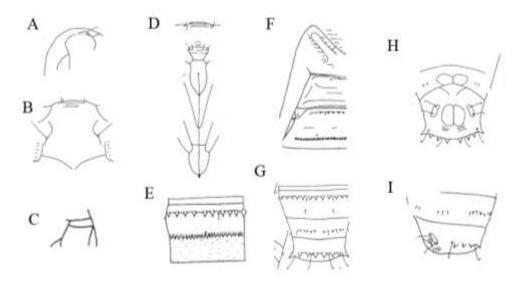


Figure 46: *Grapholita molesta* A) Head laterally, B) Frons and vicinity dorsally C) Maxillary palpus and vicinity, D) Labrum and vicinity, E) 5th abdominal segment, left half dorsally, F) Metanotum, abdomen base left half, G) Abdominal end dorsally H) Abdominal end ventrally I) Abdominal end laterally (Taken from Patočka & Turčáni, 2005; Plate 134 Figs 18-26)

3.8.4 Cryptophlebia peltastica

Williams (1953) and Timm et al., (2005) both described C. peltastica:

Williams (1953) regarded *C. peltastica* as indistinguishable from that of *C. williamsi*. The pupa of *C. williamsi* was described as follows:

10-16 mm long, 3-3.5 mm wide. Spines on 8 and 9 developed, but less so than in *A. leucotreta* Dorsum of II heavily and coarsely punctured. Segment 10 with two pairs of hooked setae and with spines, two of which (one on each side of the anus) are stout and hooked. Otherwise as *A. leucotreta*.

Timm et al. (2007) described C. peltastica pupae as follows (Fig. 47):

General. Pupa dark brown, with darker patches on the pronotum; frontal region dark brown; 8.59-10.63 mm in length (n = 10). A cocoon of soil particles and debris present if pupation takes place in soil, absent if pupation occurs in fruit. Distinct cremaster absent.

Head. Front with slight, broad mesal ridge, bearing one pair of setae. Eyes large, prominent. Antenna prominent, extending beyond mesocoxa by a length less than that of mesocoxa. Length of maxilla along midline 1.5 times that of labial palpus.

Thorax. Segments well delimited dorsally. Pronotum more than four times as long as vertexalong midline. Forewing almost completely obscuring hindwing ventrally. Protarsus extending beyond procoxa by approximately 1/5 length of mesocoxa. Metatarsus well developed, clearly delimited. Coxa of metathorax slightly exposed.

Abdomen. Spiracles oblong, indistinct. A2–3 lacking dorsal cavities and crossfolds. A4–7 with posteriorly directed dorsal spines, anterior row prominent, posterior row inconspicuous, bearing 4–12 dorsal spines between anteromesad setae. A8–10 with a single row of prominent, posteriorly directed dorsal spines. Anal rise with two pairs of thickened, distinctly curled setae medially adjacent to the ventral spines on A10; both pairs of setae situated anterior to anal pore in both males and females. Ventral spine consists of single projection enclosing bases of anal setae. Female pupa with genital opening ventromedially on anterior margins on A9 and extending to the anterior margin of A10. Male pupa with genital aperture ventromedially on A9.

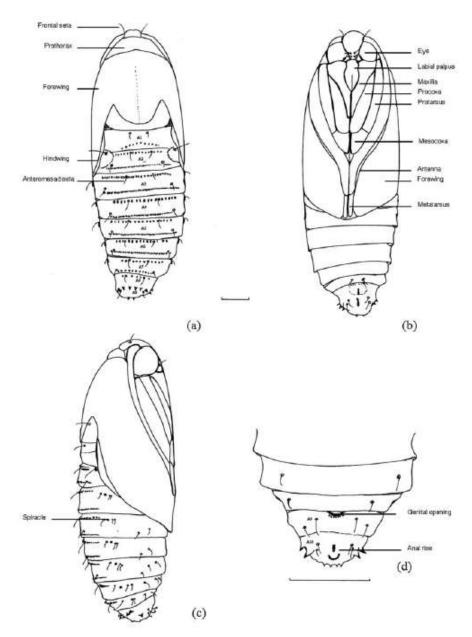


Figure 47: *Cryptophlebia peltastica* pupa. a) Dorsal aspect (male); b) ventral aspect (male); c) lateral aspect (male); d) terminal abdominal segments (female). Only prominent setae are drawn. Scale bars = 1 mm. (Taken from Timm *et al.*, 2007; Fig. 9)

3.8.5 Thaumatotibia batrachopa

Komai (1999) described *Thaumatotibia* pupae (based on *T. batrachopa, T. encarpa* and *T. leucotreta*) as follows:

Body length 6-10 mm. Body pale yellowish brown. Spiracles transversely ovate. A2-A7 with two rows of dorsal spines; A8-A10 with one row of strong spines (in male of *T. leucotreta*, A8 with two rows of dorsal spines); A10 with a pair of strong spines along anal rise, without hooked setae except two pairs along anal rise

Timm et al. (2007) described T. batrachopa pupae as follows (Fig. 48):

General. Pupa 6.14–8.29 mm in length (n = 8), uniformly dark brown; usually found in the ground encased in a cocoon constructed of soil particles, cocoon absent if pupation takes place directly in fruit. Distinct cremaster absent.

Head. Front with slight, broad mesal ridge, bearing one pair of setae. Eyes prominent, large. Antennae extending below mesocoxa (mesothoracic legs) by a length approximately equal to mesocoxa. Maxilla length along midline approximately equal to twice length of labial palpus.

Thorax. Segments well delimited dorsally. Forewing almost completely obscuring hindwing ventrally. Metatarsus (metathoracic legs) clearly delimited. Pronotum more than four times as long as vertex along midline. Protarsus (prothoracic legs) extending beyond procoxa by approximately 1/3 length of mesocoxa. Coxa of metathorax (metathoracic legs) visible in males and females.

Abdomen. Spiracles oblong, inconspicuous. A2–3 lacking dorsal cavities and crossfolds. A4–7 with posteriorly directed dorsal spines, anterior row conspicuous, posterior row inconspicuous, bearing 4–8 dorsal spines between anteromesad setae. A8–10 with a single row of prominent, posteriorly directed dorsal spines. Female pupa with broad genital opening ventromedially on anterior margins on A9, posteriorly lined with flattish white setae; anal rise with two pairs of thickened, distinctly curled setae, anterior pair situated just anterior to anal pore. Male pupa with genital aperture ventromedially on ninth segment, setae on anal rise situated adjacent to anal pore, anterior pair may occur slightly anterior to the opening of the anal pore. Lateral projections of ventral spine closer to anal rise than in *T. leucotreta*.

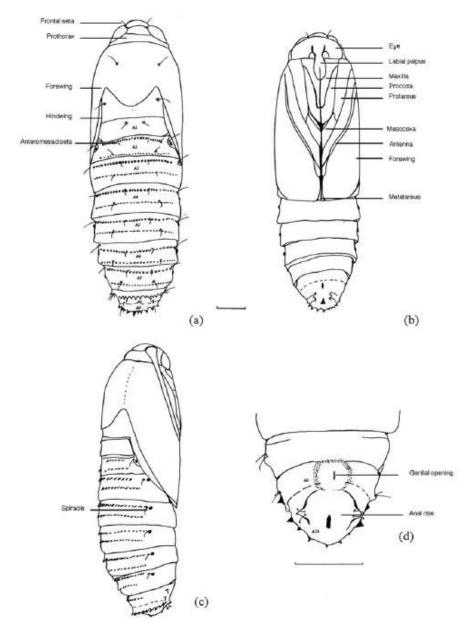


Figure 48: *Thaumatotibia batrachopa* pupa. a) Dorsal aspect (female); b) ventral aspect (female); c) lateral aspect (female); d) terminal abdominal segments (male). Only prominent setae are drawn. Scale bars = 1 mm. (Taken from Timm *et al.*, 2007; Fig. 8)

3.8.6 Epichoristodes acerbella

Nuzzaci (1973) described *E. acerbella* in Italian; part of his elaborate description has been abridged and his text freely translated (Fig 49):

Length 8-12 mm at broadest 2-2.5 mm, cremaster with 8 curved setae, of which 4 are in 2 terminal pairs and 2 pairs laterally visible on both ventral and dorsal side.

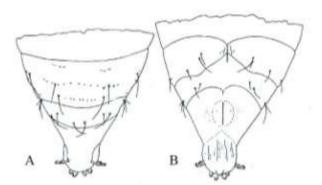


Figure 49: *Epichoristodes acerbella* A) Dorsal view B) Ventral view of A8-A10 (Taken from Nuzzaci, 1973; Fig 17). Detailed descriptions were also in Italian, provided by Fenili (1976).

Timm et al. (2008) described E. acerbella pupae as follows (Fig. 50):

General (Figs 17. Pupa uniformly yellow brown or with abdominal segments darker ventrally, 9.53-12.50 mm in length (n = 16).

Head. front smoothly rounded or with slight, broad mesal ridge, bearing one pair of setae. Maxilla along midline approximately 2.0–2.5 times length of labial palpus. Antenna extending below mesocoxa (mesothoracic legs) by a length greater than the length of the mesocoxa thickened in males relative to females.

Thorax. Segments well delimited dorsally. Pronotum along midline more than four times as long as vertex. Forewing almost completely obscuring hindwing ventrally. Protarsus extending beyond procoxa by approximately 4/5 length of mesocoxa. Metatarsus well developed, clearly delimited. Coxa of metathorax exposed.

Abdomen. spiracles prominent, oblong. A2–3 lacking dorsal cavities and crossfolds. A2 with anterior and posterior rows of dorsal spines slight. A3–7 with anterior row of dorsal spines readily apparent and posterior row slight. A8 with dorsal spines virtually absent. A4–7 with 5–12 spines between anteromesad setae. Cremaster present, well developed, elongate, length greater than width, tip broadly flattened. Four pairs of thickened, distinctly curled cremastral setae.

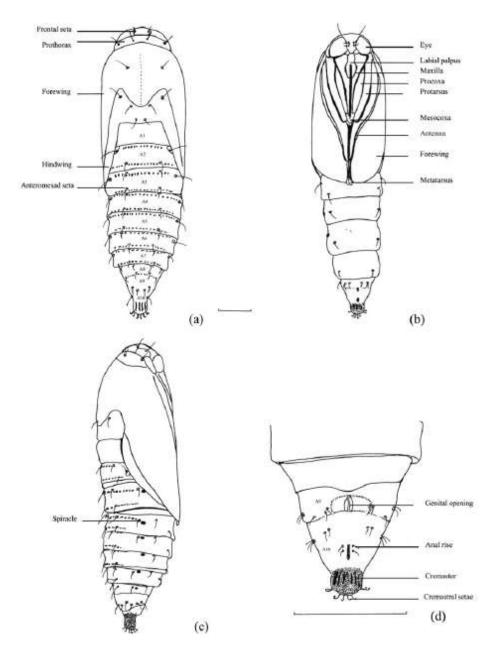


Figure 50: *Epichoristodes acerbella* pupa. a) dorsal aspect (female); b) ventral aspect (female); c) lateral aspect (female); d) terminal abdominal segments (male). Only prominent setae are drawn. Scale bars = 1 mm. (Taken from Timm *et al.*, 2008; Fig 4).

CHAPTER 4

Morphological study and development of a taxonomic key for the adult stages of economic importance tortricids on fruit crops in South Africa

4.1 Abstract

The accurate identification of economically important pest species is critical because misidentifications may lead to ineffective pest management. Currently in South Africa, no proper identification guide for adult tortricids exists. Thus, the aim of this study is to use the available literature supplemented by morphological examination of adults to compile comprehensive descriptions of the adult stage of each species, which can then be used to develop a LUCID key.

4.2. Introduction

Cydia pomonella (Linnaeus, 1758) (Codling moth), Thaumatotibia leucotreta (Meyrick, 1913) (False codling moth), Grapholita molesta (Busck, 1916) (Oriental fruit moth), Cryptophlebia peltastica (Meyrick, 1921) (Litchi moth), Thaumatotibia batrachopa (Meyrick, 1908) (Macadamia nut borer), Epichoristodes acerbella (Walker, 1864) (Pear leafroller/Carnation worm), and Lozotaenia capensana (Walker, 1863) (Apple leafroller) are South Africa's most economically important tortricid pests on fruit crops (Timm et al., 2007). Widespread damage on a variety of cultivated crops is caused by the larval feeding of these species, causing extensive loss in fruit productivity or yield (Powell, 1964; Timm, 2005). To date no proper key for identifying the adults of these species has been developed. The main objective of this study was to compile comprehensive descriptions of each species that could be used to compare and distinguish among them, and incorporated these features into a modern key, using the LUCID Key program, for the use by workers in the fruit industry. A pilot survey using specific pheromones was conducted in fruit-growing areas to determine the wider distribution of the species.

