An analysis of the stag beetle genus Lissotes in Tasmania

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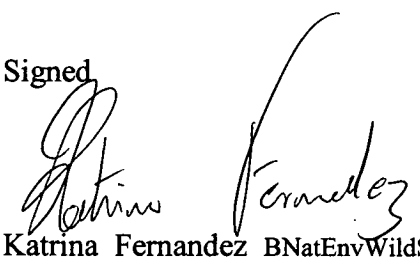
Declaration

This thesis contains no material which has been accepted for the award of any other degree or diploma in any tertiary institution, and to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

Signed



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Last but not the least, to my family, who have helped me persevere thus far. Without your strength, support and love I would not have been able to do this. Thank you all so very much.

Abstract

Little is known about the extraordinarily rich Tasmanian beetle genus *Lissotes*, belonging to the ancient Stag Beetle family Lucanidae. No less than 25 described species are known to be endemic to Tasmania, with three species known in Victoria. In Tasmania a number of rare *Lissotes* beetles are threatened due to land clearing, including forest practices and other anthropogenic effects. Potential forestry threats to one species, *L. latidens*, are subject to current challenge in the Federal Court.

The present taxonomy of the genus dates back to the last century and is based on a limited range of morphological characters. Some of these characters such as mandible size are sex-linked, subject to allometry, and are otherwise unreliable. My research aimed to bring modern methods of species discrimination to bear on this question. This study involved using a new suite of morphological characters (e.g. labrum and scutellum) as well as DNA molecular sequence data (Cytochrome b gene).

However, technical difficulties precluded good resolution in the DNA data but new insights were gained from the morphological research. These DNA approaches deserve further investigation in the future.

The study also provided an overview of the biology and distribution of the species. In doing so a more comprehensive understanding of the genus has been provided which will aid in future conservation efforts.

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Introduction

Stag beetles (family Lucanidae) are one of the most ancient beetle families of the order Coleoptera with over 1,250 species representing about 95 genera. Species are widely distributed in forests around the globe but are most abundantly found in the tropics. The Australian lucanid fauna currently comprises 17 genera and about 95 species (Moore, 1978). The small island of Tasmania is remarkable in that it supports one of the highest concentrations of stag beetle species in the world. At least 30 species of Lucanidae are present there, of which no fewer than 25 endemic stag beetles belong to the genus *Lissotes*. This genus is primarily confined to Tasmania, but three are known from wet forests in Victoria.

Interest in *Lissotes* dates from the late eighteenth century when the first specimens from southern Tasmania came to the attention of European science. Numerous species were described over the following 150 years based on comparisons of a limited range of morphological characteristics. However, there is known to be a large degree of variation in morphological features between individuals of many lucanid beetles. This in large part is related to pronounced allometry, and hence the present taxonomy is unlikely to be accurate and has been a matter of contention amongst scientists for many years. The development of an agreed phylogeny within the family has been greatly retarded by the proliferation of named taxa without adequate scientific description and differentiation.

The conservation of this important element of the Tasmanian fauna is fraught with difficulties. Today, anthropogenic alterations to their natural habitat continue to result in the decline in their numbers. The opportunity to alleviate the potential negative effects on these species has been hindered by a lack of knowledge *vis a vis* species limits. In Tasmania, land use practices, including forestry practices, may have negative effects on the future of *Lissotes* species.

Objectives

This study aims to address a number of these pressing issues. It will firstly review the current state of the taxonomy and provide an overview of *Lissotes* morphology, biology, and distribution. Then I will address aspects of its phylogenetics using contemporary scientific methods in an attempt to clarify the relationships among and between species as an aid to conservation planning.

In Chapter one is an overview on history and biology of beetles and in particular

Lissotes, based on past research. Chapter Two presents the methods used in my study, both the molecular as well as morphological components. Chapter Three presents the results of my work and Chapter Four will encompass the discussion. Chapter five is the image section of the thesis and will have maps and images of distribution and species respectively. The thesis will conclude with a restatement of the major findings.

Chapter 1

1.0 Coleoptera

The beetle order Coleoptera is the largest order of insects with about 350,000 described species (Gullan and Cranston, 2005). Within this there are four extant lineages (sub-orders): Archostemata, Myxophaga, Adephaga, and Polyphaga in evolutionary sequence. The latter contains the vast majority of beetle diversity and amounts to more than 90% of the species, including the Lucanidae.

The key feature of the adult beetle is the modification of its forewings into sclerotized rigid elytra. These elytra extend over the body to cover the metathorax and most of the abdominal segments. It is also beneath these elytra that the hind wings are elaborately folded at rest (Gullan and Cranston, 2005). The elytra may also cover the abdominal spiracles allowing the control of water loss. The hind wings are longer than the elytra when extended for flight and have comparatively less venation.

Beetle biology is highly diverse, and includes predation, herbivory and saprophagy as lifestyles. Their development is holometabolous with a variable number of larval instars depending on the group. There are three instars in Lucanidae and their relatives (Gullan and Cranston, 2005).

Beetle mouthparts are mandibulate. The compound eyes range from well developed to completely absent. In most species there are 11 segments on the antennae. Their prothorax is distinct, large and extends laterally beyond the coxae; the mesothorax is small and fused to the metathorax to form the wing bearing pterothorax. The legs are variably developed with coxae that are sometimes large and mobile. There are adaptations in the tarsi which allow for digging in soil or wood or for jumping depending on the species. The abdomen is usually nine segmented in females and 10 segmented in males with at least one terminal segment retracted. The sterna are very strongly sclerotized compared to the terga. The females have a substitute ovipositor while the male genitalia are trilobed (Gullan and Cranston, 2005).

1.1 The Stag Beetles (Family Lucanidae)

1.1.1 Adults

Stag beetles are usually black or brown in colour, but several (*Lamprima, Phalacrognathus*) are brightly metallic and glossy (The Insects of Australia, 1970). The adults are medium to large sized beetles, ranging from less than 1 cm to 9 cm in length. The body shape is usually weakly convex, sub-depressed, or cylindrical

(*Syndesus*). There can be scales (modified flattened setae) present on the dorsal surface.

Head

Lucanids generally have a transverse head characterised by elbowed antennae which have an open antennal club comprising of three or four lamellae, borne on a long scape. All have well developed mandibles that are sexually dimorphic in most species. In some males the elongated mandibles resemble the antlers of male deer hence the term stag beetles. Males use these mandibles to establish sexual dominance over other male opponents. The growth of male mandibles is typically allometric, where the size range of the mandibles in a population is not simply proportional to the body size (The Insects of Australia, 1970).

The eyes contain eucone or acone ommatidia and an eye canthus can be present or absent. The maxillae support up to four segmented palpi and the labium has a pair of palpi with up to three segments (pers. obs.).

Thorax

The pronotum is variably convex, with or without tubercles. The elytra are weakly convex, and can be with or without impressed striae. The wings are usually well developed with apical, detached veins. The coxae of the legs are transverse with the mesocoxae separated. The protibia is dentate on its outer margin and the apex posesses one spur. The meso- and metatibiae have ridges and an apex with 2 spurs. The tarsi have five segments and a pair of claws that are identical (The Insects of Australia, 1970).

Abdomen

The abdomen has five or six visible sternites. There are eight functional abdominal spiracles situated in the pleural membrane. The scutellum is exposed, triangular or parabolic. The male genitalia comprise paired symmetrical parameres and a median lobe (Ratcliffe & Paulsen, 2005).

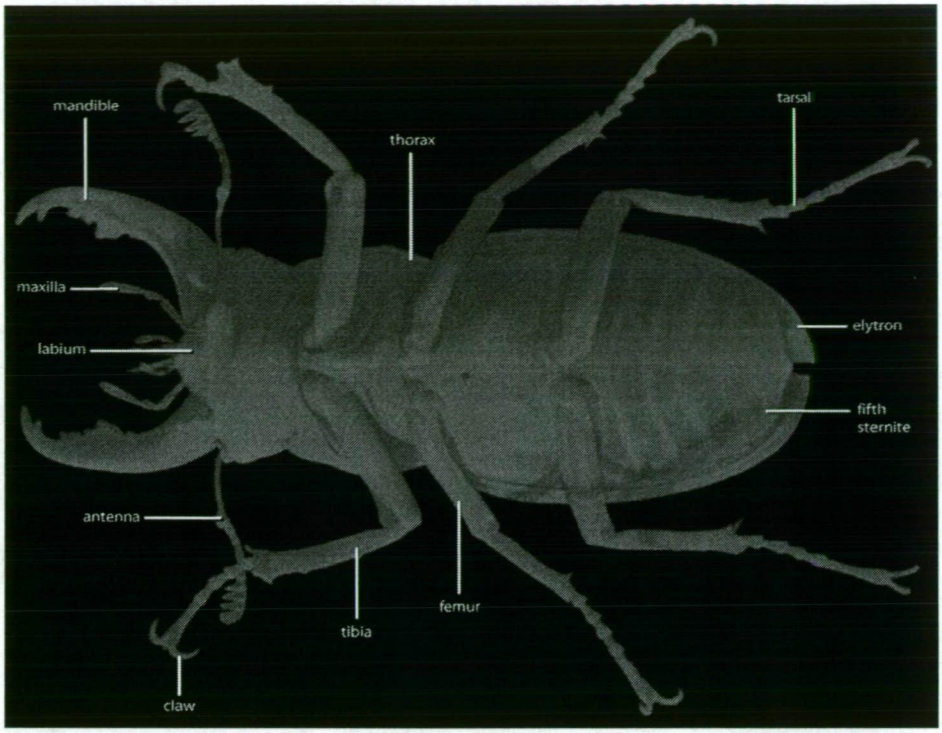


Fig. 1.1 showing the ventral view of *Lucanus* sp.,

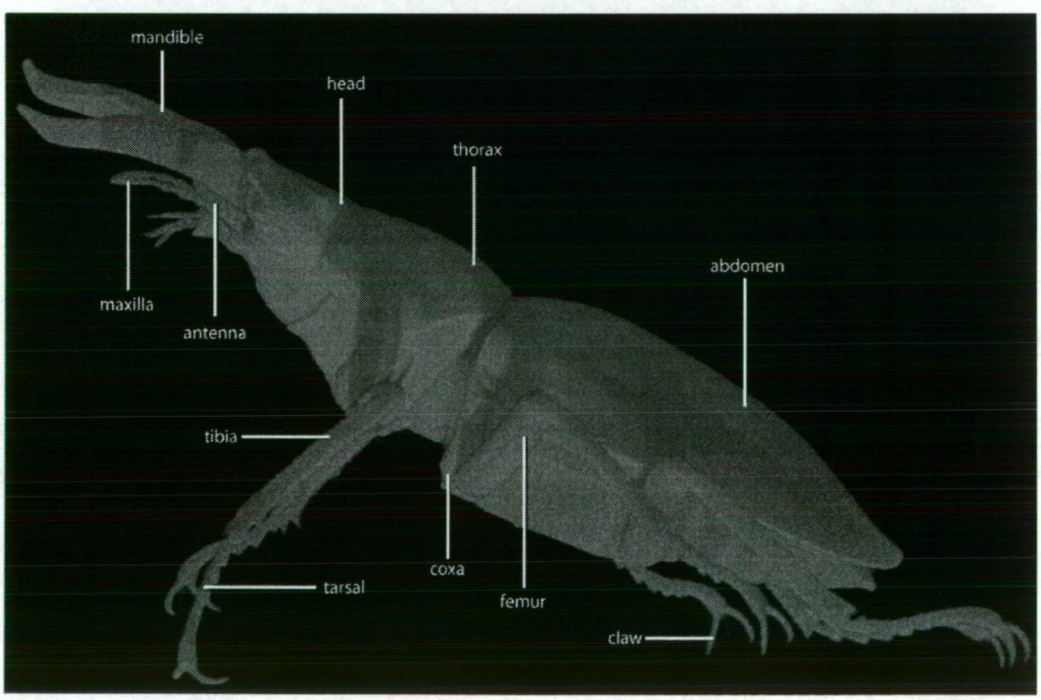


Fig. 1.2 showing the lateral view of *Lucanus* sp.,

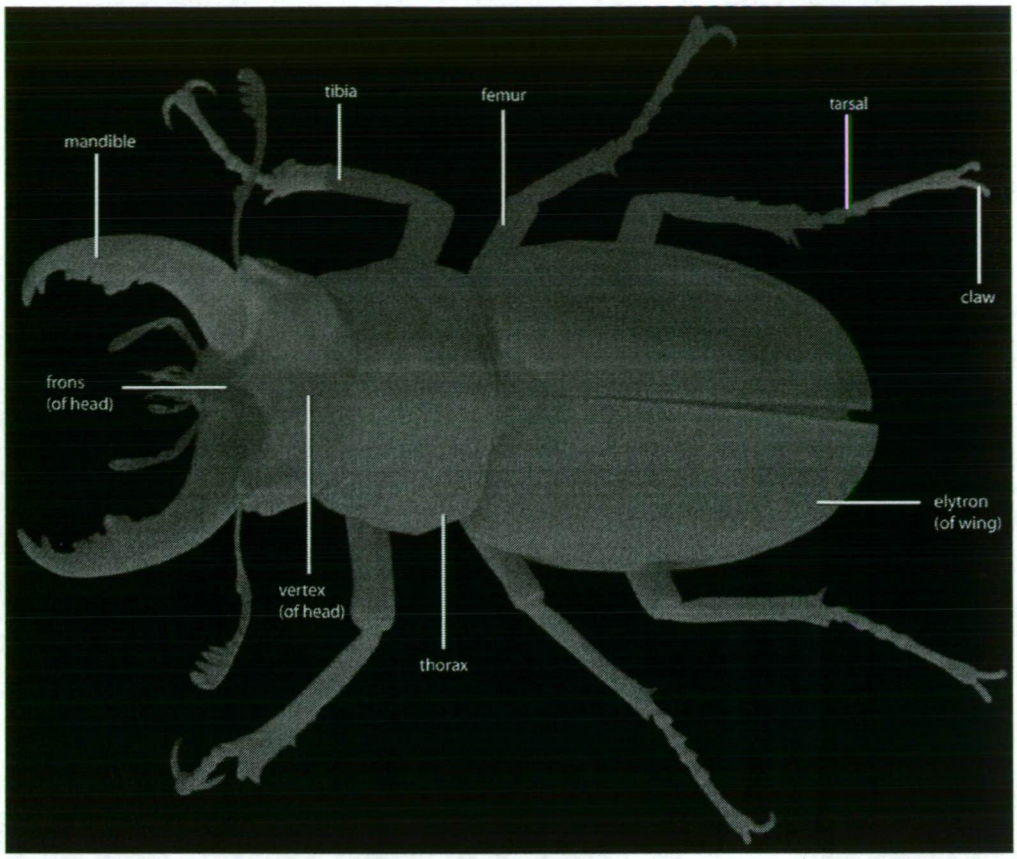


Fig. 1.3 showing the dorsal view of *Lucanus* sp.,

1.1.2 Larvae

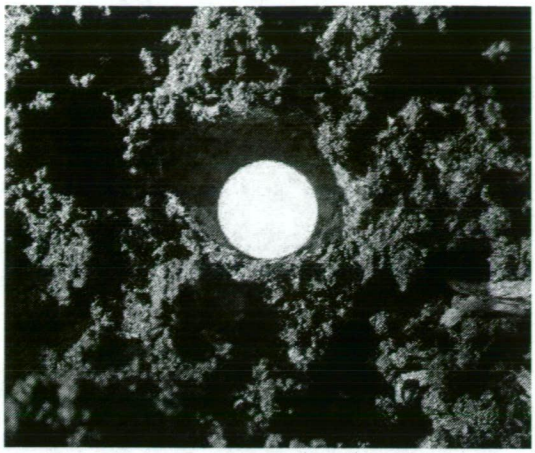
The eggs usually hatch in two to four weeks. There is no evidence of egg diapause in Tasmanian species. The larvae are scarabaeiform (sub-cylindrical and C-shaped), and are a creamy-white or yellowish colour. The cranium is heavily sclerotized and lightly pigmented. Antennae are three to four segmented with the last segment greatly reduced in size. The ocelli are absent. The frontoclypeal suture is present. Labrum at apex is rounded or weakly lobed as is the epipharynx, which bears symmetrical tormae. The maxilla has the galea and lacinia distinctly separate and maxillary stridulatory teeth are absent. The maxillary palpus is four segmented. The mandibles are elongate and asymmetrical. There are three to seven abdominal segments with two annuli, each with one or more transverse rows of short setae. The anal opening is Y-shaped or longitudinal, surrounded by two fleshy lobes. The legs are four segmented. A stridulatory apparatus is present on the meso- and metathoracic legs (Ratcliffe & Paulsen, 2005).

Across the family, larval stage duration is variable, from one to five years depending on the species, although probably one to two years in Tasmania. This stage is so long because of the slow development caused by the low nutritive quality of rotten wood

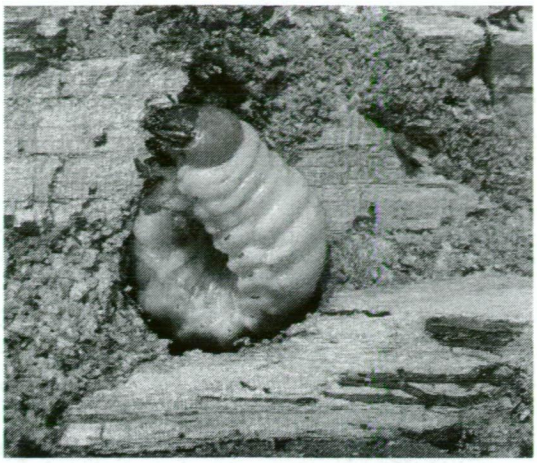
(low nitrogen content) which does not allow for rapid growth.

1.1.3 Pupae

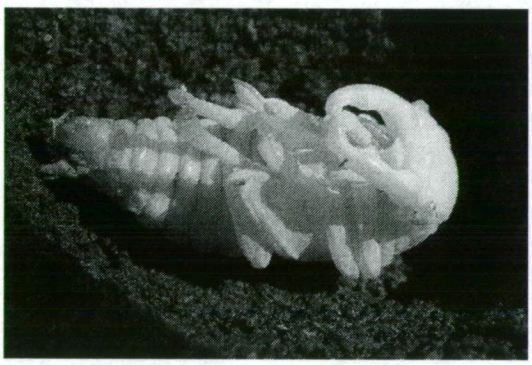
Pupation occurs in wood or in soil, near stumps, within a chamber built with wood pieces, soil and other materials stuck together with bodily secretions. It seems that metamorphosis occurs during autumn and that imagoes remain in the pupal chamber during winter (Ratcliffe & Paulsen, 2005).