4.3. Material and methods

Published descriptions of the seven tortricid species by various authors (Appendix 4.1) were consulted and relevant data were extracted for use in the development of the Lucid Key.

4.3.1. Insect material

Specimens from the Entomological Museum of Stellenbosch University (USEC), South Africa, were used for this study. Additional material of *T. leucotreta*, *C. pomonella* and *C. peltastica* were kindly donated by established South African laboratory colonies: *T. leucotreta* from XSIT, Citrusdal, and Rhodes University, Grahamstown; *C. pomonella* from Entomon, Stellenbosch; and *C. peltastica* from Bioriver Science, Addo. *G. molesta* was caught in a dry trap on Frankenhof farm, Swellendam. *T. batrachopa* adults were received from Upotn farm, Umhlali. Larvae of *E. acerbella* and *L. capensana* were collected in the field and reared to adult hood. Adults of *E. acerbella* and *L. capensana* were also caught in the Jonkershoek Nature Reserve (GPS co-ordinates: 33°55′″S 18°51′″E), South Africa, using UV light traps.

4.3.2. Preparations of specimens

Field-collected adult specimens were killed with cyanide, pinned, and set.

4.3.3. Preparation of Genitalia

Male and female abdomens were removed, placed into 10% KOH and left to soak for 24 hours at room temperature. After soaking, abdomens were rinsed with water, neutralized in 10% HCL, transferred into 30% ethanol and cleaned using fine brushes, and the genitalia carefully removed. The integument of the abdominal segments 8-10 of the females were left attached to the genitalia. Once properly cleaned, the genitalia were transferred to 50% ethanol. Male and female genitalia were stained with chlorozol black. Using forceps, a small tear was made in the corpus bursae of females, and a spermatophore, if present, was removed. Once sufficiently stained, genitalia were placed for 10 min into 70% ethanol, then 80% and finally to 96% ethanol. Broken glass chips were placed on top of the male and female genitalia to flatten them, and the genitalia were left overnight to harden. Genitalia were then mounted on slides using Euparal. To avoid distortion, a piece of thin fishing line was placed above the genitalia to raise the coverslips.

4.3.4. <u>Preparation of images</u>

Photos of adults were taken with a Canon 50D, with 100 mm macro lens (f2.8L). Higher magnification images of the genitalia were taken with a Leica MZ 16A automontage microscope for two dimensional image analysis with a Leica DFC 290 fixed digital camera and Leica Application Suite (LAS) v.2.7. software. Photos were edited in Adobe Photoshop Element v.9.0.0 (Adobe System Incorporated).

4.3.5. Nomenclature

Nomenclature and characters studied followed those of Gilligan *et al.* (2008) as these authors emphasised that adult characteristics most useful for identification. Wings provide the most important distinguishing characteristics in adult tortricids, most having clearly distinctive markings. Hence, emphasis was placed on these in the key. For detailed nomenclature of genitalia see Chapter 1.

4.3.6. Key development

A diagnostic key was developed using LUCID Key 3.5.2 (Lucid, The University of Queensland, Australia), based on the key TortAI Adult Key – CAPS program by Gilligan & Epstein (2012) for uniformity.

4.3.7. Field trapping

The field survey was conducted over an eight week period between March and end of April 2012 at six locations (traps at Porterville and Stellenbosch were only monitored from 22 March 2012 onwards) (Fig. 51). Yellow Delta traps, sticky pads (Chempac) and pheromones (Table 6) were used for trapping. One trap for each pheromone was placed in an orchard and one in natural habitat close to the orchard, except for Prince Alfred Hamlet which did not have natural habitat close by (Table 7). Traps where monitored and sticky pads replaced every two weeks. LBAM lure was used to monitor for the possible invasive species *Epiphyas postvittana* (light brown apple moth, see Appendix I) in orchards, and MNB lure was placed only

into the natural habitat to monitor the presence or absence of *T. batrachopa* and *C. peltastica* because no host fruit crop orchards for these two species are present in the Western Cape. Traps were suspended at least 50 m apart.

Light trapping was conducted in May 2012 in Swellendam (GPS co-ordinates: 34°34'S, 20°32'E) and Jonkershoek Nature Reserve, Stellenbosch.

Table 6: Pheromones used in field surveys to trap tortricid moths associated with deciduous fruit in the Western Cape

Species	Lures	Supplier	Active Ingredients
			Z-8-Dodecenyl acetate
T. leucotreta	False codling moth (FCM) Lure	Chempac	E-8- Dodecenyl acetate
			E-7- Dodecenyl acetate
C. pomonella	Codling moth (CM) Lure	Chempac	E8-E10 Dodecadienol
			Z-8-Dodecen-1 yl acetate
G. molesta	Oriental fruit mothy (OFM) Lure	Chempac	E-8- Dodecen-1 yl acetate
			E-7- Dodecenol
E. acerbella	Pear leaf roller (PLR) Lure	Chempac	
T. batrachopa/	Magadamia nut hanan (MND) Luna	Champaa	
C. peltastica	Macadamia nut borer (MNB) Lure	Chempac	
E	Light Brown Apple	Scentry Biologicals,	E-II-Tetradecenylacetate
E. postvittana	Moth (LBAM) Lure	Inc.	E, E-9, 11-Tetradecadienol Acetate

Table 7: Trap locations to survey for tortricid moths in Western Cape fruit orchards, vineyards and adjoining natural habitats (veld) (Fig. 45).

Location	Traps/Lures	Orchard	Veld	Location	Traps/Lures	Orchard	Veld
	FCM	Apple	✓	Riebeeck-	FCM	Citrus	✓
Elgin (Elg)	CM	Apple	✓	Kasteel (RBK)	CM	Plum	✓
(Rooihoogte)	OFM	Apple	\checkmark	(Wynkelder	OFM	Plum	✓
34°08'S,	PLR	Apple	✓	Berg)	PLR	Grapevine	\checkmark
19°01'E	MNB	-	✓	33°21'S,	MNB	-	✓
	LBAM	Apple	-	18°51'E	LBAM	Grapevine	-
	FCM	Peach	✓		FCM	Peach	✓
Porterville (Port)	CM	Peach	✓	Stellenbosch	CM	Peach	✓
(Rooihoogte)	OFM	Peach	\checkmark	(Stel)	OFM	Peach	✓
33°04'S,	PLR	Peach	\checkmark	(Timberlea)	PLR	Peach	✓
18°51'E	MNB	-	✓	33°54'S, 18°50E	MNB	-	✓
	LBAM	Peach	-		LBAM	Peach	-
Prince Alfred	FCM	Apple		Womaastan	FCM	Apple	✓
Hamlet (PAH)	CM	Apple		Worcester	CM	Apple	✓
(Goosen	OFM	Peach		(Wor)	OFM	Apple	✓
Boerdery)	PLR	Pear	NI/A	(Vergenoegd)	PLR	Apple	\checkmark
33°15'S,	MNB	-	N/A	33°33'S,	MNB	_	✓
19°20'E	LBAM	Apple		19°20'E	LBAM	Apple	-

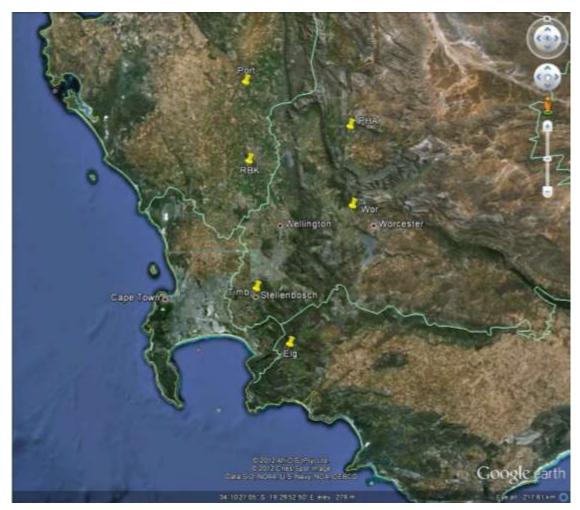


Figure 51: Google Earth map with trapping locations in the Western Cape

4.4. Results

4.4.1. Thaumatotibia leucotreta (Figs 52-53)

Sexually dimorphic.

Head: Labial palpi porrect, light brown beige with dark brown patches; proboscis smooth, unscaled; maxillary palpi reduced. Ocellus present; chaetosema present and dark in colour. Antennae with one row of scales per segment.

Forewing: Cryptic/mottled appearance, varying from greyish brown, dark brown to orange/bronze brown. Distinct "question-mark" shaped pattern along the termen and darker semi-circular band in the middle of the costal margin. White discal spot situated between the "question mark" and the semi-circle. Fringe red brown.

Hindwing: Light brown; fringe concolourous as that of forewing, slightly longer. Males with a modified scent gland represented by a dark spot on the lower margin of the hindwing (Fig. 52D).

Legs (Fig. 52C): Light brown; outer apical spur shorter on hind tibia. Males with prominent dense black tufts on inner side of hind tibia.

Genitalia: Males (Fig. 53A): Valve rounded/oval shaped with prominent darker hairs on inner surface of cucullus, phallus pointed apically, uncus absent. Females (Fig. 53B): Corpus bursae rounded to slightly

oval with two spine- or thorn-like signa, situated opposite each other; ductus bursae short and narrow, broadening towards corpus bursae.

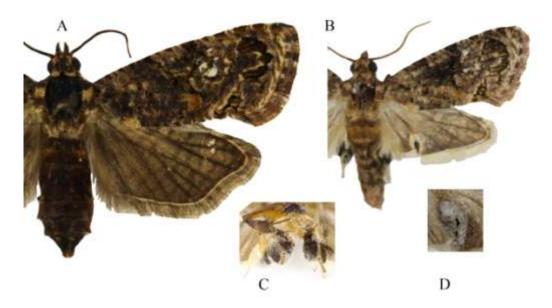


Figure 52: Thaumatotibia leucotreta adults. A) female; B) male; C) tuft on male hind tibia; D) male scent glands.

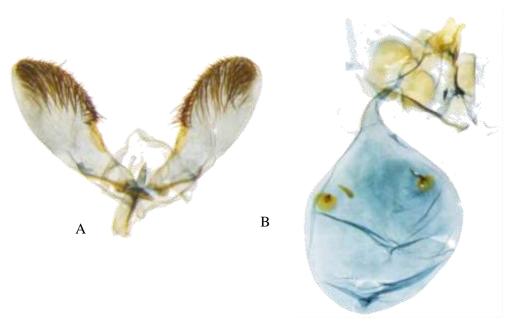


Figure 53: Thaumatotibia leucotreta genitalia. A) male; B) female.

4.4.2. Cydia pomonella (Figs 54-55)

Head: Labial palpi tusk-shaped, light brown grey; proboscis smooth, unscaled; maxillary palpi reduced. Ocellus present; chaetosema present, pale. Antennae with one row of scales per segment.

Forewing: Greyish with a grey-white striated appearance. Dark brown/metallic gold bronze ocellus in tornal region. Apical half brown. Fringe brown to dark brown-grey.

Hindwing: Brown, fringe (fringe and cilia are the same) concolourous with hindwing (the fringe looks much paler than the wing, especially along the lower margin). Males with conspicuous fascicle of darker long hairs concealed in a fold of the Cu vein (Fig. 54B) indicated by the arrow.

Legs: Light brown; apical spur on hind tibia of different length in males and females.

Genitalia: Males (Fig. 55A): Valva with costa relatively straight, venter rounded apically with a prominent indentation at neck and a ventral spine; prominent hairs on cucullus; tegument triangular-shaped; uncus absent. Females (Fig. 55B): Corpus bursae rounded (smaller than in *T. leucotreta*) with two small spine- or thorn-like signa indicated by the arrow; ductus bursae shorter and robust, with a large rounded sterigma.

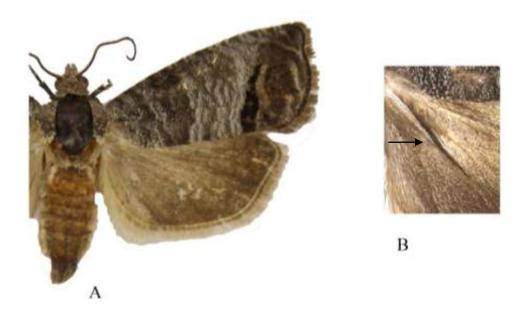


Figure 54: *Cydia pomonella* adults. **A)** females **B)** prominent darker long hairs covering the Cu vein (indicated by the arrow).

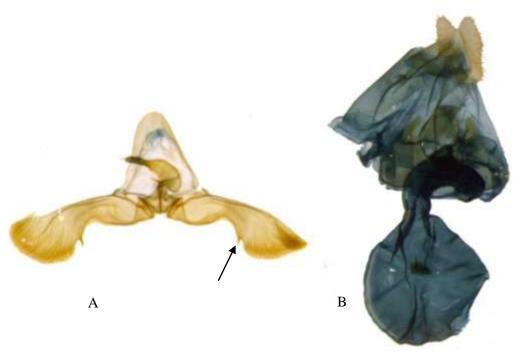


Figure 55: Cydia pomonella genitalia. A) male; B) female. Arrow indicating small spine- or thorn-like signa.

4.4.3. Grapholita molesta (Figs 56-57)

Head: Labial palpi slightly tusk-shaped, light brown proboscis smooth, unscaled; maxillary palpi reduced. Ocellus present; chaetosema present, pale brown to brown. Antennae with one row of scales per segment. Forewing: Dark grey brown to black. Row of darker spots from apex to tornus in subterminal region, forming a somewhat dotted semi-circle. Ill-defined white discal spot present. Fringe dark with white tips. Hindwing: Pale brown with fringe slightly paler than that of forewing.

Legs: Light brown; apical spur on hind tibia of different length in males and females.

Genitalia: Males (Fig. 57A): Very small; valvae elongate with conspicuous neck covered by a membrane, cucullus rounded with prominent long hairs; tegumen triangular shape. Females (Fig. 57B): Corpus bursae rounded (smaller than in *T. leucotreta*) with two small thorn-like signa; ductus bursae shorter and thick, with a large almost rectangular shaped sclerotized area around the sterigma.



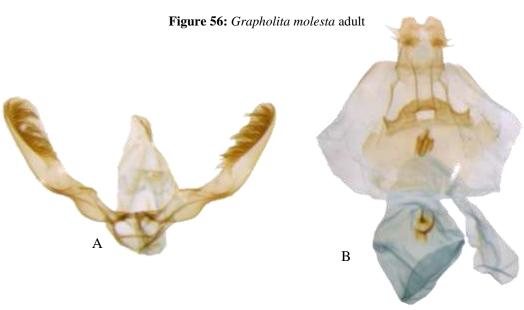


Figure 57: Grapholita molesta genitalia. A) male; B) female.

4.4.4. Cryptophlebia peltastica (Figs 58-59)

Sexually dimorphic.

Head: Labial palpi porrect, light brown beige; proboscis smooth, unscaled; maxillary palpi reduced. Ocellus present; chaetosema present, light brown to brown. Antennae with one row of scales per segment.

Forewing: Greyish bronze white, somewhat mottled in appearance. Distinct brownish black triangle (tornal spot) along dorsum with two narrow pale bronze bands crossing tornal spot horizontally. Prominent irregular fascia or spots extending diagonally from costa to termen. White discal spot present. Fringe concolourous with forewing. Forewing length 5-7 mm.

Hindwing: Pale brown to brown, fringe in females concolourous with forewing, in males hindwing darker grey brown towards outer margins with grey-brown fringe. In male, inner margin at anal angle with longer denser hair (Fig. 58B).

Legs: Scales on legs darker brown, denser on fore-, mid and hind tibia in males and females. Hind tibia of male with denser tufts than in females as in *T. leucotreta*. Apical spur on hind tibia of different lengths in males and females (Fig. 58C).

Abdomen: Male densely covered with long hair-like scales.

Genitalia: Males (Fig. 59A): Valva rectangular, cucullus rounded with short hairs and three prominent spikes indicated by the arrow; uncus absent. Females (Fig. 59B): Small oval sterigma; moderately long, narrow ductus bursae and two thick thorn- or hook-like signa in corpus bursae.

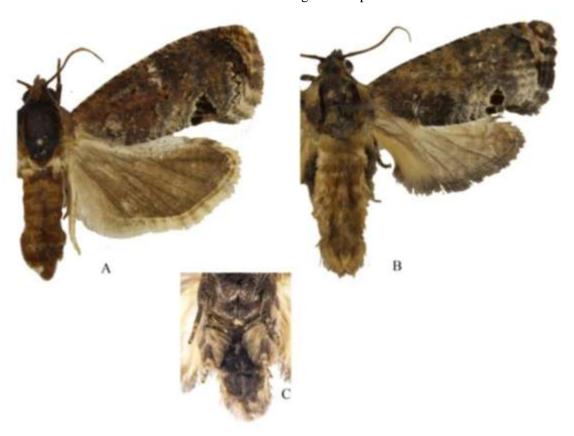


Figure 58: Cryptophlebia peltastica adults. A) female; B) male C) hind tibia of males.

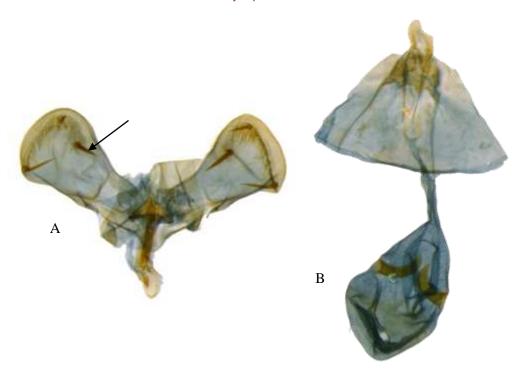


Figure 59: *Cryptophlebia peltastica* genitalia. **A)** male **B)** female. Arrow indicating prominent spikes found on male genitalia.

4.4.5. Thaumatotibia batrachopa (Figs 60-61)

Sexually dimorphic.

Head: Labial palpi porrect, longer than other species, brown grey; proboscis smooth, unscaled; maxillary palpi reduced. Ocellus present; chaetosema present, pale. Antennae with one row of scales per segment.

Forewing: Light to dark grey, with light brown "triangular" terminal area. Olive green basal and tornal spot, white discal spot ill-defined. Fringe concolourous with hindwing.

Hindwing: Uniform colour, darkening towards margin. Males with scalloped posterior margin and whitish cilia on 1A+2B (Fig. 60D). Fringe concolourous with hindwing in females; medium to dark brown in males.

Legs: Scales on legs darker brown. Hind tibia of male with denser tufts as in *T. leucotreta* (Fig 60C). Apical spur on hind tibia of different lengths in males and females.

Abdomen: Males with hairy abdomen

Genitalia: Prominent anal tuft in males, longer than in other species (Fig. 60E). Males (Fig. 61A): Valvae elongate with broadened rounded cucullus rounded, densely covered with spines; tegumen broadly rounded; uncus absent. Females (Fig. 61B): Sterigma semi-oval (almost horseshoe shaped) with sclerotized surrounding area; ductus bursae thick, moderate long; corpus bursae small, rounded, with two long thorn-like signa.

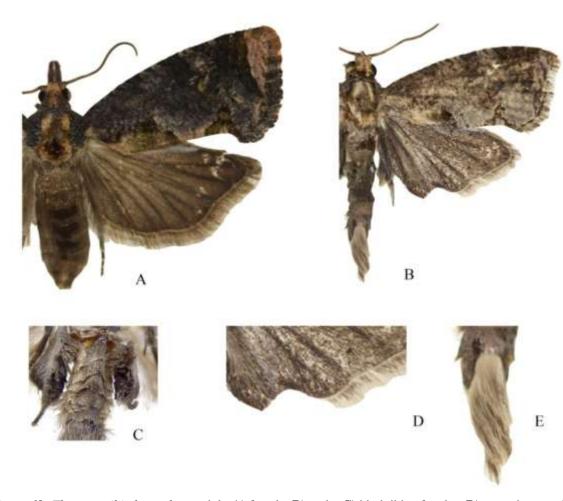


Figure 60: *Thaumatotibia batrachopa* adult. **A)** female; **B)** male; **C)** hind tibia of males; **D)** posterior margin of hindwing; **E)** male genitalia.

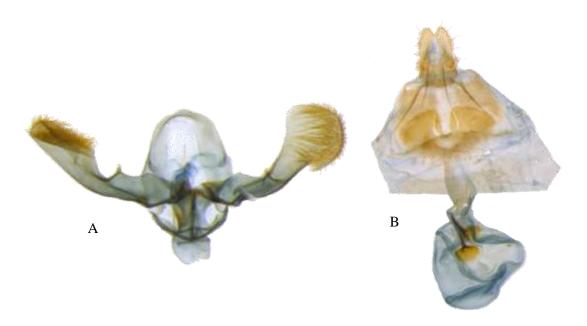


Figure 61: Thaumatotibia batrachopa genitalia A) male; B) female.

4.4.6. Epichoristodes acerbella (Figs 62-63)

Head: Labial palpi porrect, light brown; proboscis smooth, unscaled; maxillary palpi reduced. Ocellus present; chaetosema present, pale. Antennae with two rows of scales per segment.

Forewing: Light yellow to pale orange, sometimes irrorated with small brown spots. Dorsal area darker sometimes with well defined median fascia. Fringe concolorous with hindwing. Forewing length 4-7 mm, elongated, pointed appearance at tornus and termen giving the wing a pointed appearance.

Hindwing: Pale yellow beige with fringe long at anal angle (longer than in other species).

Legs: Scales on legs light brown beige in colour, fore femora with darker scales. Apical spur on hind tibia of different lengths in males and females.

Genitalia: Males (Fig. 63A): Valvae short ovoid, rounded apically; uncus spatulate; phallus tapered apically. Females (Fig. 63B): ductus bursae long, narrow; corpus bursae large, ovaoid, with one small curved signa with a small knob (capitulum) protruding from the outer surface; rectangular sclerotized sterigma surrounding small rounded ostium.

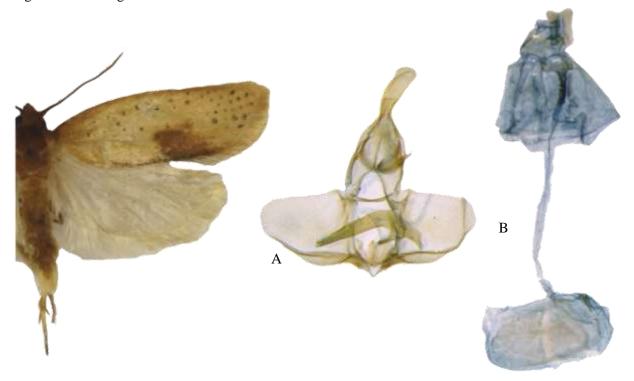


Figure 62: Epichoristodes acerbella adult.

Figure 63: Epichoristodes acerbella genitalia. A) male; B) female

4.4.7. Lozotaenia capensana (Figs 64-65)

Head: Labial palpi porrect, light brown; proboscis smooth, unscaled; maxillary palpi reduced. Ocellus present; chaetosema present, pale. Antennae with two rows of scales per segment.

Forewing: Variable in colouration, ranging from dark reddish brown, to orange-brown, to yellow. With or without various markings or pattern. Fringe concolorous with hindwing. Forewing length 8-12 mm, rectangular.

Hindwing: Light brown, darkening towards the outer edge, fringe paler than wing.

Legs: Fore legs covered with darker scales, mid legs with slightly darker scales, hind legs with light brown scales. Mid and hind apical spurs with dark scales.

Genitalia: Males (Fig. 65A): Valvae short, rounded, almost as broad as wide; uncus short, round spoonshaped; gnathos well-developed, slender arms joined distally; phallus large, tapered apically. Females (Fig. 65B): Ductus bursae short, thick; corpus bursae small, ovaoid, with one thorn-like signa.