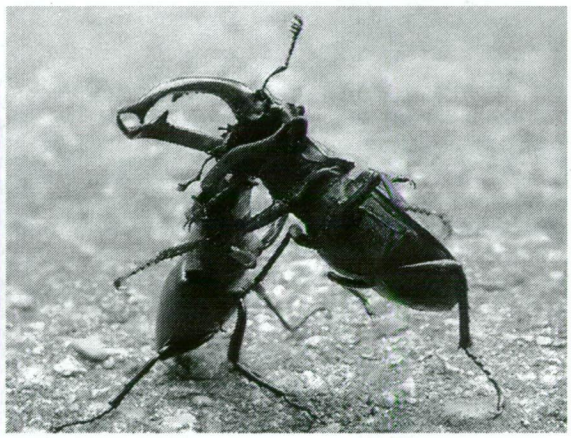
Egg



Larva



Pupa



Adult

Fig. 1.4 Generalised lifecycle of a stag beetle

1.2 A historical overview of the discovery and description of *Lissotes* in Tasmania

The discovery and description of Tasmanian stag beetles spans the history of modern biology. One of the first insects scientifically described from Australia was *Lissotes cancroides*, probably collected at Adventure Bay, Bruny Island in the 1770s on the second expedition to the Pacific of Captain Cook. It was described in 1787 by the Danish zoologist Johan Fabricius, a student of Linneaus (Fabricius, 1787).

Scientists on the French expedition of Jules Dumont D'Urville collected hundreds of insects from the Pacific area including, in December 1827, a stag beetle from near Hobart described as *Lissotes curvicornis* by Jean de Boisduval, the leading Paris-based entomologist of the day (Boisduval, 1835).

In all likelihood the next species brought to notice was collected by Charles Darwin in Hobart or Mount Wellington in February 1836. Darwin amassed a large beetle collection on the voyage of the *Beagle* most of which found its way to the Hope Museum at Oxford University where John Obadiah Westwood, a prominent beetle taxonomist, was Professor of Entomology. *Lissotes obtusatus* was the first of nine species of Tasmanian stag beetles that Westwood would describe over the next 33 years (Westwood, 1838).

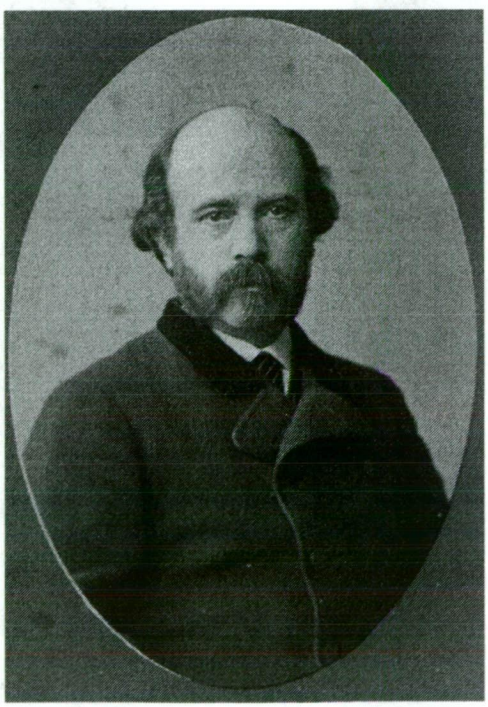
In 1910, a landmark paper by Arthur Lea, a Hobart based entomologist with the Agriculture Department, reviewed the known species and proposed six new species based on his research. This paper largely forms the basis of our present understanding of the genus.

In the last 20 years, interest has renewed in these animals on two fronts: discovery of new species and conservation policy. Since the 1980's two European scientists, Henri Bomans and Luca Bartolozzi, have published names for several new species (Bomans 1986, Bartolozzi 2003); however these are based on very small sample sizes and may not withstand more thorough scrutiny.

Johan Christian Fabricius 1745-1808

Jean de Boisduval 1801-1879

John Obadiah Westwood, 1805-1893



Henri Deyrolle

1.3 Genus Lissotes Westwood

1.3.1 The case for monophyly of *Lissotes*

Lissotes appears to be a well supported genus based on the following morphological evidence:

Lissotes has the following diagnostic features within the southern hemisphere Lucanidae (Holloway 1961, 1963a):

1. Incompletely divided eyes,

2. hooked laciniae in both sexes,

3. rounded (not truncate) third antennal club segment.

In a later paper she (Holloway 1996) expanded the diagnosis to include the following features (based on the genotype *L. menalcas* and *L. rudis*):

Major elytral vestiture.

Divided setae with the individual branches smooth-sided, cylindrical, and of similar length (Fig.1.5)

(cf *Paralissotes*: laminate, longitudinally ribbed, fan-shaped scales (Fig. 6—8))

Postocular margin of the head in males.

Strongly convex.

(cf *Paralissotes*: straight or very slightly convex)

Features of the female genitalia (Fig.1.5)

Bursa copulatrix

Much smaller than the accessory gland.

(cf *Paralissotes*: much larger than the accessory gland)

Internal surface of the bursa copulatrix

Not differently sclerotised in this area.

(cf *Paralissotes* strongly sclerotised on either side of the spermathecal duct

Insertion)

Spermathecal duct

Short.

(cf *Paralissotes*: long)

Features of the male genitalia (see Fig 1.5)

Tergite of the ninth abdominal segment

With a rounded margin (Fig 1.5)

(cf *Paralissotes* with an indented distal margin)

Permanently everted internal sac

With neither sclerites nor papillae (Fig 1.5)

(cf *Paralissotes*: with 2 pairs of sclerites externally on its apical half and with 1 or a pair of papillae about halfway along its length)

Penis

Narrow and without such lobes (Fig 1.5)

(cf *Paralissotes*: broad and with a pair of somewhat hemispherical, sclerotised, ventral lobes)

Base of the penis

With a plate-like dorsal crossbar and large dorsolateral processes (Fig 1.5)

(cf *Paralissotes*: with a rod-like dorsal crossbar and small dorsolateral processes)

Ventral surface of basal piece

Weakly sclerotised and colourless on its distal half (Fig 1.5)

(cf *Paralissotes*: uniformly sclerotised and pigmented)

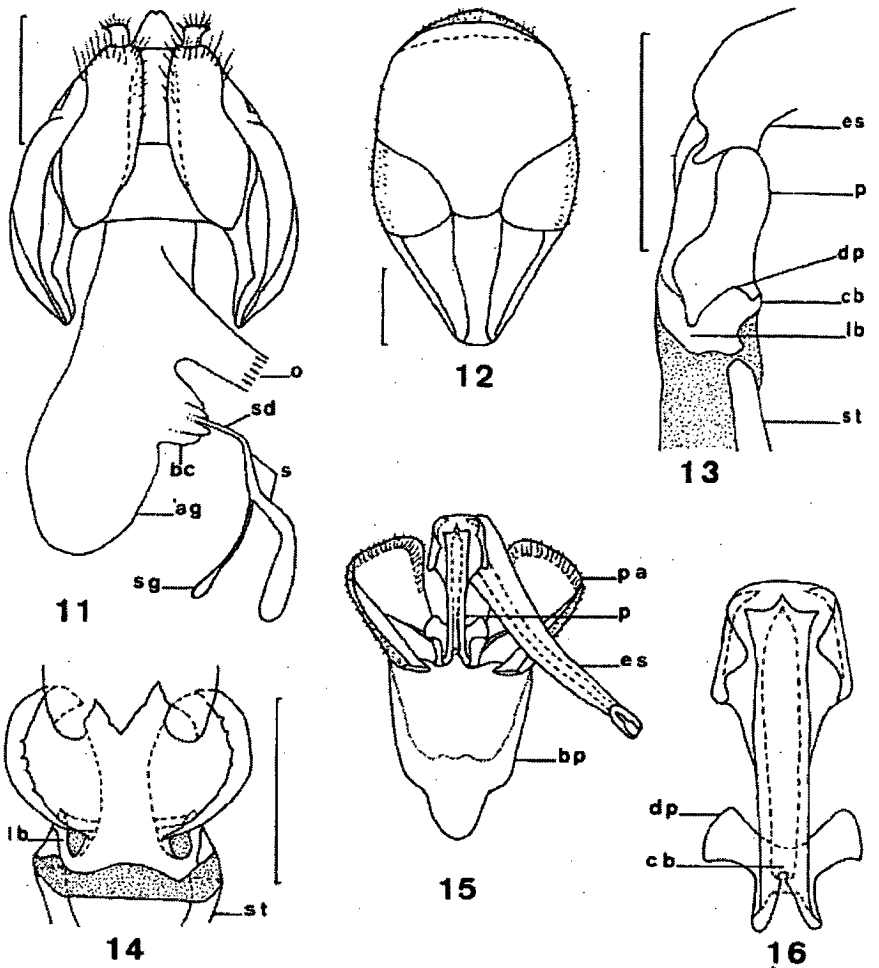


Fig. 11–16 Morphological features of *Paralissotes* and *Lissotes*. 11, Female genitalia (ventral) of *L. menalcas*. 12, Ninth abdominal segment (dorsal) of male of *L. menalcas*. 13, 14 Penis and surrounding structures (13, lateral; 14, ventral) of *P. reticularus*. 15, Male genitalia (ventral) of *L. menalcas*. 16, Penis (ventral) of *L. menalcas*. All scales lines equal 1.0 mm; Fig. 12, 13 drawn to same scale; Fig. 14, 16 same scale. Abbreviations as in Fig. 1–10.

Fig. 1.5 Genitalia of *Lissotes menalcus* compared to the related genus *Paralissotes* from New Zealand (after Holloway 1996).

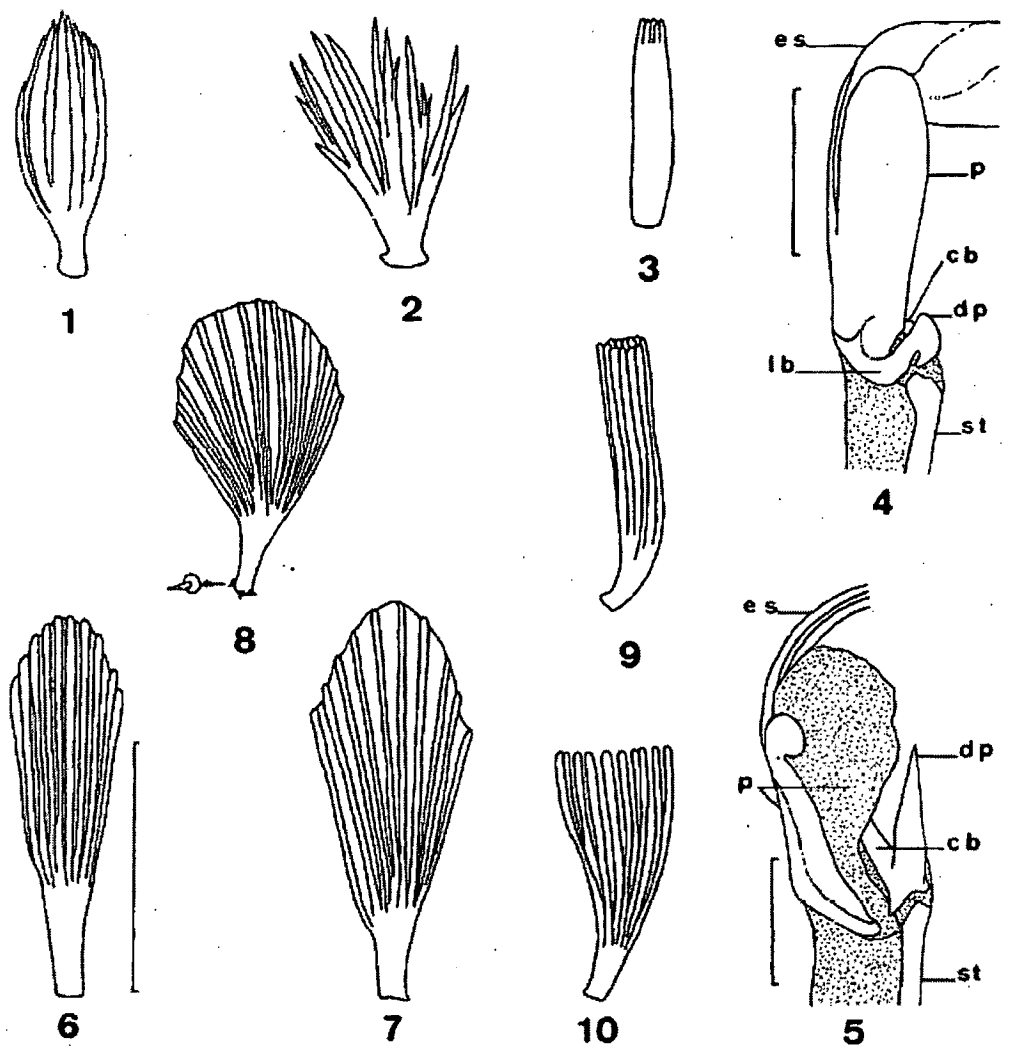


Fig. 1–10 Morphological features of *Geodorcus*, *Dorcus*, *Paralissotes*, and *Lissotes*. **1, 2** Elytral setae (1, not compressed; 2, compressed) of *G. novaezealandiae*. **3**, Elytral seta (compressed) of *D. parallelipipedus*. **4**, Penis and surrounding structures (lateral) of *G. novaezealandiae*. **5**, Penis and surrounding structures (lateral) of *D. parallelipipedus*. **6, 7** Elytral scales (6, not compressed; 7, compressed) of *P. reticulatus*. **8**, Elytral scale (compressed) of *P. stewarti* (Broun, 1881). **9, 10** Elytral setae (9, not compressed; 10, compressed) of *L. menalcas*. Lines beside Fig. 4, 5 equal 0.5 mm; line beside Fig. 6 equals 0.1 mm; Fig. 1–3 and 6–10 drawn to same scale. Abbreviations: ag, accessory gland; bc, bursa copulatrix; bp, basal piece; cb, dorsal crossbar; dp, dorsolateral process; es, permanently everted internal sac; lb, lateral bridge; o, median oviduct; p, penis; pa, paramere; s, spermatheca; sd, spermathecal duct; sg, spermathecal gland; st, strut. Stripple indicates membranous areas.

Fig. 1.6 Elytral setae of *Lissotes menalcas* compared to the related genera *Geodorcus* and *Paralissotes* from New Zealand (after Holloway 1996).

1.3.2 Relationship to other genera

Similarities in the gross form of the maxillae, eyes, antennae, wings, male and female genitalia, and body shape in *Paralissotes*, *Lissotes*, and *Pycnosiphorus* Solier, 1851 suggest that these three genera are closely related (Holloway, 1996) and reinforces the likelihood of a Gondwanan origin for them.

1.3.3 Differences between species within the genus

The most apparent differences in species in this genus relate to the shape of the male mandibles, the number of terminal lamellae on each antenna, the dorsal punctation and in the tibial dentition. Early authors, notably Lea (1910) recognised that some species were quite variable morphologically. Within some species, the mandibles are twice as large as they are on other individuals. A decrease in mandible size usually results in a decline in the number of cusps, or the cusps become less pronounced. When closed, these smaller mandibles can also look more robust. In small males, the head tends to look relatively smaller and the punctures on the head and prothorax are often larger and denser, resembling a female specimen (Lea, 1910). Larger mandibles however are associated with increased numbers of tibial teeth. The labrum is also subject to variation, especially in regard to its median prominence. Its shape is affected by the opening and closing of the mandibles and it is frequently obscured by dust and grease. Consequently, the labrum was of limited practical use in distinguishing amongst species of *Lissotes* (Lea, 1910).

This variability in morphology can give rise to the impression that there are many more species than there actually are. The consequence of this is that several of the same species have been described under different names and in all probability this mistake will continue to be made (Lea, 1910). I believe that nothing has changed in the century since Lea's insight, and new species continue to be described using unsatisfactory methods (e.g. Bomans, 1986).

1.4 Genetics

A mitochondrion contains DNA that is copied and passed down through the generations from mother to her offspring. Mitochondrial DNA contains the genes for some of the protein and ribonucleic acid (RNA) molecules needed for mitochondrial function. Because it is passed on from generation to generation it has the necessary information needed to conduct a phylogenetic study on any organism on the planet

(Ridley, 2004).

Cytochrome oxidase I

The COI gene is a good target, as it is present in all animals, in many identical copies per cell, and is known to evolve relatively quickly. It carries sufficient 'signal' to allow differentiation between closely related taxa, and in a survey of moths collected around Guelph, Ontario, show that the COI barcode can in most cases yield a reliable assignment to previously identified and sequenced species. Other insect specimens were correctly assigned to the superfamily level, and Hebert *et al* (2004) claim that in general the approach can identify which phylum a sequence derives from.

Cytochrome oxidase subunit I (COI) gene is used as a global biomagnification system of animal because of its many advantages, some of which are:

The universal primers for this gene are very robust, enabling recovery of its 5` end from representatives of most, if not all animal phyla (Folmer *et al.*,1994; Zhang *et al*, 1997).

CO1 appears to possess a greater range of phylogenetic signal than any other mitochondrial gene, its third position nucleotides show a high incidence of the substitution, leading to a rate of molecular evolution that is about 3 times greater than that of 12S or 16S rDNA (Knowlton *et al*, 1993).

Cytochrome b

The cytochrome b gene is one of the conserved genes in mitochondrial DNA. The nucleotide sequence of the COb gene generally has not been used in insect systematics, but it should be a candidate gene for studying phylogenetic relationships as it has been successfully used in other invertebrates. It is generally known that the 3rd codon of a COb sequence should not be used in a study as it is in most cases evolved to saturation (Yeh *et al*, 1998).

1.5 Vegetation types associated with *Lissotes*

This genus occupies diverse forest types, especially those dominated by *Eucalyptus obliqua*, *Eucalyptus regnans* and *Eucalyptus globulus*, which may occur either as extensive stands of wet forest or as patches in dry eucalypt forest (Bryant & Jackson, 1999). As least some *Lissotes* show a preference for forest with a well-developed overstorey and understorey, and greater than 10% ground cover of fallen dead wood (Meggs, 1999).

Dry Sclerophyll forest

Vegetation Structure

Open *E. obliqua/E. amygdalina* forest to 15m. Understorey genera include *Exocarpus* and *Banksia* and a shrub layer of *Epacris impressa*, *Pultenaea juniperina*, *Daviesia latifolia*. There can be a very sparse ground layer of *Gonocarpus teucroides* with high coverage of bare ground, rocks and litter (Reid *et al.*, 1999).

Dry eucalypt forests are identified by the dominance of eucalypts that are more than eight meters tall and associated with a multilayered understorey of shrubs that are adapted to dry conditions (xerophytic), by having hard and narrow leaves. Peppermints such as black peppermint (*E. amygdalina*) and silver peppermint (*E. tenuiramis*) typically dominate dry eucalypt forests (Reid *et al.*, 1999).