Figure 64: Lozotaenia capensana adult wing pattern variations

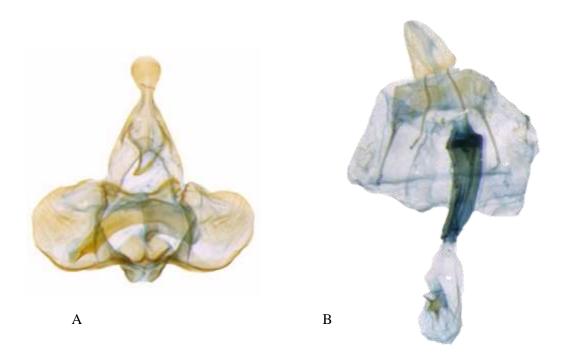


Figure 65: Lozotaenia capensana genitalia. A) male; B) female.

4.4.8. Field trapping

The results of field trapping using various pheromones are presented in Table 8. For locations specifics refer to Table 7. Only four *L. capensana* and three *E. acerbella* were caught using UV light trapping.

Table 8: Total number of adults moths captured from field surveys conducted in deciduous fruit orchards and vineyards of the Western Cape for various tortricid moths from March to end of April 2012 (For abbreviations see Tables 6 and 7).

			Orchard					Veld		
		Lure								
	FCM	CM	OFM	PLR	LBAM	FCM	CM	OFM	PLR	MNB**
Location	Total									
RBK	2*	0	0	1	0	0	0	0	0	1
PAH	0	0	0	0	0			N/A		
Port	0	0	3	0	0	1	0	0	0	7
Elg	71	21	22	0	0	1***	0	0	0	7
Wor	2	2	1	0	0	0	1	0	0	0
Stel	68	0	8	0	0	131	0	0	0	3

^{*}FCM Data received from Wynkelderberg at close of survey

4.5. Discussion and Conclusion

4.5.1. Diagnostic characters

<u>T. leucotreta</u>: Chaetosema dark. Antennae one row of scales per segment. Forewing with a distinct "question-mark" along the termen, a semi-circle along the costal margin and a white discal spot situated between these two pattern elements. Males can be easily distinguished by the distinctive scent gland (darker spot) on the hindwing and dense black tufts on the hind tibia. Forewing length: females 9-10 mm, males 6–8 mm (Gilligan *et al.*, 2011).

<u>C. pomonella</u>: Chaetosema pale. Antennae one row of scales per segment. Forewing, greyish colouration with a striped grey-white appearance. Forewing with a striped grey-white appearance, dark brown/ metallic gold bronze ocellus. Apical half medium brown. Males with prominent darker long hairs covering the Cu vein. Forewing length: 7–8 mm (Pinhey, 1975).

<u>G. molesta</u>: Chaetosema light to medium brown. Antennae one row of scales per segment. Forewing dark grey brown to black. Row of darker spots starting at apex down to tornus along termen, forming a slight dotted semi-circle. White discal spot present. Fringe dark with white tips. Hindwing medium brown with medium length hairs on fringe. Forewing length: 4–7 mm (Chapman & Lienk, 1971), distinctly smaller than the other known tortricid pests in South Africa.

<u>C. peltastica</u>: Chaetosema light to medium brown. Antennae one row of scales per segment. Forewing with clear distinct dark brown black triangle (tornal spot) along the dorsum with two light bronze thin bands crossing the tornal spot horizontally. Prominent irregular "bands" or spots crossing diagonal from costal fold to termen. White discal spot present. Fringe darker grey. Forewing length 5–10 mm.

^{**}Only *C. peltastica* was caught by MNB lure, no *T. batrachopa*.

^{***} FCM caught by OFM lure.

<u>T. batrachopa</u>: Chaetosema pale. Antennae one row of scales per segment. Forewing with light brown down-pointed acute terminal spot; olive green basal and tornal spot, white discal spot present. Sexual dimorphic. Males with scalloped posterior margin and whitish cilia on 1A+2B, prominent anal tuft, longer than in other species; hairy abdomen and hind tibia with denser tufts as in *T. leucotreta*. Prominent anal tuft in males, longer than in other species. Forewing length: 8-10 mm (Meyrick, 1908).

<u>E. acerbella</u>: Chaetosema paler. Antennae two rows of scales per segment. Forewing with light to medium yellow to orange colouration sometimes with small brown spots all over. Dorsal area darker in colouration, sometimes with a clear median fascia. Elongated shape, pointed at the apex, rounded at tornus and termen giving the wing a pointed look. Long fringe at anal angle of hindwing. Fore femora with darker scales. Forewing length: 4-7 mm, elongated, pointed apically, rounded at tornus and termen giving the wing a pointed appearance.

<u>L. capensana</u>: Chaetosema pale. Antennae two rows of scales per segment. Forewing rectangular-shaped and ranging from darker reddish to brown, to yellow, brown orange to brown. With or without various different markings or patterns. Forelegs covered with darker scales, mid legs with slightly darker scales, hind legs with light brown scales. Mid and hind apical spurs with dark scales. Forewing length: 8–12 mm, rectangular.

4.5.2. Trapping

Trapping was carried out over a wide distributional range of the fruit-producing areas in the Western Cape, but for a short period only, the timing coinciding with the late presence of adult moths, explaining low catches. The specific lures did attract the targeted species, but in two cases non-target species were trapped, indicating some similarity in the range or mixture of artificial lures. The negative results recorded in Prince Alfred Hamlet are most likely ascribable to an effective control strategy and to the absence of natural vegetation. Negative results were noted for trapping with the LBAM lure and it appears that *E. postvittana* is absent in the study area. *C. peltastica* were caught in traps containing the MNB lure instead of the *T. batrachopa* which is the target species of this lure. This corresponds with the initial notion that *T. batrachopa* does not occur in the Western Cape. Further, more extensive trapping is recommended to confirm these results.

4.6. References

- Allen, A.A. (1980) *Epichoristodes acerbella* Walker (Lep.: Tortricidae): first capture of the imago at large in Britain. In Chalmers-Hunt, J.M (1980) *The entomologist record and Journal of Variation* **92**: pp. 33.
- Bradley, J. D. (1953). Some important species of the genus *Cryptophlebia* Walsingham, 1899, with descriptions of three new species (Lepidoptera: Olethreutidae). *Bulletin of Entomological Research* **43**: 679-689.

- Forbes, W. T. M. (1920). Carpocapsa Treitschke (Cydia; Laspeyresia in part), The Lepidoptera of New York and Neighboring States 396 pp.
- Chapman, P. J. & Lienk. S. E. (1971). Tortricid fauna of apple in New York (Lepidoptera: Tortricidae); including an account of apple's occurrence in the state, especially as a naturalized plant. Spec. Publ. Geneva, NY: New York State Agricultural Experiment Station, 122 pp.
- Garman, P. (1917). The Oriental Peach pest (*Laspeyresia molesta*, Busck), a dangerous new fruit insect of Maryland. *The Maryland State Collage of Agriculture, Agricultural Experiment Station*. Bulletin No. 209, p.6.
- Gilligan, T.M., Wright, D.J. & Gibson, L.D. (2008). *Olethreutine Moths of the Midwestern United States*. An Identification Guide. Ohio Biological Survey, Bulletin New Series, Volume XVI Number 2. Columbus, USA, 334 pp.
- Gilligan, T.M., Epstein, M.E. & Hoffman, K.M. (2011). Discovery of False Codling Moth, *Thaumatotibia leucotreta* (Meyrick), in California (Lepidoptera: Tortricidae), *Proceedings of the Entomological Society of Washington* **113** (4): 426-435.
- Gilligan, T. M. & Epstein, M. E. (2012). TortAI, Tortricids of Agricultural Importance to the United States (Lepidoptera: Tortricidae). Identification Technology Program (ITP), USDA/APHIS/PPQ/ CPHST, Fort Collins, CO. [accessed athttp://idtools.org/id/leps/tortai/].
- Meyrick, E. (1905). New South African Micro-Lepidoptera, Annals of the South African Museum 5: 350.
- Meyrick, E. (1908). Descriptions of African Micro-Lepidoptera, *Proceedings of the Zoological Society of London* **1908**: 718-719.
- Meyrick, E. (1921). Descriptions of South African Micro-Lepidoptera, *Annals of the South African Museum* **8**: 57.
- Nuzacci, G. (1973). Epichoristodes acerbella (Lepidoptera, Tortricidae). Entomologica (Bari) 9: 147-148.
- Pinhey, E.C.G. (1975). Moths of Southern Africa, Tafelberg Publishers, Cape Town, pp. 37-38.
- Powell, J.A. (1964). *Biological and taxonomic studies on tortricine moths, with reference to the species in California*, University of California Press, Berkeley and Los Angeles, 317 pp.
- Timm, A.E. (2005) Morphological and molecular studies of tortricid moths of economic importance to the South African fruit industry, Phd (Agric) dissertation, Stellenbosch University.
- Timm, A.E., Warnich, L. & Geertsema, H. (2007), Morphological and molecular identification of economically important Tortricidae (Lepidoptera) on tropical and subtropical fruit in South Africa. *African Entomology* **15** (2):269-286.
- Wood, W.B & Selkregg, E.R. (1918). Further Notes on *Laspeyresia molesta*, *Journal of Agricultural Research* XIII, No. 4.
- Walker, F. (1863). Catalogue of Lepidoptera Heterocera, Fam Tortricidae, *List of the Specimens of Lepidopterous Insects in the Collection of the British Museum* **28**: 295.

4.7. Appendix 4.1

Published descriptions found in the literature.

4.7.1. Thaumatotibia leucotreta

Meyrick, (1908) described *T. leucotreta* as:

Head whitish, sides of crown suffused with achreous, palpi moderate, porrected. Second joint with long rough projecting scales above and beneath white, second joint tinged with pale brownish-ochreous. Thorax whitish irregularly tinged with brownish & spotted with dark fuscous. Abdomen fuscous more or less suffused with yellowish or whitish towards base anal tuft whitish-ochreous. Fore wings elongate somewhat dilated posteriorly. Costa slightly arched, fold extending to 2/5. Apex obtuse, termen slightly indented above middle rather oblique ferruginous-ochreous, often more or less, suffused with fuscous, variably and irregularly strigulated with white and dark fuscous. Posterior 3/5 of costa with five pairs of oblique white strigulae, whence proceed short irregularly leaden-metallic strigae. A large irregular suffused white median dorsal blotch including several grey strigulae, ocellus large, white, irregular, containing several pale metallic grey spots in two transverse series, and black dots between those, sometimes several other black dots scattered round this. Cilia dark reddish-grey finely irrorated with white, on tornus with a white patch hind wings with 3 and 4 stalkedgrey. Cilia pale grey or whitish-grey, with faint darker subbasal line

Pinhey (1975) described T. leucotreta as:

Forewings brown with black markings and white dots. Hindwing plain brown. Forewing 6-8 mm.

Gilligan et al., (2011) described T. leucotreta as:

Adults are grayish brown to dark brown with forewing length of 7-8mm for males and 9-10 mm for females. Males and females are secually dimorphic, and the two differ in overall size, wing shape, and male secondary sexual characters. The male forewing is triangular, with an acute apex, while the female forewing is more elongated with a rounded apex. Male secondary characters include tufts of modified scales on the inner side of the hind tibia, enlargment of the apical spur on the hind tibia with a batch of scales on the distal end of vein CuA2 on the Hind wing four. Forewing pattern elements: a small white dot near the end of the discal cell; a patch of raised, ususally rust colored cales near the middle of the wing; a distinct "question-mark-shaped' band of dark scales along the termen; and a semicircular band of dark scales in the middle of the costa. Male genitalia are characterized by a rounded tegumen lacking an uncus or socii, large rounded valvae, and a tapered aedeagus that is upcurved distally. Female genitalia are characterized by a semicircular sterigma, narrow ductus bursae, and large rounded corpus bursae with a pair of thorn-shaped signa.

4.7.2. Cydia pomonella

Forbes (1920) described C. pomonella as:

Gray with fine striation, showing under a lens white tips to blackish scales; base, or a broad antemedial band, slightly darker, with excurved and scalloped outer boundary; speculum of two bronze brown bars, the outer one more or less broken up, filled in with chocolate brown, the brown extending to form a large oval area almost reaching costa and outer margin; costa and extreme outer margin striate and gray like the base. No black speculum, but speculum preceded by a heavy vertical black bar, ending in a point halfway to costa. Fringe with a black line, and sometimes cut with white at M1. Hind wing brown; the enlarged scales covering Cu in the male lead-color; the hairs of the fringe on Cu blackish. 15-20 mm.