Understorey trees include wattles (*Acacia spp.*), sheoak and bulloak (*Allocasuarina spp.*) and native cherry (*Exocarpos cupressiformis*) (Kirkpatrick, 1991). It can be difficult to distinguish between wet and dry eucalypt forests, as there is often a gradual transition between the two and many species occur in both dry and wet forests.

Wet sclerophyll forest

Vegetation Structure

Discontinuing canopy of *E. obliqua* and *E. regnans*, suggesting fire events of reasonably long intervals. Canopy 60m in height with a 25-50% canopy cover

(Kirkpatrick, 1991).

Understorey of *Acacia sp.*, 25-30m in height and >75% coverage.

Ground cover, 3-4m and sparse consisting mainly of fern species (*Dicksonia antartica*)

The wet eucalypt forests of Tasmania and Victoria contain the tallest flowering plants in the world, the swamp gum, *Eucalyptus regnans*. Wet eucalypt forests are comprised of wet sclerophyll and mixed forest, and tend to be dominated by tall eucalypts, up to 90 metres high with a distinct understorey of broad-leaf shrubs. The understorey grows so densely, that very little light penetrates the forest floor, preventing the growth of eucalypt seedlings. The ash eucalypts, such as stringy bark (*Eucalyptus obliqua*), and gum-topped stringy bark (*E. delegatensis*) tend to dominate in wet eucalypt forests, with gums occurring as a sub dominant species (Reid *et al.*, 1999).

Chapter 2

2.0 Methods

2.1 Specimens

Living *Lissotes* beetles were collected in the summer and autumn 2006 from a number of forested locations in southern Tasmania. Large rotting logs were examined for the presence of specimens hiding beneath them during daylight hours. In addition, a range of specimens sampled within the last ten years ago were sourced from entomological collections, including that of Forestry Tasmania and the University of Tasmania. Molecular data is much more readily taken from recently collected material that has been well preserved, and the most recent material was collected from the field directly into vials of 100% ethanol. An attempt was made to bring together a reference collection of as many species as possible, however only ten species could be assembled for this relatively limited study. Some of the specimens were female and could not be identified because of the lack of description of these in the past.

2.2 Mapping

Distribution data was taken from the literature and from the labels accompanying preserved specimens, converted where necessary to spatial co-ordinates, and plotted on a map of Tasmania using ArcMap. These give a visual appreciation of the geographical range occupied by each species and emphasises their relative abundance. Included in the maps are rivers and lakes for reference.

Also provided are some vegetation maps to give a brief understanding of the types of forests that are habitat to these beetles. Maps were produced using the TASVEG data layer with permission of the Tasmanian Parks and Wildlife Services.

2.3 Morphological Methods

2.3.1 Character set

Morphological characters were chosen which were observed to vary across notional species, and which in many cases have been mentioned in descriptions of new

species by various authors. Character states are coded in brackets in the following list.

Head

Labium:

1. Punctations –

Absent (0); Present abundantly (1); Present sparsely (2); Present only on the margins (3).

2. Setae –

Absent (0); Present abundantly (1); Present sparsely (2); Present only on the margins (3).

3. Labial palps –

Absent (0); Present with > 1 (1); Present with 1 (2)

4. Maxilla –

Absent (0); Segments- Present with 3 or more (1); Present with < 3 (2)

Mandible:

5. Punctations –

Absent (0); Present abundantly (1); Present sparsely (2); Present only on the margins (3),

6. Setae –

Absent (0); Present abundantly (1); Present sparsely (2); Present only on the margins (3),

Eyes:

7. Dorsal view –

Absent (0); Present (1)

Labrum:

8. Dorsal view –

Present (0); Absent (1)

9. Punctations –

Absent (0); Present abundantly (1); Present sparsely (2); Present only on the margins (3).

10. Setae –

Absent (0); Present abundantly (1); Present sparsely (2); Present only on the margins (3).

Antennae:

11. Punctations on Segments –

Absent (0); Present abundantly (1); Present sparsely (2); Present only on the margins (3),

12. Setae on segments –

Absent (0); Present abundantly (1); Present sparsely (2); Present only on the margins (3),

13. Clubs –

Not fused (0); Fused (1)

14. Punctations on clubs –

Absent (0); Present abundantly (1); Present sparsely (2); Present only on the margins (3).

15. Setae on clubs –

Absent (0); Present abundantly (1); Present sparsely (2); Present only on the margins (3).

16. Medial Groove –

Absent (0); Present (1)

Pronotum

17. Medial groove –

Absent (0); Present (1)

18. Punctations –

Absent (0); Present abundantly (1); Present sparsely (2); Present only on the margins (3),

19. Setae –

Absent (0); Present abundantly (1); Present sparsely (2); Present only on the margins (3),

Elytra

20. Scutellum –

Absent (0); Present and well defined (1); Present but not well defined (2)

21. Medial suture –

Absent (0); Present (1)

22. Punctations –

Absent (0); Present abundantly (1); Present sparsely (2); Present only on the margins (3).

23. Setae –

Absent (0); Present abundantly (1); Present sparsely (2); Present only on the margins (3),

Legs

24. Punctations –

Absent (0); Present abundantly (1); Present sparsely (2); Present only on the margins (3),

25. Setae –

Absent (0); Present abundantly (1); Present sparsely (2); Present only on the margins (3),

2.4 Molecular Methods

2.4.1 DNA extractions for cytochrome oxidase I

❖ Extraction using the hot sodium hydroxide and Tris (HotSHOT) method with reference to BioTechniques, July 2000

This is a quick and inexpensive method that involves a brief incubation of tissue sample in hot sodium hydroxide and pH adjustments with a Tris solution.

Protocol

1. Collect tissue samples in small PCR tubes.
2. Add 75 µl of alkaline lysis to the tube.
3. Heat at 94 °C for an hour.
4. Then cool at 4 °C
5. Add 75 µl of neutralizing reagent to each sample

The undissolved tissue seen in the tube does not interfere with the PCR reaction.

One to five microliters of the final preparation is used in the PCR reaction.

❖ Extractions using the Cetyl Trim ethyl Ammonium Bromide (CTAB) method

Protocols

1. Less than 100 mg of tissue was placed in a micro centrifuge tube with

about 200 µl of CTAB buffer. The mesothoracic leg was used for this.

2. Grind tissues in the tube using a pestle till homogenous slurry is formed.
3. Add 300µl of CTAB buffer and grind samples a bit more.
4. Add 5 µl of proteinase K and vortex briefly
5. Incubate samples at 65 °C for 1hr. Vortex it regularly.
6. Extract the homogenate with 500µl of 24:1 Chloroform :Isoamyl Alcohol and mix well and centrifuge at 13000 rpm for 10 minutes by.
7. Following centrifugation, you should have three layers: top - aqueous phase; middle - debris and proteins; bottom - chloroform.
8. Go on to the next step quickly so the phases do not remix
9. Pipette off the aqueous phase taking care not to suck up any of the middle or chloroform phases. Pipetting slowly helps with this.
10. Place the aqueous phase into a new tube containing 500 µl of phenol/chloroform isoamyl alcohol (25:24:1)
11. Mix well before centrifuging it at 13000 RPM for 10 minutes. Repeat till the upper layer is completely clear.
12. Remove the upper aqueous layer and add it to a new tube that contains 500 µl of chloroform-isoamyl alcohol and mix well. This removes the last trace of phenol.
13. Centrifuge at 13000 RPM for 30 seconds.
14. Transfer the upper aqueous layer to a new tube that contains 1.5 volumes of cold (-20 °C) isoproponol.
15. Invert the tube a few times and a white pellet should be seen, this is the DNA.
16. Allow the DNA to precipitate at -20 °C overnight.
17. Then centrifuge the DNA at 13000 rpm for 20 minutes.
18. Remove the supernatant and add 500 µl of cold 70% ethanol. Invert the tube gently and centrifuge at 13000 rpm for 10 minutes. This removes any residual salts.
19. Dry the pellet under vacuum for about 15 minutes or in a centrifuge for 10 minutes. If in a vacuum make sure that the tube is covered with Parafilm to prevent the pellet from falling out.

20. Re-suspend the DNA pellet in 100 µl of dH20, or approximately 1 µl for every mg of tissue. Allow the DNA pellet to rehydrate for several hours at 4 deg prior to using the DNA in a PCR experiment.

❖ Polymerase Chain Reaction (PCR)

Amplification of a section of the mitochondrial DNA control region is achieved through a PCR

Protocol

Distilled H20	32.3 µl
10 x reaction buffer	5 µl
MgCl2	3 µl
Dntp`s	0.5 µl
Oligonucleotide Primer 1	1.0 µl
Oligonucleotide Primer 2	1.0 µl
BSA	5 µl
Taq DNA polymerase	0.2 µl

Amplification of each individual DNA is conducted in 48 µl volume of solution from the above mentioned protocol with 2 µl of DNA is added to each volume to make up a

total of 50 µl.

Oligonucleotide Primers used for cytochrome oxidase sub unit I:

Mitochondrial Gene	Primers	Position	Sequence
Cytochrome Oxidase I	LCO	1490	5`- GGTCAACAAATCATAAAGA TATTGG -3`
Cytochrome Oxidase I	HCO	2198	5`- TAAACTTCAGGGTGACCAA AAAATCA -3`

Oligonucleotide Primers used for cytochrome b:

Mitochondrial Gene	Primers	Position	Sequence
Cytochrome b	CB2H	15175	5`- CCC TCA GAA TGA TAT TTG TCC TCA -3`
Cytochrome b	GLUDG-L	14724	5`- TGA CTT GAA RAA CCA YCG TTG -3`

Thermal Cycling conditions for amplification of control region in cytochrome oxidase I was done with:

Initial denaturation period 94 °C for 3 minutes; 35 cycles of 94 °C for 30 seconds; 52 °C for 30 seconds and 72 °C for 60 seconds. Final extension phase of 72 °C for five minutes

❖ Gels

To ensure successful amplification of the target gene area the PCR products were

separated by electrophoresis in a 1.0 % agarose gel with ethidium bromide. Once gel was set and run the product was viewed under UV light. A negative control was included in each gel to check that there was no contamination of processing solutions.

❖ PCR clean up

The PCR products were purified using the Ultra Clean PCR Clean-up Kit (Mo BIO Laboratories, Inc)

Protocols

1. 222 µl of spin bind added to 45 µl of PCR reaction and mix well
2. Transfer PCR and spin bind mix to a spin filter unit provided in the Kit.
3. Centrifuge for 30 seconds at 13000 rpm in a tabletop microcentrifuge
4. Remove the spin filter basket and discard the liquid flow though form the tube by decanting.
5. Replace the sip basket in the same tube
6. Add 300 µl of spin clean buffer to the spin filter
7. Centrifuge for 30 seconds at 13000 rpm
8. Remove the spin basket and discard the flow thought by decanting the tube.
9. Replace the basket in the tube
10. Centrifuge for 30 seconds at 13000 rpm
11. Transfer the filter into a clean collection tube, also supplied in the kit.
12. Add 50 µl of elution buffer solution or sterile water directly into the centre of the white spin filter membrane

❖ Sequencing reactions were performed using CEQ 2000 Dye Terminator Cycle Sequencing Kit (Beckman Coulter, 2000)

Protocols

- Prepare sequencing reaction in 0.2 ml tubes
- Reagents must be kept on ice

Reagents	Volumes
dH2O	Adjust to make a total of 10 µl
DNA template	According to the quantity/quality of DNA
Primer	0.65 µl
DTCS	2.0 µl

The first sequence was run with cytochrome b primers GLudG reverse primer. The other two sequences were run using cytochrome b forward primer CB2H. A total volume of 10 µl of sequencing reaction was made for each sample. The 10 µl volume had varying degrees of DNA in the three sequences that were performed.

❖ Thermal cycling program:

96° C	20 sec
50°C	20 sec
60° C	4 min

For 30 cycles followed by holding at 4° C

❖ Ethanol precipitation

Protocol

1. In 0.5 mL microfuge tube add 4 µl stop solution and 0.5 µl of glycogen.
2. Transfer the sequencing reaction into the same tube

3. Add 60 µl of cold 100% ethanol stored at -20 °C
4. Mix well and immediately centrifuge at 14,000 RPM at 4 °C for 15 minutes
5. Remove the supernatants from the tube with a micropipette slowly so as to not disturb the pellet.
6. Rinse the pellet twice with 180 µl of the same ethanol. For each rinse centrifuge at 14,000 rpm at 4 °C for two minutes and remove the supernatant.
7. Vacuum dry for 40 minutes to remove any ethanol residues
8. Resuspend the sample in 40 µl of the sample loading solution

Sequence was automatically analysed using the CEQ 8000 sequencing machine (Beckman Coulter,2000).

All sequences were aligned using Sequencher 4.5

Chapter 3

3.0 Results

3.1 Morphological results (see Appendix 3)

In Tasmania the following *Lissotes* species have been identified and are described here in alphabetical order.

Genus ***Lissotes*** Westwood, 1855

Type species: *Lissotes menalcas* Westwood, 1855 by monotypy.

***Lissotes basilaris* Deyrolle, 1881**

Taxonomic history

Lissotes basilaris Deyrolle, 1881: 240 [Tasmania].

Benesh, 1960: 39; Maes, 1992: 48; Moore & Cassis, 1992: 12.

Diagnosis

Body length: ♂ to about 15mm. This beetle is black with a brownish tinge.

Description (See fig 5.28)

Head

The labium (Fig.3.1) has punctations that are large, deep and abundant. Setae are only on the outer margins of the labium and are abundant at the apex.

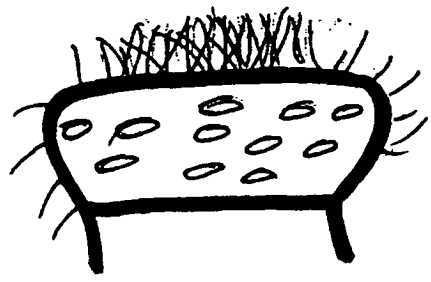


Fig. 3.1 Labium of *Lissotes basilaris*

There are three maxillae segments and a single labial palp segment that have a smooth surface with no visible punctation or setae.

The antennae are six segmented with a three-segmented club. There are setae present on the entire antennal surface but there are no visible punctations on either the antennae or the clubs.

The mandibles are very stout. There is a sub basal cusp that is visible only from the under side. There are a pair of apical cusps that are sharp and point upwards. The remaining teeth are blunt and point inwards. It is difficult to tell the exact number of cusps present, as they are very thick and not very well defined. There are no punctations or setae on the mandibles.

The head has small punctations all over its surface but there are no visible setae. There is a gap in the front of the eyes. The head has a very steep slope to the apex.

Pronotum

The pronotum is rather convex and has abrupt edges; there are no viable setae on its surface. The punctations are all over the surface and are similar to those on the head.

Elytra

The scutellum is not very well developed and has visible punctations on it. The elytra have punctations all over it the surface that are oblong and run parallel to the medial suture which is well developed and a darker colouring to the rest of the elytra. There

are no visible setae on the elytra.

Lissotes bornemisszai Bartolozzi, 2003

Taxonomic history

Lissotes bornemisszai Bartolozzi, 2003: 333-337, figs. 3-5 [Margate area].

Distribution

Southern Tasmania: Margate Area; Picton River; Picton Valley: Reuben Falls; Scott Peak Road: via L. Pedder; Snug Tiers; Scotts Peak Rd.,

Lissotes cancroides (Fabricius, 1787)

Taxonomic history

Lucanus cancroides Fabricius, 1787: 2 [Tierra Diemenii].

Olivier, 1789: 18-19, pl.4; Herbst, 1790: 314; Linnaeus, 1790: 1590; Fabricius, 1792: 239; Olivier, 1800: 69; Fabricius, 1801: 251-252; Sturm, 1802: 5, pl. 1; Thunberg, 1806: 200; Schonherr, 1817: 326; Boisduval, 1835: 234; Hope, 1837: 79

Dorcus cancroides Fabricius; Westwood, 1838: 267.

Aegus cancroides Fabricius; Burmeister, 1847: 402

Lissotes cancroides Fabricius; Westwood, 1855: 215; Parry, 1870:81; Westwood, 1871:371; Heyne & Taschenberg, 1908:56; Lea, 1910:348-349, pl.8; Boileau, 1913:263; Benesh, 1960:39, 40; Maes, 1992:48; Moore & Cassis, 1992:12-13; Mizunuma & Nagai, 1994: 277, pl. 115; Bartolozzi *et al.*, 1998: 33; Michaels & Bornemissza, 1999: 88, 89, 90; Krajcik, 2001: 2; Bartolozzi, 2003: 330-331.

Lissotes subtuberculatus Westwood, 1855:215-216, pl.12 [New Holland]; Moore & Cassis, 1992:12.

Diagnosis

Body length: ♂ to about 13mm. This is a black shiny beetle with a deep black colouring along its outer margins.

This name is also attached to many different distinct species. It is possible that this name represents a species on its own that is very rare and confined to one area or it is simply used to describe some specimens found in different areas. There is no known specimen that suits the description that Westwood made (Lea, 1910).

Distribution

Tasmania : Mt. Field : East 42°39S-146°24E, 750-1100 m; Mt. Field N.P., 42°39S-146°24E : Lyrebird Track, 650 m; Maydena : Southwest N.P. : Florentine Valley : Timbs Track; Maydena : Junee Cave St. Res., 300 m; Tahune Forest, 43°05S-146°44E.

Description (Fig.5.27)

Head

The labium (Fig.3.2) has a bluish tinge to it. Punctations are deep and visible over the entire surface. These punctations are rounded but not uniform, instead there is a mix of small and large. Setae are long and visible only on the outer margins. The apex of the labium however is abundantly covered with setae.

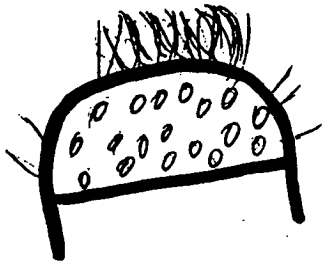


Fig. 3.2 Labium of *Lissotes cancroides*

The maxillae have four segments and the labial palps have two segments. There are no punctations or setae present on either the maxillae or the labial palps.

The mandibles in this species are very thick and have a number of cusps. There is a pair of upward pointing sharp cusps at the apex. There are three cusps that are sub basal on each mandible that face inwards. The top surface near the base of the mandible and the apex of the head there is a very blunt cusp. When clasped there is not much space between the mandibles. The mandibles have very small punctations but no visible setae.

The labrum is visible from the dorsal view of the beetle although it is remarkably small. There are setae present on the labrum but no visible punctations. The labrum is

not pointed but rather forms three curved protrusions.

The eyes can be seen from the dorsal view. There are sub conical protrusions behind the eyes and the head curves inwards behind the eyes. The fronts of the eyes have an empty space in front of it and the edges form a shelf rather than ending abruptly. These outer margins are a deep red colour. Punctations on the head are small and there are no setae present.