Pinhey (1975) also described *C. pomonella* as follows:

Forewing 7-8 mm, dark grey or pinkish-brown irrorated with white, forming sinous bands; an incomplete metallic ring, reflecting gold or copper tints, around a dark subterminal fescia. Hindwings brownish-white irrorated with brown; with a cream marginal line.

4.7.3. Grapholita molesta

Garman (1917) described *G. molesta* as follows:

Predominant color of front, antennae vertex, dorsum of thorax and front wings above dusky to blackish brown; Hind wings except the costal margin and a large spot on the caudo-mesal angles in the female, dusky drab; wings iridescent beneath, the color changing from yellowish green to a dusky brown when viewed indifferent lights. Ventral surfaces of the thorax, abdomen and legs covered with broad silvery, iridescent scales, the tarsi darker, through banded with narrow white rings. Antennae simple, with distinct whitish rings; labial palpi faintly irrorated with white; caudal half of the thoracic dorsum and the

patagia with white-tipped scales; front wings with a row of short, white double dashes on the costal margin and several very indistinct pale marks proximad of these; Black costal intervals produced into indistinct wavy lines extending across the wings; a group of white scales present distad of the centre forming the ocellus. A somewhat triangular group of white scales near the distal margin, the white scales in the latter position surrounding a row of distal margin, the white scales in the latter position surrounding a row of six small dark spots; a distinct black terminal line present proximal of the cilia. Hind wings in the female with a broad yellowish brown area occupying the caudo-mesal angles; costal margin white, the latter considerably broader proximad; hind wings of the male similar to those of the female, but without the yellowish area on the caudo-mesal angles; mesofemora with two long blunt spurs at their apices the metafemora with similar spurs at the apices and also with a pair of spurs near the middle; tip of the abdomen of the male forming a hallow cone, the genitalia composed of dark slipper-shaped harpes or claspers, uniformly covered with short setae, the lateral margins with about seven longer ones; harpes surrounded by broad scales which form the cone, and united to the tegumen by three-fourths their length; female genitalia consisting of two closely approximated egg guides, the mesal surfaces of which are densely setose; dorsad of each ventral egg guide, there is a brush of long blunt hair-like glands, presumably scent glands.

Wood & Selkregg (1918) described G. molesta as:

The head of the adult is dark, smoky fuscous; face a shade darker, nearly black; labial palpi a shade lighter fuscous; antennae simple, rather stout, half as long as the forewings, dark fuscous with thin, indistinct, whitish annulations. Thorax blackish fuscous; patagia fainly irrorated with white, each scale being slightly white-tipped. Forewings normal in form; termen with slight sinulation below apex; dark fuscous, obscurely irrorated by white-tipped scales; costal edge blackish, strigulated with obscurely irrorated by white-tipped scales; costal edge blackish, strigulated with obscure, geminate, white dashes, four very faint pairs on basal half and three more distinct on outer half besides two single white dashes before apex; from the black costal intervals run very obscure, wavy, dark lines across the wing, all with a strong outwardly directed wave on the middle of the wing. On the middle of the dorsal edge the spaces between three of these lines are more strongly irrorated with white than is the rest of the wing, so as to constitute two faint and poorly defined, white dorsal streaks. All these markings are only discernible in perfect speimesn and under a lens; Ocellus strongly irrorated with white, edge by two broad, perpendicular, faint bluish metallic lines and containing several small deep black, irregular dashes, of which the fourth from tornus is the longest and placed father outward, so as to break the outer metallic edge of ocellus; the line of black dashes as well as the adjoining bluish metalliclines are continued faintly above the ocellus in a curveto the last geminate costal spots. There is an indistinct, black apical spot and two or three small black dots below it; a thin but distinct, deep black, terminal line before the cilia; cilia dark bronzy fuscous. Hind wings dark brown with costal edge broadly white; cilia whitish; underside of wings lighter fuscous with strong iridescent sheen abdomen dark fuscous with silvery white underside. Legs dark fuscous with inner sides silvery, tarsi blackish with narrow, yellowish white annulations. Alar expanse: 10-15 mm.

4.7.4. Cryptophlebia peltastica

Meyrick (1921) described *C. peltastica* as follows:

Palpi, and thorax rather dark fuscous in male partly suffused with blackish abdomen. Grey posterior tibiae and basal joint of tarsi in male densely tufted above with long rough brownish-orchreous hairscales suffused with blackish above. Forewings elongate-triangular, costa slightly arched, apex obtuse, termen rounded, somewhat oblique, 1 c curved and nearly approximated to 2 near its origin, fuscous, somewhat mottled obscurely with brownish, towards dorsum posteriorly obscurely strigulated with whitish irroration costa somewhat marked with brownish and dark fuscous strigulae a triangular blackish-fuscous praetornal spot, cut by two brownish veins. A more or less developed blackish-brown faciate streak from beneath costa near apex to middle of termen. A transverse series of about four small irregular black dots before lower half of termen. Cilia dark grey speckled with whitish points. Hindwings rather dark grey; cilia plae fuscous with darker subbasal line in male becoming longer and pale greyish-ochreous along dorsum.

Bradley (1953) described C. peltastica as:

Forewing light brownish clay, in the female irregularly suffused with greyish fuscous which darkens the wing of some examples considerably and gives it a rather leaden and variegated appearance; pretonal spot on dorsum vinaceous, present in both sexes but usually better defined and more richly coloured in the female crossed by two light brown lines and surrounded on its inner side by a thin whitish line. Male has only a small brush of hair on the surface of the hindwings but the inner margin is fringed with long hairs and scales. The tuft on the hind tibia is considerably larger and denser. Male genitalia has small

setae between the two large, strong setae in the distal margin of the valva, the dense cluster of cornuti in the aedeagus is deciduous. Female genitalia anterior edge of eight tergite incurved.

4.7.5. Thaumatotibia batrachopa

Meyrick, (1908) described *T. leucotreta* as:

18-22 mm; Head pale ochreous, face and sides suffused with dark fuscous, palpi dark fuscous. Thorax with double posterior crest, pale ochreous, broadly suffused laterally with dark fuscous, sometimes mixed with ferruginous. Abdomen rather dark fuscous, in males with large expansible pale greyishochreous genital tuft. Posterior tibiae in male with very large dense brush of greyish-white hairs above, including a blackish-grey tuft towards apex. Fore wings elongate-triangular, costa moderatly arched, apex obtuse, termen almost straight, somewhat oblique; in male with rather strong dorsal scaleprojections towards base and beyond middle. Light brownish or brownish-ochreous, obscyrely strigulated with whitish, especially in male towards dorsum and termen; most of wing except dorsum and termen in male irrorated with fuscous-crimson and strigulated with black, posterior edge of this area in male indicated but inconspicuous. Most of wing except dorsum and termen in female largely suffused with dark fuscous sometimes mixed with dull crimson, posterior edge of this area in female well-defined and forming a blunt wedge-shape projection extending downwards from upper half to near termen before middle. Costa marked with some pale strigulae, and in male with several small fuscouscrimson and black spots, sometimes a distinct pale greenish discal dot beyond middle. A semioval olive -greenish or dark reddish-fuscous patch mixed with black occupying anterior half of dorsum, its central area more or less suffused with whitish -ochreous, especially in males and acute-triangular olivegreenish spot mixed with black on dorsum before tornus. Cilia whitish-fuscous irrorated and barred on upper half of termen with ferruginous, on lower half with blackish. Hindwings in male with a deep semi-ciricular excavation on termen above tornus, dark fuscous, darker posteriorly. In male vein 1b clothed with rough whitish hairs cilia whitish-grey, with grey subbasal shade.

4.7.6. Epichoristodes acerbella

Meyrick (1906) described E. acerbella (as Proselena ionephela) as being:

16-17 mm. Head and thorax whitsh-ocherous, more or less tinged with brownish. Palpi whitish-ochreous, second joint externally suffused with fuscous. Antennae grey. Abdomen pale whitish ocherous. Forewings elongate-oblong, costa gently arched, apex obtuse, termen nearly straight, oblique; whitish-ocherous; costa suffused with yellow-brownish towards base; a streak of ferruginous suffusion along dorsum from near base to tornus, expanding into a moderately large suffused praetornal blotch; cilia whitish-ochreous. Hindwings and cilia ochreous-whitish.

Allen (1980) described *E. acerbella* as the following:

Of about the size and shape of a small *Epinotia solandriana* L., but costa less arched; forewings noticeably narrow for a Tortricine, pale ochreous-buff, costally shaded with brown near base, more or less irregularly strwn with fuscous dots and specks; a fuscous-mixed fulvous bloch, in size about half width of wing, well defined above and inwardly, occupying the tornal area, extending outward in a point to base of termen, and inward as a narrow streak for some distance along hind margin. Hindwings pale shining whitish-brown. Palpi light fuscous, fore femora blackish above, legs otherwise pale. Patagia fulvescent in front, rest of body pale.

Pinhey (1975) briefly described E. acerbella as:

Forewing yellow with brown or violet-brown spot near anal angle; hindwing white or grayish-white. Forewing 7mm.

4.7.7. Lozoteinia capensana

Walker (1863) described *L. capensana* as an original description:

Pale fawn-colour, mostly pale cinereous beneath. Palpi porrect, rather slender, almost as long as the breadth of the head; second joint slightly fringed above; third elongate-conical about one fourth the length of the second. Antennae setulose. Abdomen extending beyond the hind wing. Fore wings acute, with very numerous transverse minute brownish streaks. Costa convex towards the base; exterior border oblique hindward. Hindwings pale cinereous. Length of the body 4 lines of the wings 8 lines.

Pinhey (1975) described L. capensana as:

Forewings with convex costa dipping before the pointed apex; pale or deeper brown with darker bands. Hidnwings cream or grey-brown. Forewings 8-12 mm.

CHAPTER 5

Determining intraspecific variation in *Thaumatotibia leucotreta* (Lepidoptera: Tortricidae) males by using shape analysis of genitalia

5.1 Abstract

Recent studies by Timm *et al.* (2010) have demonstrated that geographically isolated populations of *Thaumatotibia leucotreta* are genetically distinct. Current control methods for this important pest could be compromised if significant divergence is present within or among these separate populations. Because sexual selection can cause rapid evolution of male genitalia, differences in the morphology of these structures among different populations may be evidence of incipient or realized speciation. In this study the shape of the male valvae using geometric morphometrics to analyze variation within and among three different populations of *T. leucotreta* were examined. Although some variation was detected among mean population values for certain traits, there was no overall similarity in pattern. Principle component analysis showed no distinct clustering of valvar shape among populations, providing no evidence for divergence in male genitalia and hence, no morphological evidence of speciation.

5.2 Introduction

It has long been recognized that male genitalia undergo rapid evolution, possibly as a result of sexual selection (Eberhard, 1985). This rapid evolution can cause divergent genital morphology, even in closely related species, and in many cases, male genital morphology is a reliable way to differentiate between related species (Goulson, 1993; Arnqvist, 1998; Eberhard, 2010a, 2010b). During the last century, the use of genitalia in tortricid taxonomy has become a standard tool, following the work of Meyrick (1895). Meyrick (1895), although introducing the use of genitalia in taxonomy, later opposed their application. Kennel (1908) stated that "genitalia are so strongly diverse" that they may be useful for separating related taxa, and Dampf (1908) was the first to successfully use tortricid genitalia (of *Rhopobota naevana*, Hübner) to conduct a comparative study (Horak, 1984). Pierce &Metcalfe (1922) and Heinrich (1923) were the first to incorporate male and female genitalia into large systematic works on Tortricidae. Powell (1964) stated that the "male genitalia in tortricids form the basis for classifications," and that genitalia alone can be used to determine the identity of a species. Nearly every systematic work on tortricids published since the middle of the 20th century has used genitalia to separate species, demonstrating the importance and taxonomic value of these characters (Horak, 1984).