There is no medial suture on the head. There is a steep slope in the centre apex of the head to the base of the mandibles.

There are eight protibial teeth and these become larger distally.
The antennae have six segments with a three-segmented club. There are no punctations but there are very small sparse setae.

Pronotum

The smooth convex pronotum has a very shiny gloss. Setae are absent except for the outer margins that are a deep red and for a shelf on the edge rather than ending abruptly. The punctations on the pronotum are small and sparse with an area in the medial region having no punctuations. There is no medial on the pronotum.

Elytra

The scutellum is present but poorly defined and lacks punctations. The elytra seem to be much darker than the rest of the beetle and have very dense, large punctations that make the surface look very rough. The edges of the elytra are abrupt and end in a black lining. There are long but sparse setae present on the entire elytra. The medial suture is not very deep and is noticeable only because of a really dark line that separates it form the rest of the wing case.

***Lissotes convexus* Lea, 1910**

Taxonomic history

Lissotes convexus Lea, 1910: 349 [Tasmania: Marrawah].

Benesh, 1960: 39; Bomans, 1986: 9; Maes, 1992: 48; Moore & Cassis, 1992: 13.

Diagnosis

Body length: ♂ to about 16mm; ♀ to about 12.5mm

Distribution

North western coastal Tasmania, from Burnie to Marrawah.

Syntypes: Marrawah, 40°55′S 144°42′E, Burnie, 41°04′S-145°54′E.

Tasmania: Corinna.

Description

Individuals from this species are black and have wide heads. Their eyes are closer to the base than usual and the sides in front of eyes are narrow and flattened but not projecting. The labrum is moderately long and pointed in the middle. The mandibles are comparatively slender to other species. Each mandible has an obtuse projection at the side of the labrum. The prothorax is slightly wider than the head and its sides are finely serrated and gently rounded. The prothorax has punctures that vary from minute to large and are irregularly distributed. The elytra have dense but not very coarse punctures and feebly projecting shoulders. Females have a smaller head with denser punctures of more uniform size. The eyes are more conspicuous and the mandibles are like other lucanid females. The prothorax is also much smaller and the sides are well rounded and more strongly serrated (Lea, 1910).

Lissotes cornutus **Boileau,** 1905

Taxonomic history

Lissotes cornutus Boileau, 1905: 201 [Australia].

Lea, 1910:359, pl.9; Benesh, 1960:39; Bomans, 1986:9; Maes, 1992:48; Moore & Cassis, 1992:13; Mizunuma & Nagai, 1994:276, pl.115.

Diagnosis

Body length: ♂ to about 18mm; ♀ to about 16mm.

Distribution

Found is Stanley, Zeehan, Magnet and Waratah.

Tasmania: Luina, 41°28S-145°22E; Corinna.

Description

Individuals are usually black with a very wide head with the sides behind and in front of the eyes feebly projecting and in some cases not at all. The labrum is acutely produced in the middle. The mandibles are strongly curved and are thin, each obtusely produced close to the labrum. The lower surface has a strong cusp with other two or three more feeble cusps. The prothorax is distinctly wider near the head and sides are finely serrated and scarcely rounded. Near the base the prothorax is somewhat oblique and has dense, coarse and somewhat irregularly distributed punctures. The elytra have dense irregularly distributed punctures and the shoulders are scarcely projecting. The suture is highly polished and the striations and interstices are ill defined.

In females the head is smaller, with denser punctures that are more evenly distributed and in consistant sizes. The mandibles are the usual female type. The prothorax is no wider than the elytra and its sides are more rounded. The elytra are more coarsely and evenly punctured (Lea, 1910).

Lissotes crenatus Westwood, 1855

Taxonomic history

Lissotes crenatus Westwood, 1855: 216-217, pl. 12 [New Holland].

Benesh, 1960: 39; Bomans, 1986: 9, 16; Maes, 1992: 48; Moore & Cassis, 1992: 13.

Distribution

Souhern Tasmania: Hobart: Mt. Wellington, 42°54S-147°14E.

Description

This species was not recognised by Lea (1910), however Bomans (1986) located the types in the collection of Oxford University Museum and is of the opinion they were collected by Francois Peron in 1802 in southern Tasmania.

Lissotes curvicornis (Boisduval, 1835)

Taxonomic history

Dorcus curvicornis Boisduval, 1835: 235 [New Holland].

Dejean, 1837: 194; Reiche, 1852: 82.

Lissotes curvicornis Boisduval; Parry, 1870: 64; Lea, 1910: 348, pl. 8; Benesh, 1960:39; Bomans, 1986:9, 18; Maes, 1992:48; Moore & Cassis, 1992:13; Bartolozzi et al., 1998:33; Krajcik, 2001:21; Bartolozzi, 2003:331.

Distribution

Tasmania : St. Clair Lake, 42°S-146°E; Mt. Field : Florentine Valley, 42°36S-146°26E; Mt. Field : Lake Felton, 1000 m; Mt. Wellington, 42°54S-147°14E, 1270 m; Hartz National Park : Arve River, 43°11S-146°47E; Mt. Hartz, 43°15S-146°46E; Hastings, 43°25S-146°55E; Ida Bay Cave, 43°27S-146°50E.

Description

It is a flat looking beetle with the head and thorax having a bluish gloss, which is distinctive but does not extend to all female specimens (Bomans 1986). The mandibles are sub-basal and when clenched enclose a large apical sac. The beetle has a shining tubercle half way between each mandible and eye. The prothorax has small punctures and an apex in the middle (Lea, 1910).

Lissotes desmaresti Deyrolle, 1881

Taxonomic history

Lissotes desmaresti DEYROLLE, 1881:239, pl.5 ["Nouvelle Zélande"].

Lissotes punctatus LEA, 1910:357, pl.9 [Zeehan, Strahan, Magnet, Waratah].

Lissotes desmaresti DEYROLLE; Benesh, 1960:40; Holloway, 1961:37; Maes, 1992:48; Moore & Cassis, 1992:13; Bartolozzi et al., 1998:33; Bartolozzi, 2003:331-332.

Lissotes punctatus LEA; Benesh, 1960:41; Bomans, 1986:12; Maes, 1992:49; Moore & Cassis, 1992:15; Bartolozzi et al., 1998:33; Krajcik, 2001:22.

Distribution

Syntypes Tasmania: Zeehan, 41°44S-145°20E; Strahan, 42°09S-145°19E; Magnet; Waratah

Others: Tasmania: Tewkesbury, 600 m

Lissotes distinctus Deyrolle, 1881

Taxonomic history

Lissotes distinctus Deyrolle, 1881: 240 [Tasmania].

Lissotes distinctus Deyrolle; Benesh, 1960:40; Maes, 1992:48; Moore & Cassis, 1992:13; Bartolozzi et al., 1998:33; Krajcik, 2001:21; Bartolozzi, 2003:332.

Distribution

Tasmania: Hobart: Mt. Wellington, 42°54S-147°14E: The Springs, 700 m

Lissotes forcipula Westwood, 1871

Taxonomic history

Lissotes forcipula Westwood, 1871: 366, pl. 9; Lea, 1910: 352, pl.8; Benesh, 1960: 40; Maes, 1992: 48; Moore & Cassis, 1992: 13.

Diagnosis

Body length: ♂ to 12mm.

Distribution

All recorded specimens found in the Hobart region.

Description

Teeth on the front tibiae vary in number from three to five. The tips of the mandibles have never been the same on any two specimens of this species examined. The elytra have long hairs in five rows and some smaller hairs also in rows (Lea, 1910).

Lissotes globosus Bomans, 1986

Taxonomic history

Lissotes globosus Bomans, 1986: 3, 10 [Mt. Hartz 43°15S-146°46E].

Maes, 1992: 48; Moore & Cassis, 1992: 13-14.

Distribution

Southern Tasmania, Hartz Mountains

Lissotes lacroixi Bomans, 1986

Taxonomic history

Lissotes lacroixi Bomans, 1986: 3, 11 [Mt. Field].

Maes, 1992: 48; Moore & Cassis, 1992: 14; Bartolozzi *et al.*, 1998: 33; Krajcik, 2001: 21; Bartolozzi, 2003: 332-333.

Distribution

Tasmania: Mt. Field N.P., 42°S-146°E: Lake Fenton, 1100 m

Lissotes latidens, Westwood, 1871

Taxonomic history

Lissotes latidens Westwood, 1871: 365, pl.9 [I. Maria 42°44S-148°01E].

Lea, 1910: 349, pl. 8; Benesh, 1960: 40; Bomans, 1986: 17; Maes, 1992: 49; Moore & Cassis, 1992: 14.

Distribution

Eastern Tasmania, centred on the Wielangta area and including the northern half of Maria Island.

Description

There is a single specimen collected and belonging to the Howitt collection that matched the original description of the species. There have been several other species attached to this name and so there is no fixed description to the name. The specimen that best fits the original description was collected on Maria Island of the East Coast of Tasmania (Lea, 1910).

Lissotes laticollis Lea 1910

Taxonomic history

Lissotes laticollis Lea, 1910: 360, pl. 9 [Tasmania].

Benesh, 1960: 40; Bomans, 1986: 12; Maes, 1992: 49; Moore & Cassis, 1992: 14.

Diagnosis

Body length. ♂ to about 19mm; ♀ to about 16mm

Distribution

Western Tasmania: Corinna.

Description

Individuals are a shining black and the head is wide with the base strongly convex. The sides behind the eyes are a bit swollen but not conspicuously projecting. There are moderately large and fairly dense punctures present near the eyes but elsewhere it is much smaller, sparser and irregularly distributed. The labrum is wide and is obtusely notched in the middle. The mandibles are moderately stout especially at the base and curve inward. The prothorax is larger than usual and distinctly wider at the head. The sides are more rounded than usual and the punctures are fairly large at base and sides. Elsewhere the punctures are small and sparse. The elytra shoulders are not projecting and are less parallel than usual. The elytra have moderately dense but small punctures. In the females the head is much smaller, with coarser and more evenly distributed punctures. Tubercles are represented by slight swelling. The mandibles are the usual female type. The prothorax is smaller and no wider than the elytra at its widest and has strong punctures.

Lissotes launcestoni Westwood, 1871

Taxonomic history

Lissotes launcestoni Westwood, 1871: 365, pl. 9 [Launceston 41°27S-147°10E].

Lea, 1910: 350, pl. 8; Benesh, 1960: 40; Bomans, 1986: 12, 17; Maes, 1992: 49; Moore & Cassis, 1992: 14; Mizunuma & Nagai, 1994: 277, pl. 115.

Distribution

Widespread in northern and north western Tasmania, near Wynyard, St. Patrick's River, east and west Tamar, Strahan, Launceston, Mole Creek, Zeehan, Frankford, Burnie and Ulverstone

Tasmania : Mt. Ben Lomand, 41°33S-147°40E; Ouse Valley, 42°03S-146°42E; Musk Valley.

Description

According to Lea, 1910, this species is similar to *Lissotes obtusatus,* Westw. and is

merely a local form of the species. The only differences between the two species can be seen in the mandibles. *L. launcestoni* seems to have a strong conical projection when the mandibles are agape, and are visible even when clenched.

Lissotes menalcas Westwood, 1855

Taxonomic history

Westwood, 1855: ; Parry, 1864:63; Lea, 1910:351, pl.8; Benesh, 1960:40; Maes, 1992:49; Moore & Cassis, 1992:14; Mizunuma & Nagai, 1994:276, pl.115.

Distribution

Recorded specimens were found at Long bay, Three Hut Point and Mount Wellington , Tahune Forest, 43°05S-146°44E

Description

This species has a peculiarly shaped prothorax and a largely excavated head. Each of its mandibles has two apical cusps and a strong sub conical tubercle on the upper surface close to the apex. When the mandibles are clenched the space is large and single. There are distinct teeth on the front tibiae that vary in numbers from six to nine. However in most cases there are seven. In the females the prothorax and the head is smaller and less convex, with the median line wider and deeper. The labrum is notched and the surface behind the labrum is raised into a sub-conical tubercle (Lea, 1910).

Lissotes menalcas is listed as a vulnerable under the schedules of the Tasmanian *Threatened Species Protection Act 1995*. This is found in the wet forests types of southern Tasmania, with mature wet eucalypt forest constituting the optimal habitat of the species. Studies by Meggs & Taylor 1999, show that this species like to live in eucalypt logs containing moist red/brown clayey-rot. Given its dependence on decaying wood for all parts of its life cycle, any anthropogenic activity that will impact on the existing habitat or interrupt the future supply of decaying logs will threaten the viability of local populations (Meggs, 2002)

Lissotes obtusatus (Westwood, 1838)

Taxonomic history

Dorcus obtusatus Westwood, 1838: 267 [Van Diemen's Land].

Burmeister, 1847: 402; Reiche, 1852: 82; Westwood, 1855: 217.

Lissotes opacus Deyrolle, 1870: 97 [Van Diemen's Land].

Westwood, 1875: 244; Heyne & Taschenberg, 1908: 56.

Lissotes parvus Lea, 1910: 347, 349, 357, pl. 8, 9 [Hobart].

Benesh, 1960: 41; Bomans, 1986: 14, 18; Hawkeswood, Callister & Antram, 1991: 46; Maes, 1992: 49; Moore & Cassis, 1992: 14; Mizunuma & Nagai, 1994: 277, pl. 115.

Diagnosis

Body length. ♂ to about 19mm; ♀ to about 16mm. This is a black beetle with a visibly smooth surface.

Distribution (Fig. xx)

Widely distributed throughout Tasmania, from sealevel to over 1000m.

Tasmania : Launceston, 41°27S-147°10E; Mathinna, 41°29S-147°53E; Mt. Ben Lomand, 41°33S-147°40E; Hobart, 41°53S-147°14E. Mt. Wellington, 42°54S-147°14E; Blackman's Bay, 43°00S-147°58E.

Description

Head

Punctations on the labium (Fig. 3.3) are round deep and abundant. They are spread across the labial surface in uniformity. The setae on the labium are small and thin and are visible only on the outer margins of the labium. Setae become extremely abundant at the apex of the labium.

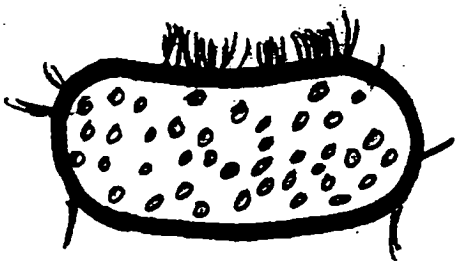


Fig. 3.3 Labium of *Lissotes obtusatus*

The maxilla has three segments each that are visible from the dorsal view. Each labial palp consists of a single segment. There are no punctations or setae on the palps, but instead are rather smooth and reflective.

On each mandible there are two upward facings cusps at the apex, two sub apical cusps facing inward and another single basal cusp close to the labium. Mandibles are very thick, have sparse punctations that are small and setae on the outside of the base near the head.

Labrum is visible from the dorsal view and is a sharp triangular shape. The labrum has setae and punctations that are abundant all over.

There are short fine setae present all over the antennal surface.

The head has punctations all over the surface, and especially on the outer margins. There are no setae on the upper surface of the head and the medial suture is absent. The head has two subconical tubercles in the medial section. The head is highly convex and dips downwards to the anterior margins of the head and the base of the mandibles.

Pronotum.

Medial suture is present but not very prominent. The pronotum like the head is very convex. There are dense punctations along the margins but become sparse over the rest of the pronotum.

Elytra

The scutellum is present but it's not a very sharp triangle. Medial suture present, deep and a dark brown to black look. The elytron has setae on the outer margins that are abundant and small. There are punctations that are abundant.

There are seven protibial teeth and these become larger distally.

Lissotes parvus Lea, 1910

Distribution

Hobart

Description

Individuals in the species are black and shining. The head is wide and moderately convex with the front slightly concave. The sides behind the eyes are sometimes rounded and this pattern is sometimes followed to the front. Sometimes a tubercle is present towards each side of the mandible. The labrum is feebly produced towards the centre. The mandibles are short and stout with a sub conical projection near the base. The prothorax is wider than the head and a little if at all wider than the elytra. The sides of the prothorax are finely serrated and feebly incurved towards the base. The punctures are coarse and dense on the sides and base but elsewhere fairly dense and smaller. The elytra are parallel for most of its length and the shoulders are slightly projecting with dense, coarse punctures. Males grow to about 11mm and females to 10mm. the females head is much smaller and has smaller, coarser and denser punctures. The prothorax is less transverse with sides more rounded and punctures somewhat coarser (Lea, 1910).

Lissotes politus Lea, 1910

Tanonomic history

Lissotes politus Lea, 1910: 361, pl. 9 [Tasmania].

Benesh, 1960: 41; Bomans, 1986: 14; Maes, 1992: 49; Moore & Cassis, 1992: 14-15.

Distribution

Found in the Hobart and Kingston area.

Tasmania: Cockle Bay (42°42S-147°56E) to Ida Bay; Mt. Hartz, 43°15S-146°46E.

Description

Individuals are black and shining. The head is large and longer than usual with the frontal slope unusually long and feebly concave. The sides behind the eyes are evenly rounded. A greater portion of the surface is smooth with a few small punctures towards each side. The labrum is acutely produced in the middle. The mandibles are stout at the base, strongly angular and close to the labrum. The mandibles strongly curve to the apex where each is narrowly notched. The prothorax is slightly wider than the head with the sides smooth and parallel for most of the length. The sides and base have dense and rather coarse punctures while elsewhere there are small and minute punctures. The elytra have shoulders that are rounded off with dense and comparatively small punctures. The striations and interstices are fairly distinct. The female of this species is unknown.

Lissotes punctatus Lea, 1910

Distribution

Western Tasmania: Zeehan, Strahan, Magnet and Waratah.

Diagnosis

Body length: ♂ to about 17mm; ♀ to about 15mm.

Description

Individuals in the species are black and have a wide head that is moderately convex, with the sides slightly projecting behind the eyes. The labrum is strongly but obtusely produced in the middle. The mandibles are slender, each with a projection closer to the labrum. The prothorax is slightly wider than the head with the sides feebly serrated and gently rounded. The elytra with feebly projecting shoulders are very densly and coarsely punctate (Lea, 1910).

Lissotes rodwayi Lea, 1910

Taxonomic history

Lissotes rodwayi Lea, 1910: 363, pl. 9 [Tasmania].

Benesh, 1960: 41; Bomans, 1986: 10; Maes, 1992: 49; Moore & Cassis, 1992: 15; Mizunuma & Nagai, 1994: 277, pl. 115.

Diagnosis

Body length: ♂ to about 18mm; ♀ to about 15mm. These individuals are shining black with some parts a dark reddish brown.

Distribution

Tasmania: Mt. Hartz, 43°15S-146°46E., Zeehan

Description (see Fig. 5.23)

Head

Punctations on the labium (Fig. 3.4) range from round to oblong and in some cases are curved. Though these punctations can be seen all over the labial surface, they are rather sparsely presented. The setae are short, fine and visible only on the outer margins of the labium. Setae become extremely abundant at the apex of the labium.

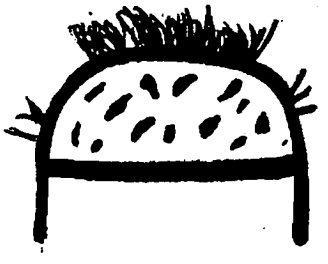


Fig. 3.4 Labium of *Lissotes rodwayi*

The maxilla has three segments each that are visible from the dorsal view. There are no punctations or setae on the maxillary palps. Each labial palp consists of two segments that can be seen only from a ventral view.

This species has two apical cusps and one sub conical tubercle near the apex of each mandible. Another single cusp is present at the base of each mandible, close to the labium, that can be seen from the underside. There are no setae on the mandibles but there are small shallow punctations. The mandibles are not very stout except at the base near the labrum.

There is a sub conical tubercle on either side of each eye. The head is large and moderately wide with the head projecting in both the front and behind the eyes. The antennae have fine setae along its segments and clubs. There are punctations on the antennal clubs but none on the segments. The clubs have three segments that are fused together.

Labrum feebly incurved to middle of apex with projection over the middle.

Punctations on the surface of the head are much larger and less dense than the punctations on the rest of the body. Setae are present on the outer margins of the head especially where the head meets the pronotum. There is no medial suture on the head. It has a convex surface that slopes downwards to the base of the mandibles at the apex of the head.

Pronotum

The highly convex pronotum has no medial suture. There are setae along the outer margins but none on the medial surface. Punctations are present all over the pronotum especially on the outer margins. The outer margins of the pronoun do not end abruptly but instead have a jagged edge that form a shelf.

Prothorax is not much wider than the head and a little wider if wider at all than the widest part of the elytra. The sides of the prothorax are feebly serrated and the apical two thirds are parallel. Punctures are dense and coarse along the sides and base but sparser along the middle.

Elytra

The scutellum is present but not very well defined. The elytra have setae on the outer margins. Punctations are abundant all over the convex surface of the elytra and especially abundant on the outer margins. The elytra are not parallel and the

shoulders are rounded. Females have smaller heads with denser and coarser punctures. The medial suture is well defined. There are seven protibial teeth and these become larger distally.

Lissotes rudis Lea, 1910

Taxonomic history

Lissotes rudis Lea, 1910:354, pl.9 [Denison Gorge, Lottah, George Bay, Frankford, Sheffield, Forster River, Ullerstone].

Benesh, 1960:41; Bomans, 1986:14; Hawkeswood, Callister & Antram, 1991:46; Maes, 1992:49; Moore & Cassis, 1992:15; Mizunuma & Nagai, 1994:276, pl.115; Bartolozzi, 2003:339.

Diagnosis

Body length: ♂ to about 20mm; ♀ to about 15mm. It is black and has a shiny gloss.

Distribution

Found in parts of Tasmania- George's Bay, Forester River, Dennison Gorge, Lottah, Sheffield, Frankford, Wilmot, Ulverstone

Tasmania : Derby, 41°09S-147°48E; Mt. Blue Tier, 41°13S-147°58E; Goulds Country, 41°14S-148°03E; Via Pyengana : St. Columbas Falls, 41°19S-147°57E.

Syntypes: George Bay, 41°19S-148°17E; Frankford, 41°20S-146°46E; Sheffield, 41°23S-146°20E. Lottah, 41°14S-148°02E Denison Gorge, 41°11S-147°15E; Forster River; Ulverstone, Launceston, 41°27S-147°10E; Mt. Ben Lomond, 41°33S-147°40E; Targa.

Description (Fig.5.25)

Head

Punctations on the labium (Fig. 3.5) are large dense and deep. They vary in size from round to slightly oblong. These are seen all over the labial surface. The setae on the labium are short and fine and are visible only on the outer margins of the labium. Setae become extremely abundant at the apex of the labium.

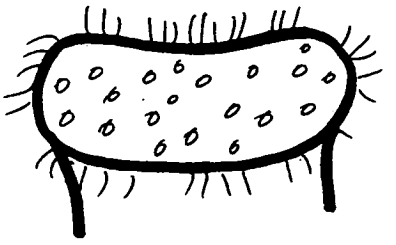


Fig. 3.5 Labium of *Lissotes rudis*

The maxilla have three segments each that are visible from the dorsal view. The labial palp consists of a segment each and can be seen only from a ventral view. There are no puntations on the maxillae or the labial palps but there are very fine setae that are visible although very sparse.

The mandibles on this species are very thick and complex and are about the same length of the head and in some cases longer. Each mandible has a single apical cusp that point upwards and another sub conical tubercle on each mandible just near the apex. Three sub apical cusps that point inwards are also present on each mandible. At the base of the mandibles near the labium there are a set of cusps that point upwards. There are no setae on the mandibles but there are small shallow scattered punctations.

The eyes are visible from the dorsal view and have a strong projection behind each eye.

The labrum is visible from the dorsal view and more visible in the female. It is an acute triangular shape with no punctations and bears very fine long setae.

The antennae have 6 segments with 3 segmented clubs. There are no visible punctations or setae on the antennae or clubs. Punctations on the surface of the head are larger and dense There are setae present sparsely on the outer margins of the head.

Punctations on the head are small and round. They are present all over the rather convex surface. Setae can be seen all along the outer margins of the head. Medial

suture on the head is absent and the male head is broader that the female.

Pronotum:

The pronotum has no medial suture. There are setae along the outer margins but none on the medial surface. There are punctations all over the pronotum surface especially on the outer margins where they get denser.

The prothorax is small and usually its sides are finely serrated and gently rounded. The prothorax is no wider than elytra and the sides are more rounded with denser more uniform punctures (Lea, 1910).

Elytra

The scutellum is very well defined and has small, shallow round punctations. Setae present on the outside margins of the elytra. The elytral surface is moderately convex and the medial suture is well defined. Punctations are abundant all over the surface of the elytra and especially on the outer margins. The elytra have shoulders that are slightly projecting laterally.

There are seven protibial teeth and these become larger distally.

Lissotes subcaeruleus Bomans, 1986

Taxonomic history

Lissotes subcaeruleus Bomans, 1986: 3, 14 [George Bay, Mt. Wellington, Mt. Hartz].

Maes, 1992: 50; Moore & Cassis, 1992: 15.

Distribution

Eastern Tasmania (George Bay 41°19S-148°17E, Mt. Wellington 42°54S-147°14E, Mt. Hartz 43°15S-146°46E).

Lissotes subcrenatus Westwood, 1871

Taxonomic history

Lissotes subcrenatus Westwood, 1871:368, pl.9 [Tasmania].

Lea, 1910: 353; Benesh, 1960: 41; Maes, 1992: 50; Moore & Cassis, 1992: 15.

Diagnosis

Only a female individual has been recorded of this species and so there is no know description of the species.

Lissotes urus Bomans, 1986

Taxonomic history

Lissotes urus Bomans, 1986: 3, 15 [Mersey River Valley 41°25S-146°28E].

Maes, 1992: 50; Mizunuma & Nagai, 1994: 277, pl. 115.

Diagnosis

Body length: ♀ to about 15mm. This species is black with a brownish orange tinge.

Distribution

Tasmania: Liffey Falls, 41°41S-146°45E.

Description

Head

The labium (Fig. 3.6) has large deep punctations all over. Punctations are round and uniform. The setae are visible sparsely along the margins and are abundant at the apex.

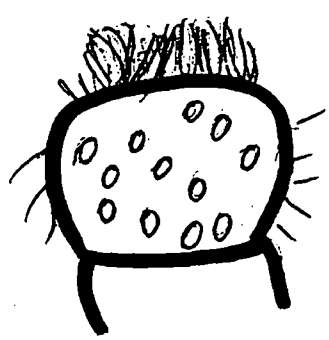


Fig. 3.6 Labium of *Lissotes urus*

Each maxilla has three segments and each labial palpi two segments. The maxillae as well as the labial palps have finely visible setae, but no punctation. The maxillae can be seen from the dorsal view

The mandibles have large punctations seen on the surface and sparse setae, the female mandibles are small with two apical cusps on either side. Each mandible has a sharp cusp that points upwards and another that points inwards.

The antennae are six segmented and have three-segmented clubs that are fused. There are small thin setae sparsely distributed on the antennae and clubs and small punctations as well.

The eyes can very distinctly be seen from the ventral view, a characteristic that was not so visible in other species.

There is a subconical tubercle in the central apex of the head between the mandibles. Punctations on the head are large and abundant, but setae are absent on the head. A medial suture is absent. The head is rather flat and there is a very gradual slope towards the apex.

Pronotum

The outer margins of the pronotum have an orange tinge. The punctations are large, abundant and uniform all over the surface. There is no medial suture on the pronotum. The setae on the pronotum only occupy the outer margins.

Elytra

The elytra have oblong punctations that run parallel to the medial suture. The triangular scutellum is well defined, deep and darker than the rest of the body. The medial suture is very well defined and deep and has a darker colour. Setae are present all over the surface and are especially abundant on the outer margins. The elytra have an orange tinge.

Other *Lissotes* of uncertain identification used in the DNA study:

Lissotes DNA 37 (Fig. 5.22)

The male in this species grows to approximately 14 mm in length from the apex of the mandibles to the posterior tip of the elytra. It is a deep brown to black beetle with a distinct red tinge.

Head:

A noted characteristic of this species is the head that is distinctly narrower than the rest of the body. And slopes down towards the base of the mandibles and the apex.

Punctations on the labium (Fig. 3.7) are large and spread abundantly across the labial surface. Punctations are rounded and deep and tend to be filled with dust. The setae, though short and fine, are visible through the entire surface of the labium. Setae become extremely abundant at the apex of the labium.

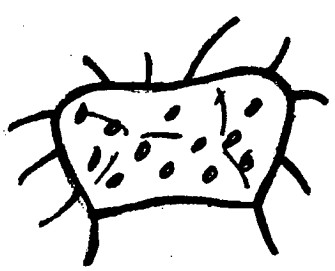


Fig. 3.7 Labium of *Lissotes DNA no.37*

The maxillary palps have four segments each that can be seen from the upper side of the beetle. There are short, fine sitae on the maxilla palps and no puntations. Each labial palp consists of two segments and can be seen only from the underside of the beetle when looking at the labium.

A single apical cusp and a single sub basal cusp make up each mandible. Very fine setae are visible on the mandibles along with small rounded punctations that are not

very abundant.

There is a distinct hollow space seen after the eye towards the apex.

Punctations on the head are small and abundant across the entire surface and are uniform across the entire beetle. Setae are visible abundantly on the outer margins. There are setae present on the outer margins of the head especially on the anterior margins where the head meets the pronotum.

There is no medial suture on the head.

The labrum is not visible from the top.

The antennae have 6 segments that have short, fine setae and no punctations. The clubs have 3 segments that are fused and have small punctations and small setae on it.

Pronotum

In this species the pronotum seems to be separated from the head and the area where the head fuses with the prothorax can be seen clearly. The anterior region of the pronotum has no setae but the outer margins of the rest of the pronotum have short, fine setae that increase distinctly towards the posterior margins. There are punctations on the pronotum that are abundant especially on the outer margins. Medial groove is absent.

Elytra

There appears to be a gap between the elytra and the pronotum. The scutellum is small, triangular and has punctations on it. The elytron has setae on the outer margins. There are punctations that are abundant all over the surface of the elytra and especially abundant on the outer margins. The elytron also has a medial suture present that is a deep and is reddish brown.

There are seven protibial teeth and these become larger distally.

Lissotes DNA 32

This beetle is approximately 14 mm in length from the apex of the mandibles to the posterior tip of the elytra. This is a back beetle with a slight gloss on its surface.

Head:

Punctations on the labium (Fig. 3.8) large, dense and deep, round to oval and all over the labial surface. The setae on the labium are long and thin and are visible only on the outer margins of the labium. The apex of the labium does not seem to have the cluster of setae present in most of the other species.

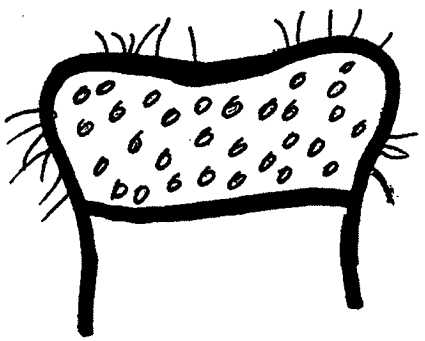


Fig. 3.8 Labium of *Lissotes DNA no. 32*

The maxilla has three segments each that are visible from the dorsal view. The labial palps have two segments. There are no visible punctations or setae on the maxilla palps or the labial palp.

Mandibles broad at the base and more slender towards the apex. Large pair of teeth at the top and a smaller pair at the base, both of which face inwards. There are no setae or punctations on the mandibles. The mandibles are smooth and shiny.

The eyes on this specimen can be seen from the top and seems to have two sub conical tubercle protrusions in the front of each eye, making it look like another set of eyes.

The labrum can be seen from the dorsal view. It is not a triangular shape but rather

forms a hexagon with the top section jutting out like a square. There are no punctations on the labrum but there are setae present all over the surface.

The head has abundant punctations on the entire surface with the exception of the medial section, which is a smooth surface. Setae are present over the entire surface of the head with a few bald spots through the medial section. The head is relatively convex and there is no medial suture present.

The antennae are six segmented and have three segmented clubs that are fused. There are no punctations on the antennae or the clubs but there are short fine setae present on both.

Pronotum:

Medial suture on the pronotum is absent. Setae are visible beneath the pronotum plate but not on the dorsal surface. There are small punctations abundant all over. The pronotum has a dark outer margin that does not end abruptly but rather forms a shelf.

Elytra

The elytra have punctations that are small and abundant, the medial suture is present. The setae are sparse through the middle but dense on the outer margins. The elytra shape differs from the other species in that it is not long and oval but more long and triangular. The scutellum is not very deep and distinct.

There are seven protibial teeth and these become larger distally.

Lissotes DNA 31 (Fig. 5.25)

This beetle is approximately 14 mm in length from the apex of the mandibles to the posterior tip of the elytra. This is a reddish brown beetle.

Head

Punctations on the labium (Fig. 3.9) are large, round, deep and abundant. Although punctations are abundant, it is not densely presented on the labial surface. Punctations are spread across the labial surface in uniformity with large gaps between each

punctation. The setae on the labium are long, fine and visible on the entire labial surface. Setae become extremely abundant at the apex of the labium and on the outer margins compared to the surface.

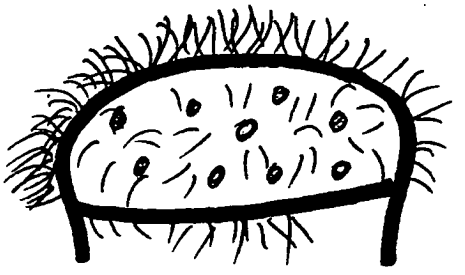


Fig. 3.9 Labium of *Lissotes DNA no. 31*

The maxilla has three segments each that are visible from the dorsal view. Each labial palp consists of a three segments as well. There are no punctations on the palps, but there are short fine setae present.

The mandibles on this species are very simple, having two apical cusps on each mandible. The mandibles are relatively thick and when clenched do not have a large space in-between. The mandibles have no setae but do have sparsely presented punctations that are round and small.

Labrum is visible from the dorsal view and appears as a square protrusion. The labrum has setae that are abundant all over but there are no visible punctations.

The antennae have six segments and three the clubs each is fused. There are short fine setae present all over the antennal surface. Punctations present, small and round.

The head has punctations all over the surface, and especially on the outer margins. There are setae on the outer margins of the head and the medial suture is absent. The head has sub conical tubercles in the medial section just above the labrum. The head is moderately convex and dips down wards to the anterior margins of the head and the base of the mandibles.

Pronotum.

Medial suture is absent; the pronotum is highly convex with dense punctations along the margins. The rest of the surface has abundant punctations that are round and uniform. There are setae only on the outer margins.

Elytra

The scutellum is a very sharp triangle. It is a deeper colour than the rest of the elytra. In most species in the genus the scutellum comes across as being engraved in the elytra. In this species however it seems to just be a marking and does not have an actual indentation. Medial suture present, deep and dark brown to black. The elytron has setae on the outer margins that are abundant and small. The punctations on the elytra are abundant, small and seem to be dense compared to the pronotum and head. The elytron also has a medial suture present.

There are seven protibial teeth and these become larger distally.

Lissotes DNA 39 (Fig.5.26)

This beetle is 17 mm in length from the apex of the mandibles to the posterior tip of the elytra. These are black beetle with a slightly brownish tinge.

Head:

Punctations on the labium (Fig. 3.10) are large and not very dense. The punctations are round and uniform across the surface. Setae are very sparsely distributed along the outer margins of the labium. They do not become abundant at the apex as they do in most other species.

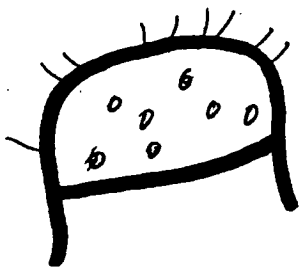


Fig. 3.10 Labium of *Lissotes DNA no 39*

The maxilla has three segments. Each labial palp consists of a single segment. There are no visible punctations or setae on the palps.

Each mandible has two apical cusps facing upwards and three apical teeth facing inwards. There are no setae on the mandibles but there are small punctations. These are not very deep or dense. The labrum is not visible from the top.

The antenna has five segments and a three segmented club. There are no visible punctations or setae on the clubs or antennal segments.

Punctations on the surface of the head are small and abundant. No setae. There is a slope in the front of the head as it goes towards the base of the mandibles. There is no medial suture on the head.

Pronotum:

The mildly convex pronotum has no medial groove. There are setae along the outer margins but none on the medial surface. There are small punctations all over the pronotum surface especially abundant on the outer margins. The apex of the pronotum however has no punctations.

Elytra

The scutellum is very well defined and deep. The elytron has setae on the outer margins. There are punctations that are abundant all over the surface of the elytra especially on the outer margins these punctations are oblong and are parallel to the

suture. A dark medial suture is present.

There are seven protibial teeth and these become larger distally.

Lissotes DNA 33 (Fig.5.24)

This beetle is approximately 15 mm in length from the apex of the mandibles to the posterior tip of the elytra. These beetles are black with a grey tinge.

Head:

Punctations on the labium (Fig. 3.11) are round, large and sparse. The setae on the labium are small and thin and are visible only on the outer margins of the labium. Setae become extremely abundant at the apex of the labium.