Thaumatotibia leucotreta (Meyrick) is one of the major fruit pests in South Africa. Larvae of this species, which can cause serious economic damage, have been recorded from on a wide variety of host plants (see Chapter 1, Table 3). Recent studies have demonstrated that geographically separated populations of *T. leucotreta* show minor, but statistically significant genetic differences (Timm *et al.*, 2010). These differences are likely due to limited dispersal abilities among widely separated populations (Timm *et al.*, 2010). Genetic divergence in *T. leucotreta* could influence alternative pest management practices such as sterile insect technique and monitoring systems using sex pheromones, emphasizing the need to determine

if target populations are being influenced by speciation processes. Because of rapid evolution in male genitalia (Eberhard, 1985, 2010a, 2010b), genital morphology in different populations of a single species can be used to infer if speciation is occurring. Geometric morphometrics is one method that has been used to quantify genitalic differences between species (Mutanen & Kaitala, 2006). Morphometrics is the "measurement and analysis of a form" (Daly, 1985) and was traditionally based on size, ratios and linear measurements (Daly, 1985; Mutanen & Pretorius, 2007). The study of geometric morphometrics has become easier as computer programs such as SHAPE (Iwata & Ukai, 2002) enable users to quantify complex shapes (Mutanen & Pretorius, 2007). The aim of this study was to determine if there is morphological evidence of speciation occurring in *T. leucotreta* by examining differences in the shape of the valvae of the male genitalia in different populations.

5.3 Material and methods

5.3.1 <u>Study organism</u>

Thaumatotibia leucotreta was studied from three geographically separate populations: Addo, Citrusdal, and Mbombela (Nelspruit). Larvae were obtained from captive colonies at Rhodes University, Grahamstown (Addo and Mbombela populations) and XSIT, Citrusdal (Citrusdal population). The Rhodes University colonies were established in 2008 and no additional genetic material was added after establishment (Opoku-Debrah and Moore, 2008). The Citrusdal colony was established in 2010 (R. Stotter, pers. comm., August 2011).

5.3.2 Preparation of material

Larvae from each population were used to establish new colonies. Twenty adult males were selected from each colony. Abdomens were removed and placed into 10% KOH and left to soak for 24 hours at room temperature. After soaking, abdomens were rinsed with water, neutralized in 10% HCL, transferred into 30% ethanol, and cleaned using fine brushes. The genitalia were carefully removed, cleaned, and transferred to 50% ethanol. The valvae were separated completely from the tegumen, placed into xylene for ten minutes, and then mounted on slides using Euparal (Bioquid Products. Inc). Valvae were mounted in a consistent manner to avoid any artefacts of preparation, and only 14 of the 20 genitalia where used due to damage or mounting errors. Genitalia were photographed using a Leica DM 2000 automontage microscope with a Leica DFC 295 fixed digital camera and Leica Application Suite (LAS) v.4.0.0. Using Adobe Photoshop Element v.9.0.0 (Adobe System Incorporated), the valvae were each outlined separately, aligned in a single file, and a black and white bitmap image was created for analysis using SHAPE (Fig. 66) (Iwata & Ukai, 2002).

5.3.3 **SHAPE and Data analysis**

SHAPE was used to determine the contour shapes of the left and right valvae by calculating Elliptic Fourier descriptors (EFDs), which were then converted to principal components for a principal components analysis. SHAPE analysis follows the procedure outlined in Gilligan & Wenzel (2008). SHAPE is

composed of three modules: 1) Chaincoder, 2) Chc2Nef, and 3) PrinComp. The first module, Chaincoder, converts the outline of the valve into a chaincode which is then used by the second module, Chc2Nef, to calculate normalized EFDs based on the first harmonics (Iwata & Ukai, 2002). A total of 30 harmonics were included. The third module, PrinComp, is used to conduct a principle components (PC) analysis of the coefficients of the EFDs (Iwata & Ukai, 2002). PrinComp provides a summary and visualization of the variation in shape for each PC axis. To determine the proportion of variance explained by each principle component axis, the eigenvalue for each axis was assessed. Principle component scores from axes 1 (PC1) to 5 (PC5) were used for further analysis as these accounted for more than 80% of the total variation in both valvae, and the PCs for each of the populations overlap, indicating that there is no significant variation in valvae shape. The mean PC scores were calculated and plotted together to determine the overall variance among populations. To analyse the amount of variation among the three populations, a one-way ANOVA was calculated for the principle components (PC1 - PC5) of both the left and right valvae (Table 9). A Bonferonni adjustment for multiple comparisons was applied by dividing the significance level (0.05) by the number of comparisons (five) to obtain an adjusted significance level of 0.01. Further analyses to determine intraspecific variance in both valvae were conducted in SAS Enterprise guide and Excel, Microsoft Office 2010.



Figure 66: Outlines of left and right valva of *T. leucotreta* used for shape analysis: Specimens arranged by populations: Row 1: Citrusdal (14, 15, 16, 18, 19, 22, 23); Row 2: Citrusdal (5, 7, 9, 10, 11, 12, 13); Row 3: Addo (9, 10, 11, 12, 13, 14, 17); Row 4: Addo (2, 3, 4, 5, 6, 7, 8); Row 5: Mbombela (12, 13, 14, 15, 16, 18, 19); Row 6: Mbombela (2, 6, 7, 8, 9, 10, 11).

5.4 Results

5.4.1 Principle components

Principle component 1 (PC1) and Principle component 2 (PC2) together accounted for 53.5% and 58.4% of the total variation for the left and right valvae, respectively (Figs. 67-68). Combined, PC1 to PC5 accounted for more than 84.9% of variation in the left valvae and 82.3% in the right valvae. Reconstructed shape contours presented in Figs. 2 and 3 allow for visual examination of the variation of the valvae shape. For the left valvae (Fig. 67), PC1 and PC4 represent the overall size, PC2 represents the overall width, PC3 represents the curvature of the costal margin, and PC5 represents the width at the proximal end. For the right valvae (Fig. 68), PC1 represents the overall size, PC2 represents the width at the proximal end, PC3 represents the overall width, PC4 represents the curvature of the costal margin, and PC5 represents the

width at the proximal end and curvature of the costal margin. Because more than 50% of the variation was accounted for by PC1 and PC2, and because other PCs accounted for only minor (>15%) variation, only these two axes were used to provide a visual representation of the overlap in variation among the populations (Polihronakis, 2006). As shown in Fig. 69, PC scores across the three *T. leucotreta* populations overlap on both PC axes, with high levels of intraspecific variation and no distinct grouping formed by any single population. The means for each population are displayed in Fig. 70.

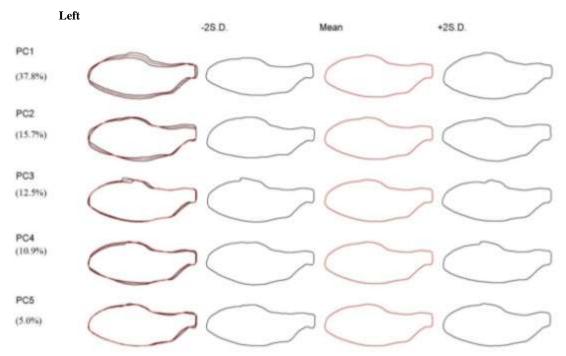


Figure 67: Reconstructed shape contours of the principle components analysis and corresponding percentages for left valva. Superimposed outlines on the left are combined on the mean and ± 2 standard deviations. Mean is outlined in red.

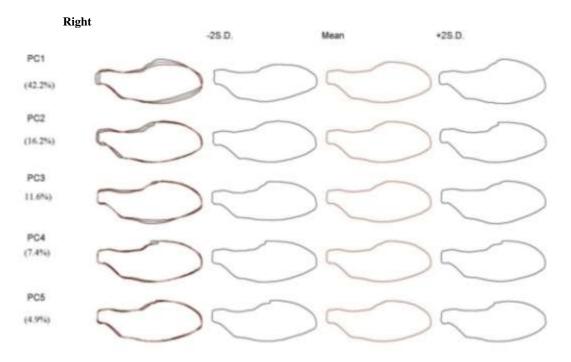


Figure 68: Reconstructed shape contours of principle components analysis and corresponding percentages for right valva. Superimposed outlines on the left are combined on the mean and ± 2 standard deviations. Mean is outlined in red.

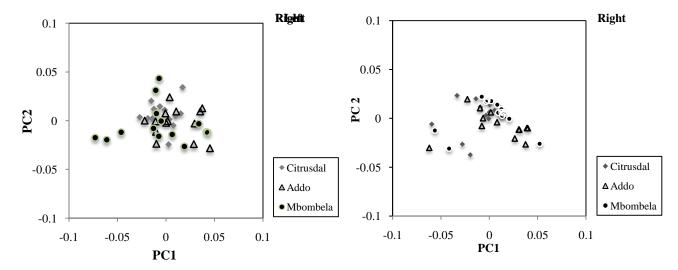


Figure 69: Plot PC1 vs. PC 2 for both left and right valvae. Three *T. leucotreta* populations from different locations were compared.

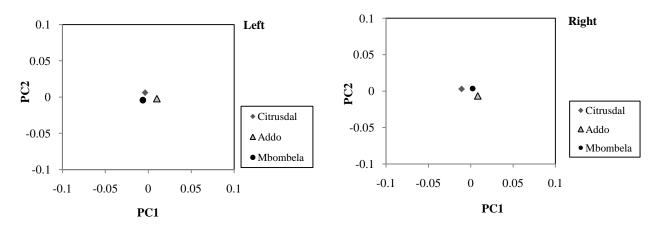


Figure 70: Mean of PC1 vs. mean of PC 2 for each population, indicating the centre of distribution.

5.4.2 Analysis of Variance

To determine if there was a statistically significant difference among PCs in each population, standard errors of the mean for left and right valvae were plotted in Fig. 71. In both, the left and the right valvae, the PCs for each of the populations overlap, indicating that there is no significant variation in valvar shape. For the left valvae, significant variance was demonstrated in PC4 (df = 2; P= 0.0069) and PC 5 (df = 2; P= <0.0001); however, together these two PCs contribute only 15% of the total variation (Figs 67-68), compared to PC1 and PC2 which account for more than 50% of the total variation. For the right valvae, none of the PC scores showed significant variation.

Table 9: Analysis of variance of *T. leucotreta* male valvae. Values in bold indicate statistical signicficance ($p \le 0.01$; df= 2).

			PC1			PC2			PC3			PC4			PC 5	
Population	Nr	Mean	SD	SE	Mean	SD	SE	Mean	SD	SE mean	Mean	SD	SE	Mean	SD	SE
<u>Left valve</u>																
Addo	14	0.0100	0.0214	0.0057	-0.0021	0.0152	0.0041	0.0047	0.0152	0.0041	-0.0077	0.0115	0.0031	-0.0049	0.0065	0.0017
Citrusdal	14	-0.0037	0.0135	0.0036	0.0063	0.0132	0.0035	-0.0014	0.0140	0.0038	0.0080	0.0116	0.0031	-0.0030	0.0076	0.0020
Mbombela	14	0.0063	0.0353	0.0094	-0.0042	0.0196	0.0052	-0.0033	0.0145	0.0039	-0.0002	0.0138	0.0037	0.0079	0.0082	0.0022
		df	F ratio	P	df	F ratio	P	df	F ratio	P	df	F ratio	P	df	F ratio	P
Variance		2	1.70	0.1962	2	1.63	0.2099	2	1.15	0.3267	2	5.67	0.0069	2	12	<0.0001
Right valve																
Addo	14	0.0084	0.0290	0.0077	-0.0065	0.0139	0.0037	0.0027	0.0156	0.0042	0.0019	0.0139	0.0037	0.0014	0.0086	0.0023
Citrusdal	14	-0.011	0.0194	0.0052	0.0030	0.0167	0.0045	0.0040	0.0128	0.0034	-0.0019	0.0101	0.0027	0.0027	0.0080	0.0021
Mbombela	14	0.0023	0.0261	0.0070	0.0035	0.0163	0.0043	-0.0067	0.0099	0.0026	0.00004	0.0079	0.0021	-0.0040	0.0088	0.0024
		df	F ratio	P	df	F ratio	P	df	F ratio	P	df	F ratio	P	df	F ratio	P
Variance		2	2.10	0.1356	2	1.83	0.1732	2	2.84	0.0706	2	0.42	0.6609	2	2.47	0.0977

Based on the non-overlap between error bars in Fig. 71, it is evident that the Citrusdal population differs from the Addo population using PC1 and PC2 for both the left and right valvae. For the left valve, the Addo population differs significantly from the other populations for PC3. All three populations differed significantly from each other for PC4 and Mbombela from the other two populations for PC5, however, it should be noted that PC3-PC5 together contributed less than 30% to the variation in shape. For the right valvae, PC3and PC5 of Mbombela differed significantly from the other two populations. PC4 showed no significant difference for any population. Again it should be noted that PC3-PC5 accounts for less than 25% of the variation in the shape of the valvae. Comparing these to Fig. 69, it is evident that no clear clustering or pattern is formed, thus only limited variation exists.