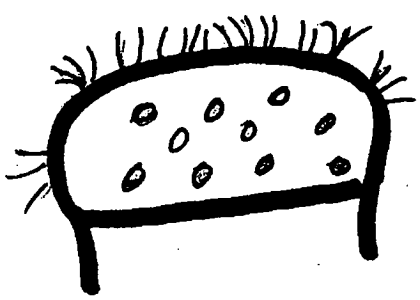


Fig. 3.11 Labium of *Lissotes DNA no 33*

The maxilla has three segments that are visible from the dorsal view. Each labial palp consists of one segment. There are no visible punctations or setae on the palps.

On each mandible there are two upward facings cusps at the apex, two sub apical cusps facing inward and another single basal cusp close to the labium. Mandibles are very thick, have sparse punctations that are small and visible only at the base. There are no visible setae.

The eyes specimen can be seen from above.

The triangular labrum is visible from the top. Setae present, a subconical tubercle on

the labrum. No punctations and a very glossy finish.

Large punctations abundant on the head and no setae present. Small sub conical tubercle present on either side of the head between the eyes and each mandible. There is no medial suture on the head. It has a convex surface that slopes downwards to the base of the mandibles at the apex of the head.

The antennae have 6 segments that have small setae and punctations. The clubs have 3 segments that are separated. There are small punctations and fine setae present on the antennal segments and clubs.

Pronotum:

The anterior region of the pronotum has no setae. Medial suture present. Setae on the outer margins of the rest of the pronotum. The pronotum has a convex surface.

Elytra

The scutellum is present but not a sharp triangle. The elytron has setae all over its surface. There are punctations that are abundant all over the convex surface of the elytra and especially abundant on the outer margins. Punctations on the elytra are oblong and parallel to the medial suture.

The elytron also has a medial suture present but it is not very well defined. It is visible mostly due to its darker colouring when compared to the rest of the beetle.

There are seven protibial teeth and these become larger distally.

Lissotes DNA 40

This beetle is approximately 16 mm in length from the apex of the mandibles to the posterior tip of the elytra. It is black with a reddish tinge.

Head:

Punctations on the labium (Fig. 3.12) are round, large and sparse. The setae on the labium are short, fine and visible only on the outer margins of the labium. The setae

are marginally more abundant at the apex.

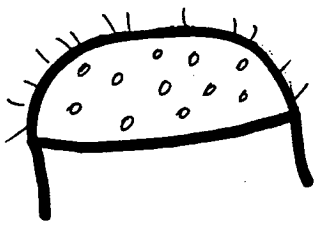


Fig. 3.12 Labium of *Lissotes DNA 40*

The maxilla has three segments each and each labial palp consists of a single segment. There are no punctations visible on any of the palpi but there are short fine setae present.

The mandibles have three apical cusps on either mandible that face upwards. A single cusp facing down and a basal cusp facing upwards on each mandible, close to the labium, that can be seen ventrally. There are no setae on the mandibles but there are small punctations. These are not very deep or dense.

The labrum is visible from the top and resembles three joined semicircles. There are no punctations on the labrum but there do appear to be short fine setae.

The antennae have six segments that have small setae and no punctations. The club has small punctations and small setae.

Punctations on the surface of the head are large and dense. There are no setae present.

There is no medial suture on the head. It has a convex surface that slopes downwards to the base of the mandibles at the apex of the head.

Pronotum:

The pronotum is very convex and has a medial suture. There are setae along the outer margins but none on the medial surface. There are small punctations all over the surface especially on the outer margins. The outer margins do not end abruptly but

instead have jagged edge.

The scutellum is present but not very well defined. There are no punctations present on the scutellum but there do appear to be very fine setae. The elytron has setae on the entire surface as well as oblong punctations that are parallel to the medial groove.

There are seven protibial teeth and these become larger distally.

3.2 Molecular results

3.2.1 Hotshot method

The results of the first extraction using the hotshot method were encouraging (figure 3.1). An interesting aspect of this first extraction was that the tissue sample from the leg of species one and the head tissue from species two yielded result. The remaining samples of leg and thorax both drew a blank. It seemed that the gene of choice COI and the universal primers COIL and COIH (see Chapter 2) used to amplify this region could be used in the beetles if a sufficient quantity and quality of DNA was used.

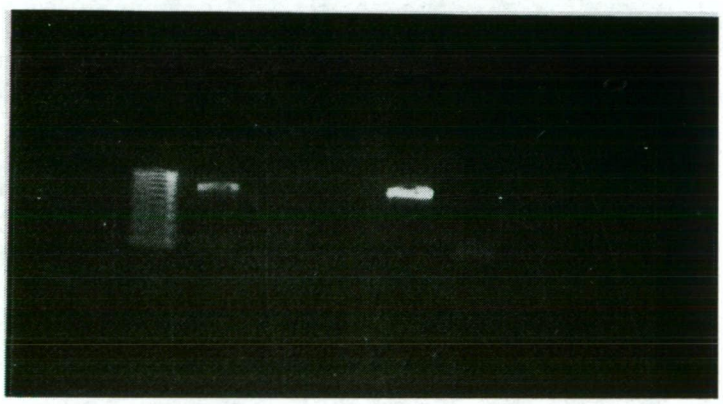


Figure 3.1 DNA yield from beetles using the hotshot method. Left band is from leg of beetle A; right band from head of beetle B.

Using the same extraction technique a set of *Lissotes* specimens were used. Both 100% ethanol-stored as well as dried specimens were used. A positive and negative control was used to monitor contamination. In this case the positive control was the positive DNA template from the previous result.

The result after this PCR was disappointing as the entire process showed no results including the positive control. The conclusion was that there was a failure in the

PCR process and so the entire process was repeated. This time the results drew a blank except for the positive control (figure 3.2). This demonstrated that the process did work but in all probability the PCR controls did not allow the primers to anneal to the particular samples. Before this method was completely dismissed the PCR was run once again under more relaxed PCR conditions. Again there were no results.

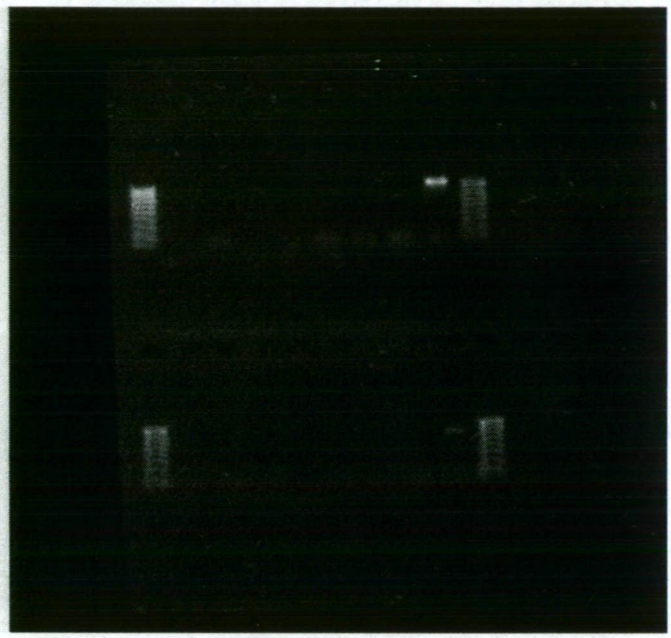


Figure 3.2 Hotshot extractions of *Lissotes* showing failure to amplify required DNA region (except for control at right).

3.2.2 The CTAB method

The next approach was to pursue COI but using a different extraction technique. The CTAB method is known to yield better DNA quality though the process is time consuming. Once the extraction was completed the PCR was run using the same COI primers and under the same conditions. Once again a positive control was used and in this case it was a known fish DNA, the orange roughy. The positive control worked but the COI did not have very good results (Figure 3.3).

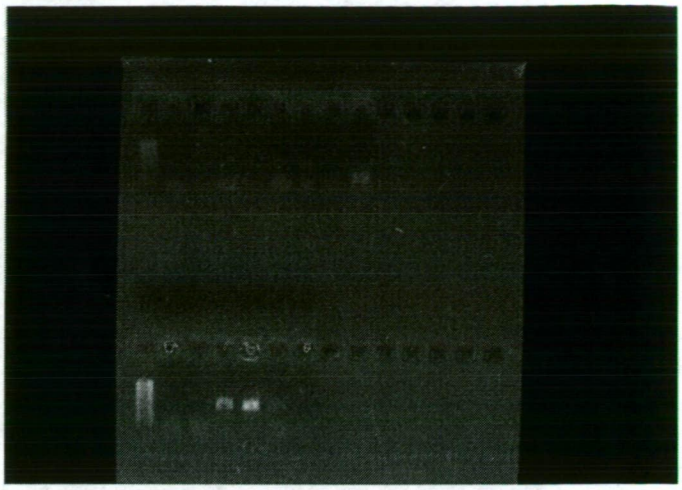


Figure 3.3 CTAB extractions of *Lissotes* showing failure to amplify required DNA region (except for control).

3.2.3 Comparing different primers

By this stage bearing in mind the time constraint for this project and seeing that things were not going too well something had to be done to check the quality of DNA and the quality of the primers. To check what the potential problem might be a PCR was run that included 3 separate batches of the same DNA templates all run with different primers. Primers included the dloop primers, COI primers and Cytochrome b. Orange roughy was used as the positive control. Of the three groups, dloop did not show a result, COI returned a few results but they were weak, and the Cytochrome b came up with a very strong result (figure 3.4).

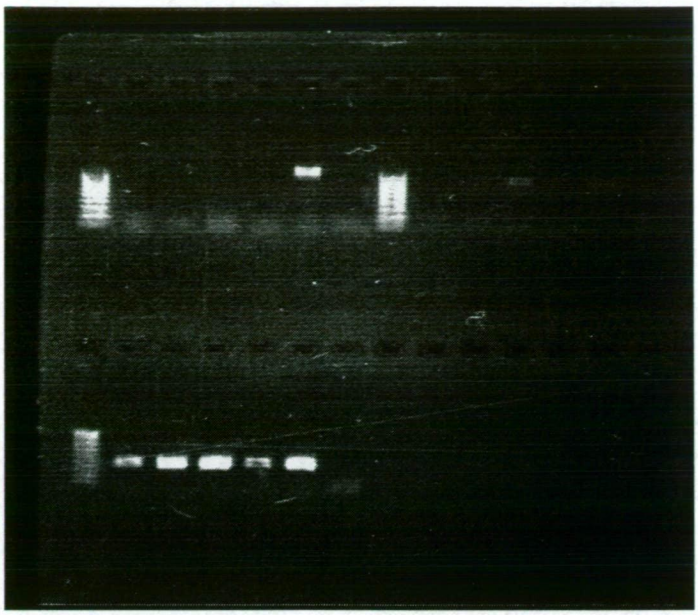


Figure 3.4 DNA yields for 3 primers from 4 *Lissotes* beetles plus orange roughy control: dloop (top left), COI (right), cytochrome b (bottom).

3.2.4 Switching primers and genes

The next step involved switching the primers and changing the gene that the project was targeting.

Reactions were run under different conditions than the PCR for COI. However, the results on the PCR were not as good as expected. Of the 18 specimens that were run, and after many attempts of different PCR conditions, a total of 11 specimens showed some product yield although none of them were very strong.

These 11 products were then processed for sequencing and the first sequence was run using the GLudG reverse primer of cytochrome b. A total volume of 10 microliters made up the sequencing reaction. Of the 11 products submitted for sequencing, only three showed a result. The sequences were about 350 bp but they were not very strong signals and the data not good enough to align and go on.

Another sequencing reaction was set up using cytochrome b primer CB2H which is a forward primer. This called for using the previously extracted DNA templates. This yielded six useable results (See Appendix 4 for the full sequences).

The six samples were cleaned using Sequenture 4.5 and a search was run on the World Wide Web for a sequence match. The closest BLAST matches to the *Lissotes* sequences on the web were to butterflies and mosquitoes. Because of the low signal strength in the signals a cleanup was done of the sequence and then an alignment. Sequencher 4.5 was able to align just two of the total of six sequences. A phylogenetic study was therefore not able to be completed at this stage.

Chapter 4

4.0 Discussion

4.0 Morphology Study

Differences between species of *Lissotes* are most apparent in features of the mandibles, labium, labrum, antennal clubs, scutellum and in the shape of the head. However, variation within species is also considerable, and here remain difficulties in recognising the limits of species based on external characters.

Mandibles display allometry, a phenomenon where the body parts of an organism grow at different rates. This is a common feature of male stag beetles . The varying degree of size in the mandible (modified mouth parts) of individuals of the same species relates to allometric growth. Differences in body size may relate to food intake in the larval stage which can last up to five years and during this period they feed on dead wood. If the food supply runs out then the larvae may pupate prematurely at a smaller size. Another determinant of small mandibles could be the nutrient quality of the dead wood.

However if the quality and quantity of dead wood is not limiting, then the mandibles tend to grow relatively larger than the body does. Studies by Hung and Simmons *et al*, 1999, on dung beetles show that the regulation of horn size by food is mediated through the prepupal endocrine system, wherein the hormone ecdysone synthesis stimulates horn growth.

Enlarged mandibles are a secondary sexual character and males use them to fight off opponents. One would assume that this would put males with smaller mandibles at a disadvantage. However, smaller beetles may have a behavioural advantage whereby they sneak in and mate with the females while the males with the larger mandibles are fighting each other.

Environmental conditions may also contribute to morphological variation among individuals by affecting body size and the expression of certain morphological traits; however the scaling relationship between a morphological trait and body size over a

range of body sizes is generally assumed not to change in response to environmental fluctuation (allometric plasticity), but instead to be constant and diagnostic for a particular trait and species or population.

The *labium* offers some potential to separate species as it varies widely as demonstrated in this study. Lea (1910) noted the labium was often obscured, with dirt that obscured it use as an identifying character. Today, microscopes and a dab of ethanol help us use the labium as a diagnostic character. The labium displays a variety of shapes and sizes (see chapter 3). The surfaces of the labia have punctations and setae that seem to differ from species to species as do the number of segments on the palps. Moreover, the variability within species is negligible for these characters. This being the case, the labium should be used as a reliable character in lucanid identification.

The *labrum* is not always conspicuous. It is visible between the mandibles and also varies in shape and size between species. Other variables associated with the labrum include the size and shape of punctations and the presence and relative length of setae. Because the labrum is obvious only in some species it can not be used as a universal feature for identifying *Lissotes*. In the species examined in this project there were no two that had the same shape of the labrum.

Though all the specimens displayed a *scutellum* once again there are individual differences in the shape size and external characteristic of the scutellum. In most cases it is well defined. In some it is smooth and triangular in others there are punctations and in others still there are setae. Amazingly there is difference in the shape. Though it is commonly triangular, the point of the apex can be acute or rounded.

The shape and size variation of the head among species is very prominent. In some species the margin of the head is expanded behind the eyes while in others the margin just curves into the pronotum smoothly. In most cases the head is transverse (i.e. wider than long).

In summary, it is clear that some previously neglected features of the external morphology have excellent utility as diagnostic features for separating the species.

4.1 Molecular Study

The universal DNA primers, LCO 1490 and HCO 2198 (Folmer *et al*, 1994) that were used in this project, have been used to amplify a 710 bp region of the mitochondrial gene COI from a broad range of invertebrates. A number of conclusions might be drawn from the lack of results achieved through these COI primers in this study.

In all probability the COI primers that were used in the PCR were not sufficiently specific to *Lissotes* and the region that was being targeted. DNA amplification can also be sensitive to PCR conditions, e.g. annealing temperature and the number of cycles could influence the final outcome. Though a number of different conditions were trialled, there was unfortunately no improvement in the outcome.

The options were to look out for a specific primer combination that has been used in the past and to get them synthesised. This would have taken a long time and is potentially expensive.

Of the 18 specimens for which DNA was extracted in this study, 11 were amplified using cytochrome b (Palumbi, 1996), but results were poor. There are several possible reasons for this:

(i) Insufficient homology between template and primers

(ii) Potential fault with DNA template

(iii) Insufficient amounts of DNA- quantity and quality

(iv.) Contamination from outside especially in the case of the dried specimens where the specimens were handled without gloves before the extractions.

An internet database of DNA sequences called BLAST was used to match up the sequenced results so as to have an idea of the basic alignment of nucleotides in beetle DNA. Unfortunately there were very limited sequences on cytochrome b but a match was found for the three sequences. The most common closest sequence that aligned with the beetles was that of butterflies and mosquitoes.

Because the signal of the sequences was low, cleaning up these sequences meant that there is a lot of subjectivity with regard to what nucleotide region has to be removed and what can be put in.

4.2 Conservation aspects

Lucanids are saproxylic beetles which mean that they depend on dead, moribund and decaying wood. Lucanids are also xylophagous and are most likely also stenophagous for the tree type and decay type in particular (Meggs, 1999). It is clear from this that various stages of decay of various forest types are very important for the survival of the species. A continuous supply of this fallen decaying wood is essential for the survival of stag beetles and in return these beetles contribute to the regrowth of forests by breaking down wood, releasing nutrients, and priming old logs for a succession of other plants and animals.

Today, two of Tasmania's *Lissotes* species, *L.latidens* and *L. menalcas,* are formally listed as threatened. Although Tasmania was one of the last Australian states to pass a Threatened Species Act in 1995, it is notable for giving due emphasis to a range of rare and threatened invertebrates. It has been known for many years that some Tasmanian stag beetles have restricted ranges and, given their profile among collectors and others, it was inevitable that some would receive conservation assessment (Meggs, 1999). An acceleration in the rate of wood chipping and conversion of natural habitats to plantations since the early 1990s has further focussed attention on forest dwelling species.

Lissotes latidens is one of the few invertebrates listed as endangered under the Commonwealth *Environment Protection and Biodiversity Conservation Act 1999.* It is also listed as endangered under the state's *Threatened Species Protection Act 1995* due to the restricted distribution of the species, continued threatening processes throughout its distribution and small population density. The species satisfies the criteria for Endangered under the State Act because:

- Its extent of occurrence is less than 500 km2 (Criterion B2)

- There is a potential decline in numbers due to the operation of threatening processes

throughout its range (Criterion A1)

- it has a restricted range (Criterion B)

L. latidens is found almost exclusively in the Wielangta forests of southeast Tasmania. The species was first described in 1871 and has since been recorded at about 40 sites scattered across an area of 280 square km, including Maria Island (Meggs & Munks, 2003).

At a regional level, the restricted distribution of *L. latidens* appears to be partly due to an interrelationship between climate, topography and rainfall (Meggs & Munks, 2003). The Wielangta forests occupy an area of relatively high rainfall for southeast Tasmania (Meggs & Munks, 2003). Preferred habitat for *L. latidens* includes wet forest with well-developed canopy cover and a ground cover of CWD (greater than 10%) where CWD is dominated by small (>10 cm) and medium (10-50 cm) diameter logs (Meggs & Munks, 2003). The preference of *L. latidens* for wet forest with these characteristics may relate to a requirement of the beetle for a relatively cool and moist micro-climate (Meggs and Munks, 2003). Although the quantity of CWD appears to be an important component of the habitat of *L. latidens*, the species is not a log dweller (Meggs & Munks, 2003). However, these logs may help to provide a cool microclimate, protection from predators and adverse weather conditions. They may also place high quality nutrients into the soil for larvae to feed on. However the exact nature of this relationship between beetle, soil and logs is unknown (Meggs & Munks, 2003).

Only broad forest type is a reliable predictor of the likely presence of *L. latidens*. Within its current known range it occupies a range of wet forest communities including wet eucalypt forest, damp eucalypt forest and rainforest (Meggs & Munks, 2003). One essential habitat requirement of the species is a moist environment with good soil quality (Meggs & Munks, 2003). This may be why *L. latidens* prefers riparian environments surrounded by otherwise unsuitable drier forest types.

The main threatening process that will affect the survival of *L. latidens* is the loss of its forest habitat (Micheals & Bornemissza, 1999). This habitat loss is caused predominantly by land use practices (both forestry and agriculture). Approximately

20% of forest cover within the species area of occurrence has been cleared since European settlement, mostly for agricultural purposes. The Wielangta forests are now threatened by commercial logging. Bulldozing and burning disturbs litter and logs, dries out forest floor by opening the canopy and kills regenerating trees that are potential habitat logs in years to come. Replacement of these forests with plantations will completely destroy habitat for the species.

Little is known about the population size of *L. latidens* but there are indications that it occurs at lower densities in comparison to some other *Lissotes* species and over half of its suitable habitat is in State forest potentially available for production forestry.

The species is identified as a Category 6 fauna species (those species believed to be at risk but whose conservation needs cannot be assessed without further research on their distribution and/or habitat requirements) under the *Tasmanian Comprehensive Regional Assessment* process (Tasmanian Public Land Use Commission, 1997).

It was also identified as a priority species "requiring recovery action" under the *Tasmanian Regional Forest Agreement* (Attachment 2, Part A.1) signed between the Commonwealth of Australia and the State of Tasmania in November 1997.

Land Tenure	Potential *L. latidens* Habitat (ha)	Potential *L. menalcas* Habitat (ha)
Formal reserves	780 (19%)	16097 (13%)
Informal reserves	470 (11%)	8805 (8%)
State forest (couped)	2360 (56%)	65127 (53%)
Other public land	0 (0%)	1005 (1%)
Private Property	600 (14%)	30275 (25%)
Total	4210 (100%)	121308 (100%)

Table 4.1 The distribution of potential habitat for *L. latidens* (taken from Meggs & Munks 2003) and *L. menalcas* (Meggs pers. data 2000) based on land-use categories.

Another species *Lissotes menaclas*, also known as the Mt Mangana stag beetle, has come to the attention of various natural resource management bodies in recent years.

L. menalcas is classified as vulnerable under the Tasmanian *Threatened Species Protection Act (1995)* because:

- there is a predicted extent of habitat loss over the next 10 years (Criteria A1);
- it is predicted the species will undergo a population loss of 20% in the next 10 years (Criterion A2).

Since its nomination to the act, surveys have determined an increased range for the species and its status could be reassessed, especially if recovery actions are successful.

It is identified as a Category 3 fauna species (those species whose conservation needs are to be met by management prescriptions) under the *Tasmanian Comprehensive Regional Assessment* process (Tasmanian Public Land Use Commission, 1997) due to its restricted habitat range and threatening process such as silviculture (depletion of CWD).

It was also identified as a priority Ssecies "requiring recovery action" under the *Tasmanian Regional Forest Agreement* (Attachment 2, Part A.1) signed between the Commonwealth of Australia and the state of Tasmania in November 1997.

L. menalcas can occur in a broad range of wet forest communities ranging from old-growth mixed forest to 23 year old wet silvicultural regrowth forest (Meggs & Taylor, 1999). The species may also occupy rainforest because it has been recorded in logs of rainforest species (Meggs & Taylor, 1999). It occurs below about 650 m and prefers a rainfall between 700 mm to 1200 mm.

L. menalcas occupies a total predicted range of 3050 km square (Gove *et al.*, 2002) in south eastern Tasmania, including the Tasman and Forestier Penninsulas, parts of the Wellington Range and Mount Mangana on Bruny Island. This habitat is threatened by potential human developments

Meggs & Taylor (1999) consider that plantation development in native forest will almost certainly lead to the local extinction of *L. menalcas* populations due to the

depletion of rotting logs suitable as habitat. The removal of these rotting logs is the single most threatening process because both adults and larvae live within them. By the second plantation rotation most of this resource has rotted away and there is little or no replacement of logs on the ground. It is important that appropriate prescriptions for the management of these logs (CWD) be implemented to manage rotting log resources when large areas of native forest are converted to plantation (Meggs & Taylor, 1999). Any silvicultural intervention (other than tree thinning which may in fact increase CWD (Mark Wapstra, quoted in Yaxley 2004) will lead to the long-term depletion of CWD habitat relative to natural disturbances, particularly large diameter logs.

The conservation status and potential threatening processes for Tasmanian stag beetles were identified by Jackson & Taylor (1995); *L. latidens* by Meggs & Munks (2003), Michaels & Bornemissza (1999); and *L. menalcas* by Meggs & Taylor (1999).

In Tasmania the removing of complex vegetation and ecology to be replaced by single aged monospecific stands can threaten the existence of many of Tasmania's endemic species.

Neither the Tasmanian nor the Commonwealth governments have a recovery plan in place for the species. Senator Bob Brown on the 30th of May 2005 launched legal action in the Federal Court against Forestry Tasmania in order to test the efficacy of the interagency agreement between Forestry Tasmania and DPIWE as an instrument delivering protection as required under the EPBC 1999 Act. At time of writing the judge has still reserved his decision.

There has been a vast amount of interest in beetle ecology throughout the world. Simon Grove, one of Australia's foremost researchers in the field of log associated beetle ecology conducted a study on Queensland's rainforest beetles and the effect of logging on their population numbers. He (Grove, 2002) encountered 17,000 individual beetles of which 500 were saproxylic species. Form this mass of data certain patterns emerged:

- Old growth logged and regenerated forests hardly differed in the numbers of species they support

- Old growth forests yield twice as many individuals per trap as logged regrowth

- Old growth, logged and regrowth forests support different combinations of species

The Warra log decay study conducted by Grove and Bashford (2003) demonstrates a rich composition of saproxilic beetle diversity in *Eucalyptus obliqua* logs in an early stage of decay. The study aimed at providing answers with regard to the divergence in assembledge composition between old growth and regrowth logs. The study showed that more individuals were collected in old growth forest than in new growth but in all probability this was because of the differences in the surface area or volume of logs available. Plantations have fewer logs on the ground and will provide fewer in the future. This will disadvantage stag beetles by denying them access to breeding sites.

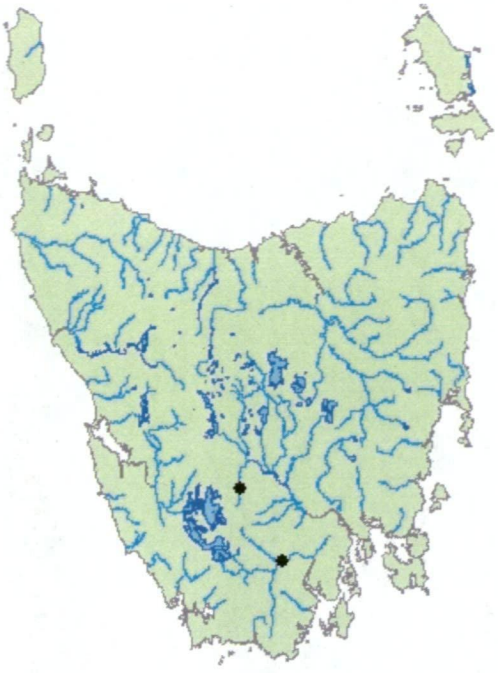
Chapter 5

5.0 Maps

Figures 5.1 to 5.17 are the distribution maps of *Lissotes* in Tasmania. These maps are based on the data presented in chapter one from previously recorded specimens.

Figures 5.18 to 5.21 are the distribution of some majore forest types preferred by stag beetles (TASVEG data with permission of DPIWE).

Figures 5.22 to 5.30 are photographs of some of the specimens used in this study.



Lissotes cancroides
Figure 5.1

N

Lissotes convexus
Figure 5.2

N

Lissotes cornutus
Figure 5.3

N

Lissotes crenatus
Figure 5.4

N

Lissotes curvicornis
Figure 5.5

N

Lissotes desmaresti
Figure 5.6

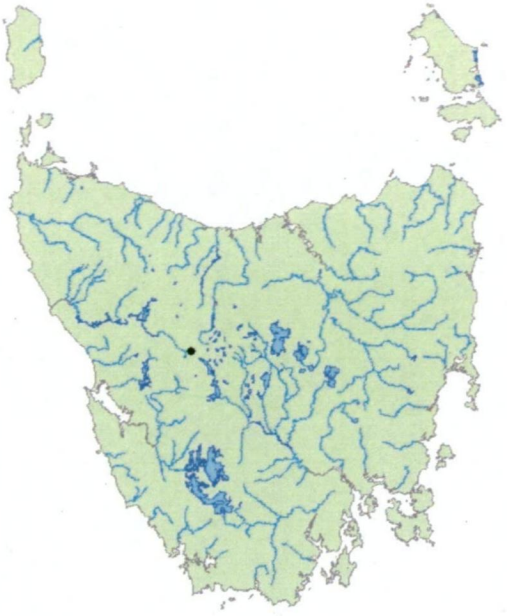
N

Lissotes distinctus
Figure 5.7

N

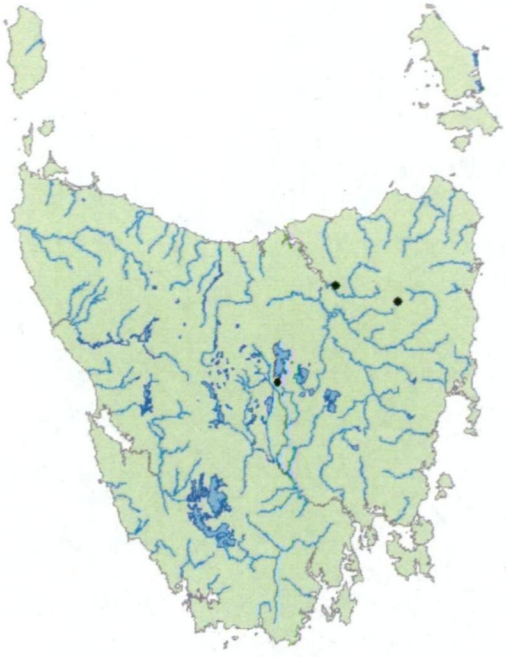
Lissotes globosus
Figure 5.8

N



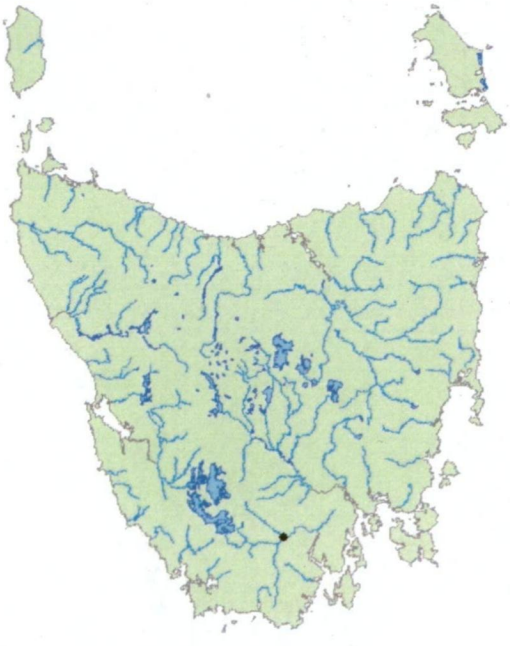
Lissotes lacroixi
Figure 5.9

N



Lissotes launcestoni
Figure 5.10

N



Lissotes menalcas
Figure 5.11

N

Lissotes obtusatus
Figure 5.12

N

Lissotes politus
Figure 5.13

N

Lissotes rodwayi
Figure 5.14

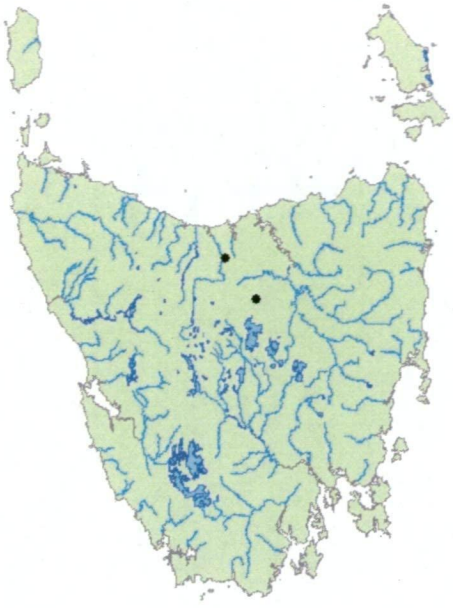
N

Lissotes rudis
Figure 5.15

N

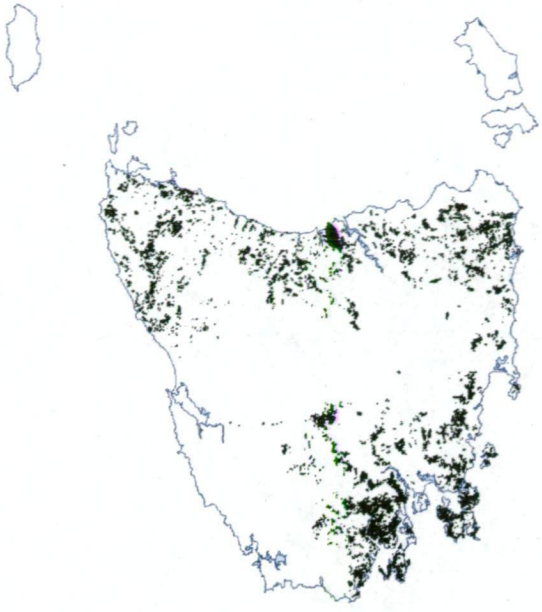
Lissotes subcaeruleus
Figure 5.16

N



Lissotes urus
Figure 5.17

N

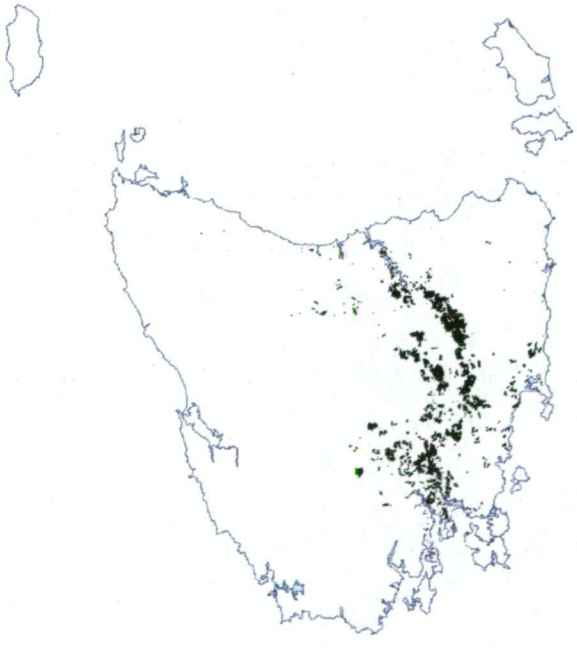


Dry *Eucalyptus obliqua*
Figure 5.18

N

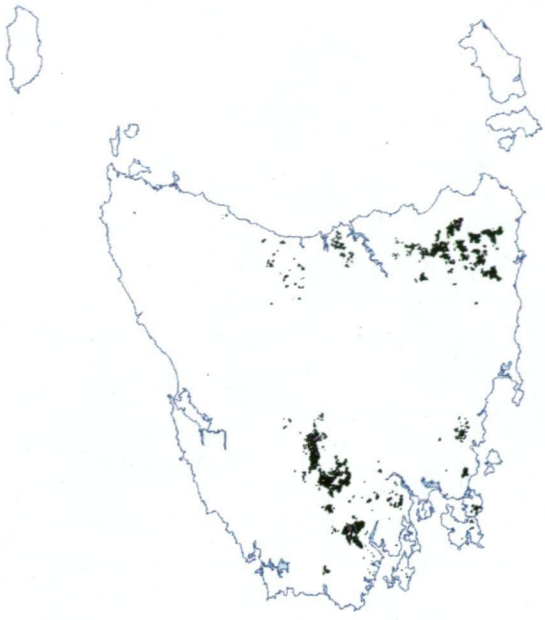
Wet *Eucalyptus obliqua*
Figure 5.19

N



Eucalyptus viminalis
Figure 5.20

N



Eucalyptus regnans
Figure 5.21

N

Fig. 5.22 *Lissotes DNA no.37*

Fig. 5.23 *Lissotes rodwayi*

Fig.5.24 *Lissotes DNA no 33*

Fig. 5.25 *Lissotes DNA no 31*

Fig.5.25 *Lissotes rudis*

Fig.5.26 *Lissotes DNA 39*

Fig.5.27 *Lissotes cancroides*

Fig.5.28 *Lissotes basilaris*

Fig.5.29 *Lissotes obstutatus*

Fig.5.30 *Liissotes DNA 32*

Chapter 6

6.0 Conclusion

This study has assembled most of the relevant literature associated with *Lissotes* taxonomy, biology, distribution and conservation from over the last two centuries. It highlights the fact that Tasmania has a unique fauna of stag beetles some of which are highly threatened by current land use practices.

Although the attempt at molecular resolution of species boundaries was ultimately unsuccessful due to technical difficulties following DNA extraction, resource constraints did not permit the process to continue. Nevertheless, it may be a productive line of future research.

It is still unclear what the population sizes are for species in this genus. More field sampling needs to be done and distributions related to environmental features in order to model distributions more accurately. This will in the long run aid future conservation efforts.

References:

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Appendix 1 - Stocks

Alkaline lysis

25 mM NaOH

0.2 mM disodium EDTA

pH of 12- prepared by dissolving the salts in water and not adjusting the pH.

Neutralizing regeant

40 mM Tris- HCL

pH of 5 – prepared by dissolving Tris HCL in water without adjusting the pH.

CTAB buffer

1 M Tris-HCL with pH of 8

0.5 M EDTA

5 M NaCL

10 gm hexadecyltrimethylammonium bromide

10 x Reaction buffer

67 mM Tris-HCL with pH of 8.8

16.6 mM (NH4)2 SO4

0.4 % Triton X-100

0.2 mg/ml Gelatine

Stop Solution

1.5 M NaOAc + 50 mM EDTA

Appendix 2 - Glossary of Terms :

Abdomen

The hindmost of the three main body divisions of an insect: head, thorax and abdomen.