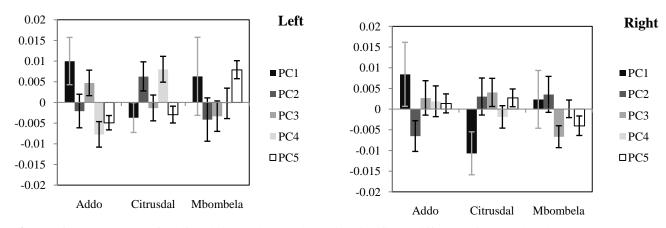


Figure 71: Means (\pm SE) for left and right valvae to determine significant differences in PC values between populations.

5.5 Discussion and Conclusion

The principle component scores significantly differed for only two out of 10 axes (Table 9), however, these two axes (PC4 and PC5 for the left valvae) contributed only 15% of the variation, insufficient to be described in standard morphological terms. By examining the mean variation in PC axes, certain significant differences were determined among populations, but no clear pattern emerged. The PC score plots (Figs 69-70) show overlap across all populations with no clustering that would allow distinction across the groups. Without any distinct morphometric differences among populations for both the left and right valvae, it is unlikely that male genitalia can be used to determine slight population differences, let alone if speciation events have occurred in *T. leucotreta*. In a study conducted by Gilligan & Wenzel (2008) on four populations of *Hystrichophora stygiana* (Dyar), high variation in valvar shape was demonstrated within a single population. Similarly, a study on the butterfly *Maniola jurtina* found extreme variability in the valvae of individuals from different populations (Goulson, 1993). Male genitalia are influenced by sexual selection and can evolve at a faster rate than many other traits, thus, genital traits are not always as strongly constrained (Lorkovic, 1953 *in* Goulson, 1993; Eberhard, 1985). The same argument could be used to explain the limited

variation in valvae observed in this study. Although Timm *et al.* (2010) found evidence that populations of *T. leucotreta* from different geographic locations were genetically distinct; no evidence for population-level divergence was found in the morphology of the valvae of the male genitalia. If genitalia diverge and evolve more rapidly than any other body part (Eberhard, 2010a, 2010b), variation among populations would have provided morphological evidence that speciation has occurred.

The results may be explained, in part, by "lineage separation" (Fig. 72) (de Queiroz, 2007). De Queiroz (2007) proposed that a single species can split into two lineages over time (grey gradient) as they acquire different properties (horizontal SC lines). This split can be the result of various biological, ecological, evolutionary or other factors (de Queiroz, 2007). It has been assumed that geographic barriers have resulted in genetically distinct populations of *T. leucotreta* (Timm *et al.*, 2007). Recently, variation in wing colouration has also been seen in *T. leucotreta* (see Chapter 4), which could indicate that *T. leucotreta* has not fully undergone speciation because no clearly definable variability has been found, and interbreeding in geographically isolated populations is still possible. This may be an indication that *T. leucotreta* is situated in the grey zone of Fig. 72, having undergone slight genetic and morphological divergence but not enough to be recognized as clearly separate lineages.

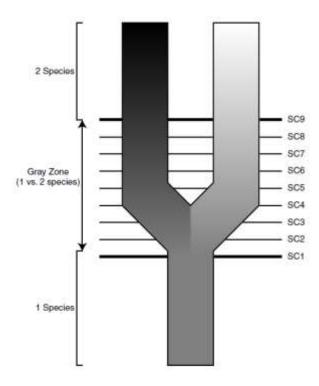


Figure 72: A single species can split into two lineages over time (grey zone) as they acquire different properties (horizontal SC lines) (Taken from de Queiroz, 2007, Fig. 1).

These results are of significance for alternative pest management practices such as sterile insect technique (SIT) and monitoring systems using sex pheromones. SIT relies on the mating of sterile males from the rearing facility in Citrusdal with wild females in areas throughout the Western Cape

Province. If the male genitalia of the wild population used for SIT differ from those of a different wild population in a different geographic location, the SIT program could fail due to mating incompatibility. For the present, it can be concluded that *T. leucotreta* of different populations are able to mate with each other and therefore without major implication on alternative pest management programs. From these results it is recommended that *T. leucotreta* populations be monitored over time, and that several factors be taken into account to determine if speciation events take place in future, as suggested by de Queiroz (2007).

5.6 References

- Arnqvist, G. (1998). Comparative evidence for the evolution of genitalia by sexual selection. *Nature*, **393** (25):784-786.
- Daly, H.V. (1985). Insect morphometrics. Annual Review of Entomology 30: 415-438.
- Dampf, A. (1908). Über den Genitalapparat von *Rhopobota naevana* Ha. (Lep., Tortricidae) nebst Bemerkungen zur Systematik der Olethreutinae. *Deutsche Entomologische Zeitschrift Iris* **21**: 304-329.
- de Queiroz, K. (2007) Species Concept and Species Delimitation, Systematic Biology 56 (6): 879-886.
- Eberhardt, W.G. (1985). *Sexual selection and animal genitalia*, Harvard University Press, Cambridge, Massachusetts, 244 pp.
- Eberhardt, W.G. (2010a). Evolution of genitalia: theories, evidence, and new directions, *Genetica* **138**: 5–18.
- Eberhardt, W.G. (2010b). Chapter 4, Rapid Divergent Evolution of Genitalia Theory and Data Updated. pp. 40-78 In: Leonard, J.L. & Córdoba-Aguilar. (2010). *The Evolution of Primary Sexual Characters in Animals*. Oxford University Press, Inc, New York, 537 pp.
- Goulson, D. (1993) Variation in the genitalia of the butterfly *Maniola jurtina* (Lepidoptera: Satyrinae). *Zoological Journal of the Linnean Society* **107** (1): 65-71.
- Gilligan, T.M & Wenzel, J.W. (2008) Extreme intraspecific variation in *Hystrichophora* (Lepidoptera: Tortricidae) genitalia questioning the lock-and-key hypothesis, *Annales Zoologici Fennici*, **45**: 465-477.
- Heinrich, C. (1923). Revision of North American moths of the subfamily Eucosminae of the family Olethreutidae. *Bulletin of the United States National Museum* **132**: 1-128.
- Horak, M. (1984). Assessment of taxonomically significant structures in Tortricinae (Lep., Tortricidae), *Mitteilungen der Schweizerischen Entomologischen Gesellschaft* **57**: 3-64.

- Iwata, H. & Ukai, Y. (2002). SHAPE: A computer program package for quantative evalution of biological shapes based on elliptic Fourier descriptors. *Journal of Heredity*, **93**: 384-385.
- Kennel, J. (1908) Die Palaearkischen Tortriciden [1. Teil] Zoologica 54, 727 pp.
- Lorkovic, Z. (1953) L'accouplement artificiel chez les Lepidoptères et son application dans les recherches sur la fonction de l'appareil genital des insectes. *Physiologia comparata et oecologia*, **3**: 313-320.
- Meyrick, E. (1895). *A Handbook of British Lepidoptera*. Macmillan and Co, London and New York, 743 pp.
- Mutanen, M. & Pretorius, E. (2007). Subjective visual evaluation vs. traditional and geometric morphometrics in species delimitation: a comparison of moth genitalia. *Systematic Entomology*, **32**:371-386.
- Opoku-Debrah, J.K & Moore, S.D. (2008). Geographic variation in the susceptibility of *Thaumatotibia leucotreta* (FCM) populations to a granulovirus (CrleGV-SA) in South Africa. *Citrus Research International (CRI), Group Summarised Annual Research Report* pp. 104-110.
- Pierce, F. N. & J. W. Metcalfe. (1922). The genitalia of the group Tortricidae of the Lepidoptera of the British Islands. Oundle, Liverpool, England, 101 pp.
- Polihronakis, M. (2006). Morphometric Analysis of Intraspecific Shape Variation in Male and Female Genitalia of *Phyllophaga hirticula* (Coleoptera: Scarabaeidae: Melolonthinae). *Annals of Entomological Scoceity of America* **99** (1): 144-150.
- Powell, J.A. (1964). *Biological and taxonomic studies on tortricine moths, with reference to the species in California*, University of California Press, Berkeley and Los Angeles, pp. 317.
- Timm, A.E., Geertsema. H. & Warnich. L. (2010). Population genetic structure of economically important Tortricidae (Lepidoptera) in South Africa: a comparative analysis. *Bulletin of Entomological Research* **100**: 421-431.

Conclusion

The primary goal of this study was to establish a user-friendly tool to aid the identification of the seven major tortricid pests in the South African fruit industry: *Cydia pomonella, Thaumatotibia leucotreta, Grapholita molesta, Cryptophlebia peltastica, Thaumatotibia batrachopa, Epichoristodes acerbella* and *Lozotaenia capensana*. The specific objectives were to 1) collate and analyse all available literature on the aforementioned seven species and to supplement this information with new morphological analyses to establish more comprehensive descriptions; 2) develop an interactive key based on LUCID software based on morphological data of local and potential invasive tortricid moth species for use by fruit industry stakeholders; and 3) using shape morphometrics of the male genitalia, determine whether geographically isolated populations of *T. leucotreta* can be distinguished.

1) Identification of all life stages

Correct identification of species, especially economically important pests of crops, is of utmost importance. Accurate morphological descriptions are necessary to facilitate such identifications. For the adults of the seven pest species, morphological descriptions are available in the literature. However, for the larvae and pupae of *C. pomonella*, *T. leucotreta*, *G. molesta*, *C. peltastica*, *T. batrachopa* and *E. acerbella* the literature is incomplete; and for *L. capensana*, there are no published descriptions of the early stages. The main objective of this part of the study was to collate all available literature supplemented by new morphological studies of the different life stages of these tortricids to develop comprehensive descriptions of the larval stages for comparison and identification purposes (Chapter 2-4). No larval specimens of *L. capensana* were obtained for this study, and the larval morphology of this species remains vacant.

There are distinct differences in the larvae of the remaining species. The main characteristics used for larval identification are: body and pinaculum colour; markings on the prothoracic and anal shields; the position of the L-group on T1, and whether or not the pinaculum extends below the spiracle; the position of the spiracle on A8 (i.e., whether it is located in the anterior two-thirds or posterior third of the segment); the number of setae in the SV group on A1, 2, 7, 8, and 9; whether or not D¹ and SD¹ share a common pinaculum on A9; D²s sharing a pinaculum ("saddle") on A9; L-group trisetose on one pinaculum or trisetose on separated pinaculum on A9; and the presence or absence of an anal comb. A complete list of characteristics is summarized in Chapter 2, Table 4.

The pupae of *L. capensana* are described for the first time. Tortricinae pupae can be separated from those of Olethreutinae by the presence of a cremaster in Tortricinae. The shape of the cremaster, the antenna, the labial palpus, the spiracle and spines enable separation between Tortricinae and Olethreutinae. Variation among the morphological details of pupae of different species of Olethreutinae is subtle and laypersons are unlikely to recognize these differences without a microscope. Certain minor differences are: sexual dimorphism in the antennae and spines of males;

length of the outer clypeal setae compared to the inner setae; the length of the maxilla compared to the labial palpus; and the number of spines on A5-A9.

Of the seven species, the adult stages are the easiest to distinguish, the wing pattern providing the most reliable feature for identification. The antennae, labial palpi, fore and hind legs, different structures on the hindwings, and genital segments also can be used for identification purposes. Dissections of genitalia might be difficult for inexperienced users, however, these structures should be used by specialists because they provide compelling confirmation of the species' identity.

2) Development of an interactive key

The aim of this section was to use the information compiled in Chapters 2-4 to develop an interactive key, using Lucid 3, which can be used by workers in the fruit industry (Appendix 1). Identification keys for the larvae and adults were developed, using the most important and most easily recognized characteristics. However, no key was developed for the pupae as their morphological details are mostly inconspicuous and/or difficult to observe. The larval and adult keys have been developed in such a manner that users with limited experience should be able to use and follow them; however, some taxonomic background is advisable.

3) Species status of *T. leucotreta*

Because recent studies have demonstrated that geographically isolated populations of *Thaumatotibia leucotreta* are genetically distinct, the question has arisen whether or not these populations are distinct or at least incipient species. This is an important issue in need of resolution, especially with regard to alternative pest management strategies such as the Sterile Insect Technique. Male genitalia are considered to be a reliable way of determining at what stage of speciation or lineage separation (Chapter 5. Figure 72) *T. leucotreta* may be experiencing. Although molecular evidence indicated that populations of *T. leucotreta* from different geographic locations were genetically distinct, no evidence for speciation was found in the morphology of the valvae of the male genitalia. This indicated that *T. leucotreta* males of different populations should be able to interbreed with females of different locations, barring the occurrence of other specific mate recognition systems.

This study provides additional information on the morphology of tortricid moth pests in the Western Cape and a more visual, interactive key for making accurate identifications. Furthermore, additional evidence is presented regarding the species status of *T. leucotreta*, confirming that, at this stage, pest management practitioners can still consider this moth as being a single species.

Future investigations

Further research should focus on obtaining details on the larval morphology of *L. capensana*. In terms of speciation of *T. leucotreta*, cross-mating trials and or more geometric morphometric analyses using different sections of the genitalia might be of benefit. *Epiphyas postvittana* and *Lobesia botrana* are of utmost concern to the South African fruit industry as potential invasive species and extensive monitoring for these two tortricids should be carried out and maintained.

Appendix 1

1. Introduction

Interactive matrix keys, such as Lucid keys, enable the user to select more than one character to examine, or to omit characters which are not conspicuous to them, and still reach a positive identification (Amante & Norton, 2003; Walters & Winterton, 2007). The Lucid3 keys provided here have been developed to assist the fruit industry stakeholders to distinguish among seven South African tortricids of economic importance. Keys have been developed for tortricid larvae and adults, but pupae were excluded as the diagnostic characters of pupae are often extremely subtle. In addition, two potential invasive species, *Epiphyas postvittana* (Light brown apple moth) and *Lobesia botrana* (European grape berry moth), have been included in the keys. Short morphological summaries of these two species are given in this chapter. For uniformity, the keys are based on TortAI Adult Key – CAPS program, and TortAI Larval Key – Port Intercepts, compiled by Gilligan & Epstein (2012).

2. Instructions

Features can be chosen in any order, it is not required to select all features and the user is able to skip features, if unsure. The user is successful if one species remains in the entities pane (right hand side). However, this is not always possible, and in the latter case the user is referred back to Chapter 2 for the larval and Chapter 4 for adult morphological characteristics. If all taxa (entities pane) are discarded, it could mean that either the specimen in hand is not represented in the key or that during the keying process, a mistake was made.

For detailed instructions on how to use the keys, see:

http://idtools.org/id/leps/tortai/larvalinstructions.html or http://aphid.aphidnet.org/how to use.php.

3. Morphological summary of two possible invasive species

Epiphyas postvittana (light brown apple moth/ LBAM)

LBAM classified as Tortricinae: Archipini (Venette *et al.*, 2003; Brown *et al.*, 2010). It is native to southeastern Australia, but has successfully invaded New Zealand, the USA, and Europe. LBAM is polyphagous and has been recorded on more than 500 plant species including pome and stone fruits, citrus, avocado, macadamias, grapes and strawberries (Suckling & Brockerhoff, 2010). LBAM feeds on leaves, shoots and the surface of fruit (Wearing 1991; Brown *et al.*, 2010; Suckling & Brockerhoff, 2010). Morphological characters are summarized in Table 10-12. Figure 73 shows the variation in forewing pattern, the pupae, and in early and final instar larvae of *E. postvittana*.



Figure 73: *Epiphyas postvittana* **A)** Forewing pattern; **B)** Pupa; **C)** Early instar larva; **D)** Final instar larva (Photos from Gilligan & Epstein, 2012).

Lobesia botrana (grape vine moth, EGVM)

EGCM is classified as Oletreutinae: Olethreutini (Roehrich & Boller, 1993; Gilligan *et al.*, 2011). It is native to the Palearctic region and has been introduced to eastern Asia, Africa and northern and southern America. EGVM is a polyphagous pest feeding on more than 40 plant species including carnations, persimmons, olives, pomegranate, grapes and some pome and stone fruits (Gilligan *et al.* 2011). First instars feed on flower buds, and later instars usually feed on fruit (Roehrich & Boller, 1993; *Gilligan et al.*, 2011). Morphological characters are summarized in Table 10-12. Figure 74 shows three lifestages of *E. postvittana*.

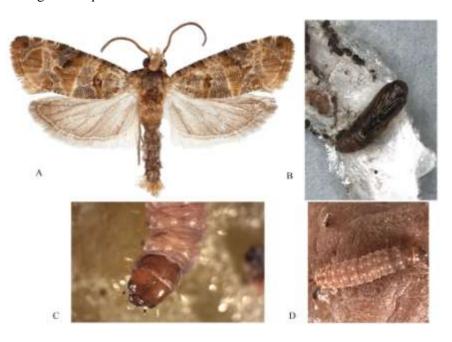


Figure 74: Lobesia botrana A) Adult (male); B) Pupa; C & D) Larvae (Photos from Gilligan & Epstein, 2012).

Table 10: Summarized larval morphological characteristics for *E. postvittana* and *L. botrana* (Venette *et al.*, 2003; Brown *et al.*, 2010; Gilligan *et al.*, 2011).

General	E. postvittana	L. botrana
Size	10-20 mm length	10-15 mm length
Colour	Yellowish green	Yellowish green to pale brown
Setal pinacula colour	Paler than surrounding integument	
<u>Head</u>	E. postvittana	L. botrana
Head colour	Pale brown	Light yellowish brown to light yellow brown to honey colour
Head pigmentation	Genal dash absent or present.	Dark stemmatal area and genal dash
Adfrontals	Sharply pointed	
P ¹ position to Adf1 & P1	Equidistant	Equidistant
Mandibles	2 inner teeth (sometimes only 1)	No inner teeth or retinaculum
<u>Thorax</u>	E. postvittana	L. botrana
Prothoracic shield colour	Pale brown, slightly darker than rest of integument.	Variable shaded – dark to black
Prothoracic shield pattern	Unmarked	Posterior & lateral margin shadings
L group pinaculum on T1		Not extending below spiracle
SD shape on T2 & T3	Rounded	
SV group on T1 & T2	Unisetose	
Thoracic legs	Pale brown	Brown to black
<u>Abdomen</u>	E. postvittana	L. botrana
$SD^2 A1-A8$	Fused to SD ¹	Absent
L group on A9 A9 D ¹ & SD ¹		Trisetose
A9 D ¹ & SD ¹		On same pinaculum
A9, D ² s position		Sharing single pinaculum forming a "saddle"
V ¹ on A9 compared to A8	Equidistant	Further apart
Anal comb	Present	Present
Number of prongs	7-9 prongs	5-8 prongs
Anal shield colour	Pale brown	Light brownish yellow
D¹s distance compared to SD¹	Closer to SD ¹ than each other	Closer to SD ¹ than each other
Proleg crochets arrangement		Biordinal, circle
Crochets number		35
Anal proleg crochets		Biordinal
arrangement		Dividinal
Crochet numbers	24-32	25

Table 11: Summarized pupal morphological characteristics for *E. postvittana* and *L. botrana* (Venette *et al.*, 2003; Brown *et al.*, 2010; Gilligan *et al.*, 2011)

General	E. postvittana	L. botrana		
Length (male & females)	7.6 mm & 9.8 mm respectively	5.5 & 7.0 mm, respectively		
Colour	Greenish brown to reddish- dark brown	Greenish brown – dark brown		
Cremaster	Present	Absent		
<u>Head</u>	E. postvittana	L. botrana		
Head projections	Absent	Absent		
Clypeal setae	2 pairs	2 pairs		
Maxilla/ labial palpi index	2.4-2.7			
<u>Thorax</u>	E. postvittana	L. botrana		
Prothoracic legs	Extends past femur by more than half the length of mesocoxae			
Abdomen	E. postvittana	L. botrana		
Dorsal abdominal pits	Absent			
Spines between D ² on A4-5		22-24 spines between D ² setae		
Anal rise		No setae		
Dorsal spines on A10		Patch or weakly defined row of spines		
Spine sizes		Increasing to A10		
Cremaster setae		Absent		

Table 12: Summarized adult morphological characteristics for *E. postvittana* and *L. botrana* (Brown *et al.*, 2010; Gilligan *et al.*, 2011)

Forewings	E. postvittana	L. botrana
Size	♂: 5.3-11.1 mm; ♀: 5.4-12.5 mm	4.5-8.5 mm
Wing pattern	Sexually dimorphic; Colour varies from rust-brown to pale yellow with brown markings; Males more variable than females; although the basal half slightly to markedly paler; the median fascia well defined, and a dark mark on the costa distal to the median fascia. Specimens could have a pale basal half and darker distal half. Female colours more uniform, with poorly defined fascia and more speckled appearance. Speckled underside	Little variation & no sexual dimorphism. Cream; interfacial areas overlaid with leaden grey; costal strigulae cream; fasciae brown to dark brown; subbasal fascia with black scaling medially; median facia with triangular medial projection often suffused with black scaling; postmedian fascia broken, forming pretonal patch along dorsum with cluster of black scales; postmedian band forming large brown patch along termen; apex often with conspicuous black dot; termen outlined in cream
Costal fold	Present in males, with flared scale fringe along distal half	Absent
Hindwings	E. postvittana	L. botrana
Males	Mottled with dark speckles	Whitish with a brown periphery
Females	Mottled with dark speckles	Brown

4. LUCID KEY - CD

5. References

- Amante, V. dR. & Norton, G.A. (2003). Developing interactive diagnostic support tools for tropical root crops. Third Taro Symposium, Proceedings of an International Scientific Meeting jointly organized by the Secretariat of the Pacific Community and the International Plant Genetic Resources Institute, Fiji Islands.
- Brown, J. W., Epstein, M. E., Gilligan, T. M., Passoa, S. & Powell, J. A. (2010). Biology, identification, and history of the light brown apple moth, *Epiphyas postvittana* (Walker) (Lepidoptera: Tortricidae: Archipini) in California: an example of the importance of local faunal surveys to document the establishment of exotic insects. *American Entomologist*, **56** (1): 34-43.
- Danthanarayana, W. (1975). The bionomics, distribution and host range of the light brown apple moth, *Epiphyas postvittana* (Walk.) (Tortricidae). *Australian Journal of Zoology*, **23**: 419-437.
- Dugdale, J.S., Gleeson, D., Clunie, L.H. & Holder, P.W. (2005). A diagnostic guide to Tortricidae encountered in field surveys and quarantine inspections in New Zealand: Morphological and Molecular characters. Ministry of Agriculture and Forestry, Wellington, New Zealand, pp. 24-28.
- Gilligan, T. M. & Epstein, M. E. (2012). TortAI, Tortricids of Agricultural Importance to the United States (Lepidoptera: Tortricidae). Identification Technology Program (ITP), USDA/APHIS/PPQ/CPHST, Fort Collins, CO. [accessed at http://idtools.org/id/leps/tortai/].
- Roehrich, R. & Boller, E. (1991). Tortricids in vineyards, pp. 507-514. In L. P. S. van der Geest and H. H. Evenhius [eds.], *Tortricid Pests: Their Biology, Natural Enemies, and Control.* World Crop Pests, Vol. 5. Elsevier, Amsterdam, 808 pp.
- Suckling, D. M. & Brockerhoff, E. G. (2010). Invasion biology, ecology, and management of the light brown apple moth (Tortricidae). *Annual Review of Entomology*, **55**: 285-306.
- Venette, R. C., Davis, E. E., DaCosta, M., Heisler, H. & Larson, M. (2003). Mini risk assessment. *Lobesia botrana* (Walker) [Lepidoptera: Tortricidae]. USDA/APHIS/PPQ Pest Risk Assessment.Dept of Entomology, University of Minnesota, Saint Paul.
- Walters, D. E. & Winterton, S. (2007). Keys and the Crisis in Taxonomy: Extinction or Reinvention? *Annual Review of Entomology*, **52**: 193-208.
- Wearing, C. H., Thomas, W. P., Dugdale, J. W. & Danthanarayana, W. (1991). Tortricid pests of pome and stonefruits, Australian and New Zealand species., pp. 453-472. In L. P. S. van der Geest and H. H. Evenhius [eds.], *Tortricid Pests: Their Biology, Natural Enemies, and Control. World Crop Pests, Vol. 5.* Elsevier, Amsterdam, 808 pp.