Antenna (plural antennae)

The sensory organs on the head. Stag beetles have two antennae or 'feelers' with a characteristic shape at the end, which is the same for all the beetles that belong to the Lucanidae family. It is with their antennae that stag beetles pick up important pheromone signals.

Coxa (plural coxae)

The first segment of the insect leg, often firmly attached to the body.

Dorsal surface:

Surface on the back of the body.

Elytron (plural elytra) :

The hard wing case of a beetle.

Femur (plural femurs or femora) :

The third, and often the largest segment of the insect leg.

Instar:

The stage in an insect's life history between two moults.

When a stag beetle larva first comes out of the egg is said to be the first instar.This tiny larva has a rigid head so, in order to grow, it has to shed its skin by moulting. This way the second instar is the larva after the first moult. The third instar is the larva after the second moult and so on until it gets to the fifth instar; sometimes in captivity a sixth instar might happen. At this stage the fully grown larva pupates and the imago is called the last instar.

Labrum:

The 'upper lip' of the insect mouth-parts: a small hardned body part on the front of the head. In stag beetles it is clearly seen between the jaws. Its shape can be a good identification clue as it varies quite a bit between species and gender.

Mandible:

The jaw of an insect. In male stag beetles they are very large and look like antlers, hence the name "stag beetle".

Palps:

Short feelers coming from the mouth. There are two pairs of palps in stag beetles; they are mostly for tasting food.

Pronotum:

The back surface of the first part of the thorax. In a stag beetle, looking from above, it is the body part between the head and the wing cases.

Pupa (plural pupae):

The third stage in the life cycle of a stag beetle while it undergoes a complete metamorphosis. It is during the pupal stage, which does not feed and does not move about, that the larval body of a stag beetle is rebuilt into that of an adult insect.

Saproxylic Invertebrates:

Species of invertebrates that are dependent, during some part of their life cycle, upon the dead or dying wood of moribund or dead trees (standing or fallen), or upon wood-inhabiting fungi, or upon the presence of other saproxylics.

Scutellum:

The part of the middle segment of the thorax, which you can see from above. In stag beetles it is the triangular bit at the top of the wing cases.

Stridulation:

The production of sounds by rubbing two parts of the body together; best known in crickets, grasshoppers and cicadas. Larval Lucanidae, also stridulate, rubbing a series of ridges on the coxa of the middle legs with the plectrum on the trochanter of the hind leg.

Tarsus:

The 'foot' of the insect: primitively a single segment but now usually divided up into several sub-segments.

Thorax:

The middle of the three major divisions of the insect body. The wings and the legs always come from the thorax. The thorax is itself divided into three segments, one for each pair of legs. In stag beetles the first segment is called the pronotum and is easily seen from above. The other two segments are hidden by the wing cases, except for a triangular bit of the middle segment, the scutellum. Seen from below each thoracic segment has a pair of legs attached to it.

Tibia:

One of the segments of the leg, between femur and tarsus.

Trochanter:

A segment of the insect leg between the coxa and the femur, often very small and easily overlooked. However the trochanter in the hind legs of stag beetle larvae is very well developed.

Appendix 3 – Morphology results

Species Name	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
Lissotes basilaris	1	3	1	2	2	2	1	0	0	0	0	2	1	0	2	0	0	1	0	2	1	1	0	2	1
Lissotes cancroides	1	3	2	2	2	0	1	1	0	2	0	2	1	0	0	0	0	2	3	2	1	1	2	0	1
Lissotes obtusatus	1	3	1	2	2	3	1	1	1	3	0	1	0	0	1	0	1	1	3	1	1	1	2	1	1
Lissotes rodwayi	1	2	2	2	0	0	1	0	0	0	0	2	1	1	1	0	0	1	3	2	1	1	3	1	1
Lissotes rudis	1	3	1	2	3	0	1	1	2	2	2	2	0	1	1	0	1	1	3	1	1	1	3	0	2
Lissotes urus	1	3	2	2	1	2	1	1	1	0	1	2	2	2	2	0	0	1	3	1	1	1	1	0	0
Lissotes 31	2	1	2	2	3	0	1	1	0	0	2	2	1	2	2	0	0	1	3	1	1	1	3	0	1
Lissotes 32	1	3	2	2	0	0	1	1	2	2	0	1	1	0	1	0	0	1	0	1	1	1	2	0	0
Lissotes 33	2	2	1	2	3	0	1	1	0	2	2	2	0	1	1	0	1	1	3	1	1	1	1	0	0
Lissotes 39	1	2	1	2	2	0	1	0	0	0	1	0	1	0	0	0	1	1	3	1	1	1	3	1	0

Appendix 3

Presenting data that corresponds to the morphological character set. This worksheet provides a brief overview of the variation of characteristics between species in the genus *Lissotes*.

Appendix 4 - Sequencing results

Sequenced Sample number	Species
KF3.C11_060520017Q	DNA 39
KF5.E11_060520017Q	DNA 45
KF6.F11_060520017Q	*Lissotes basilaris*
KF2.B11_060523104K	*Lissotes obsutatus*
KF6.F11_060523104K	DNA 32
KF7.G11_060523104K	*Lissotes cancroides*

Sequence Results: KF6.F11_060523104K

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el Algorithm Type:			
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uffer Part Number:			
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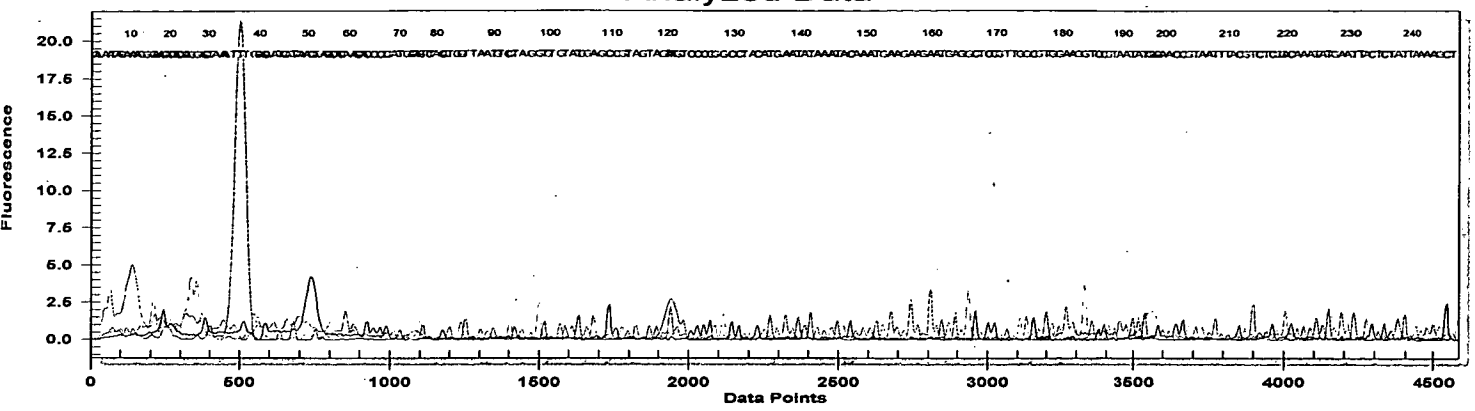

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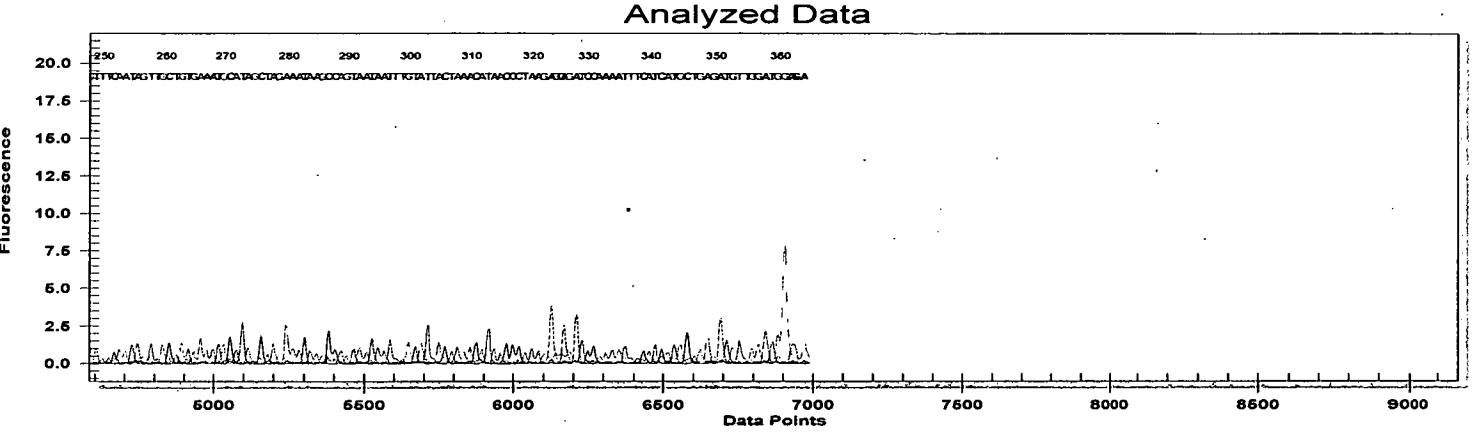

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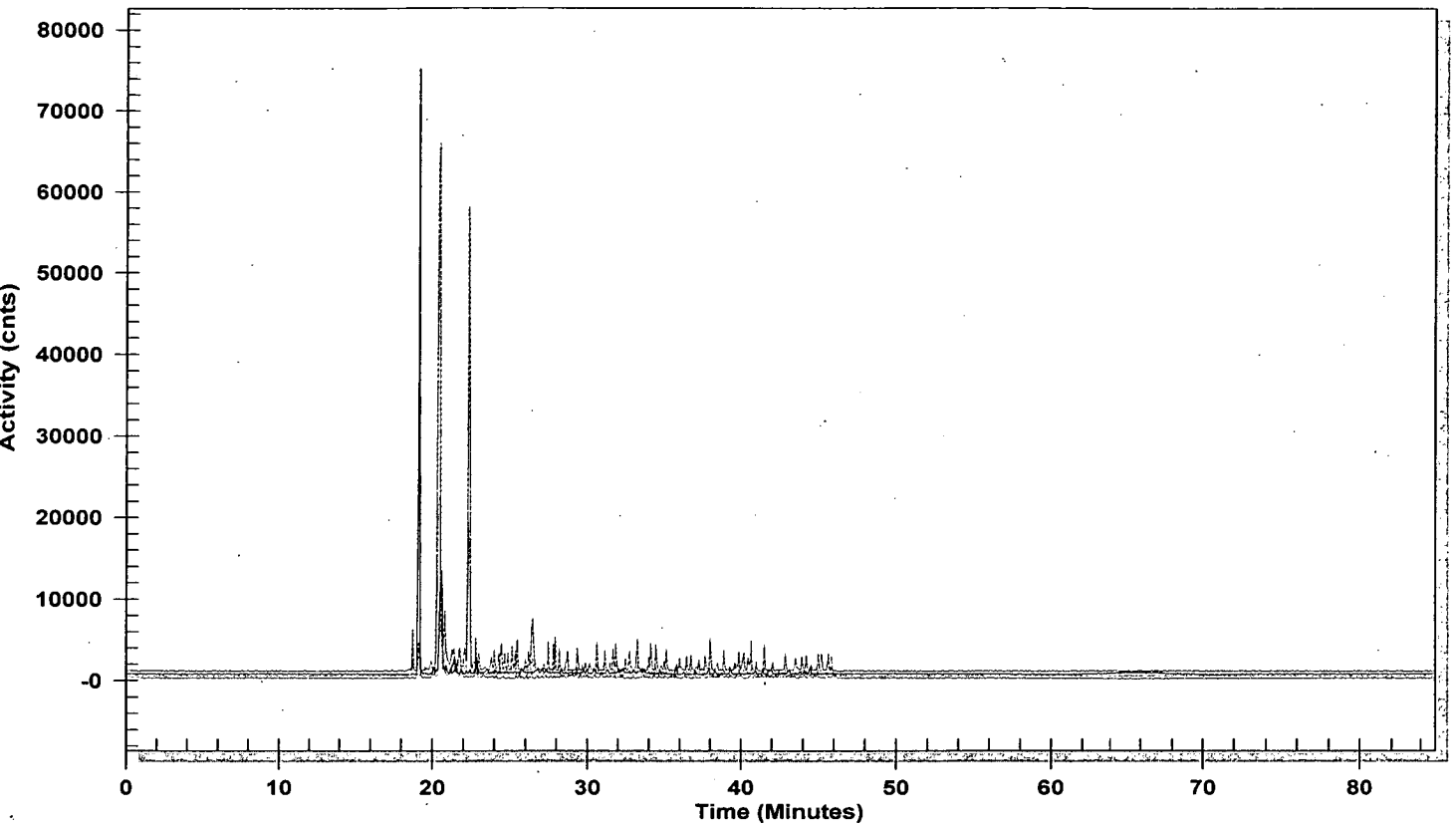
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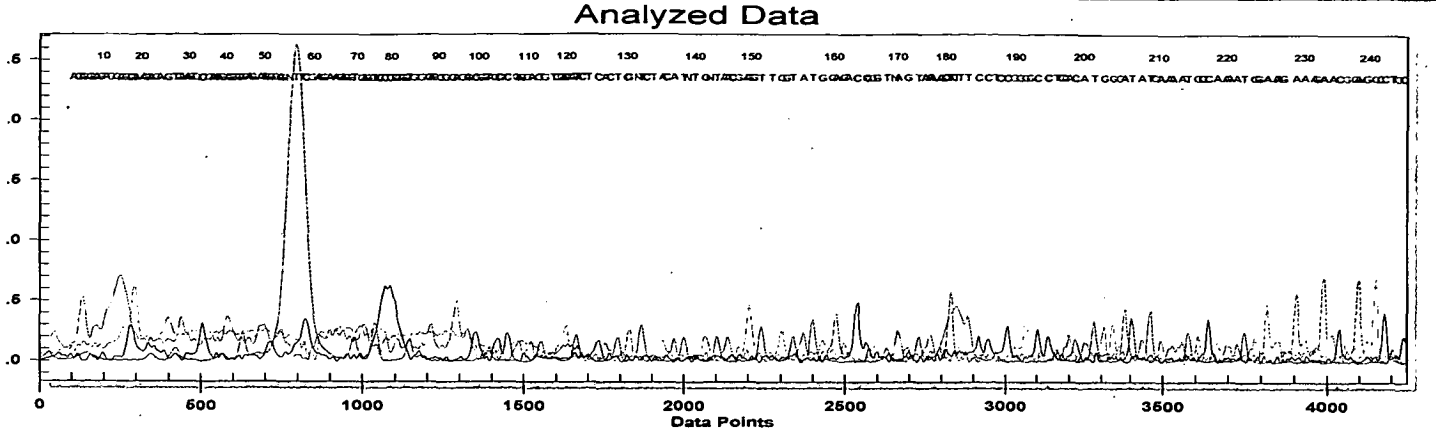

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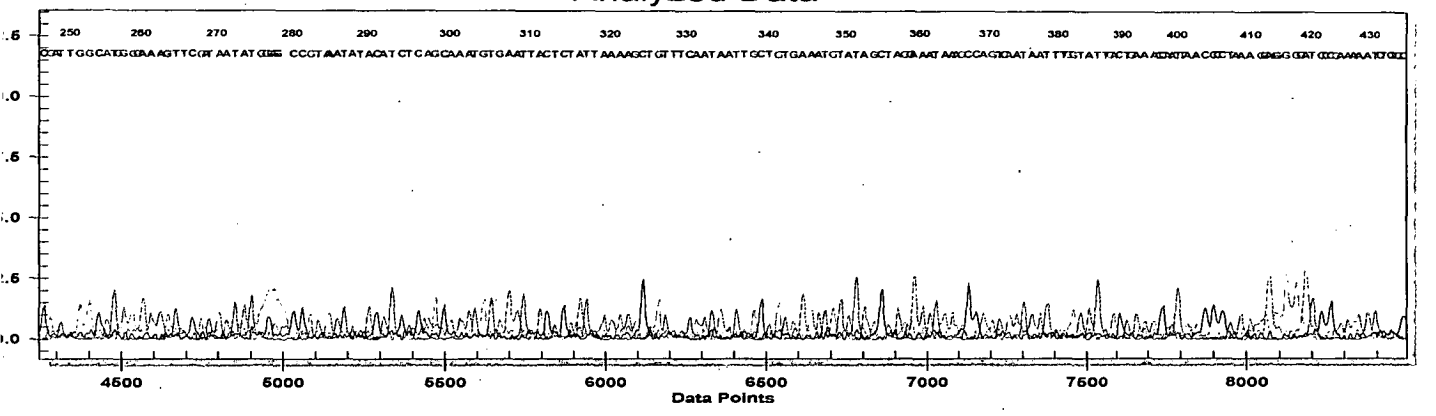

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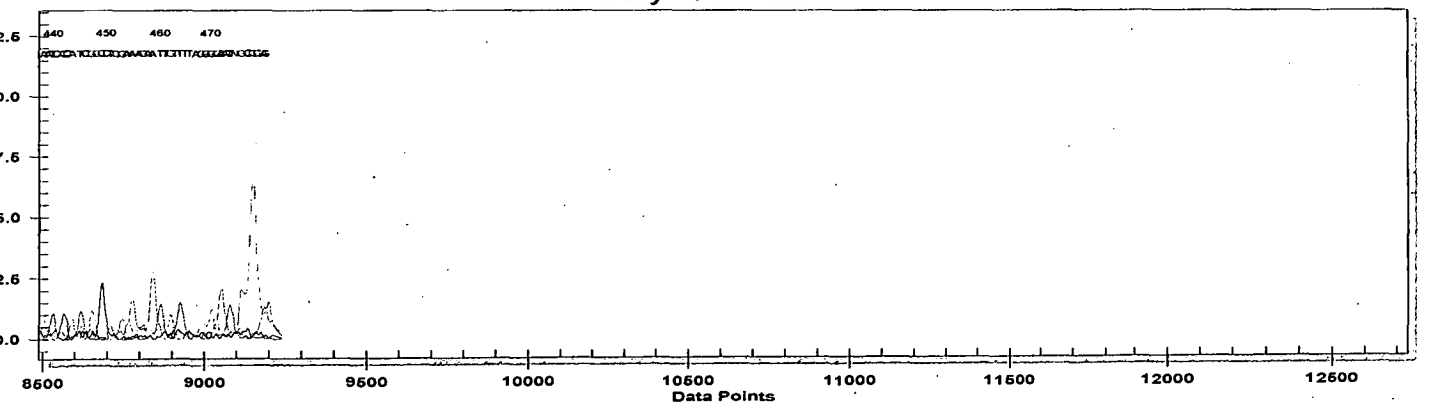

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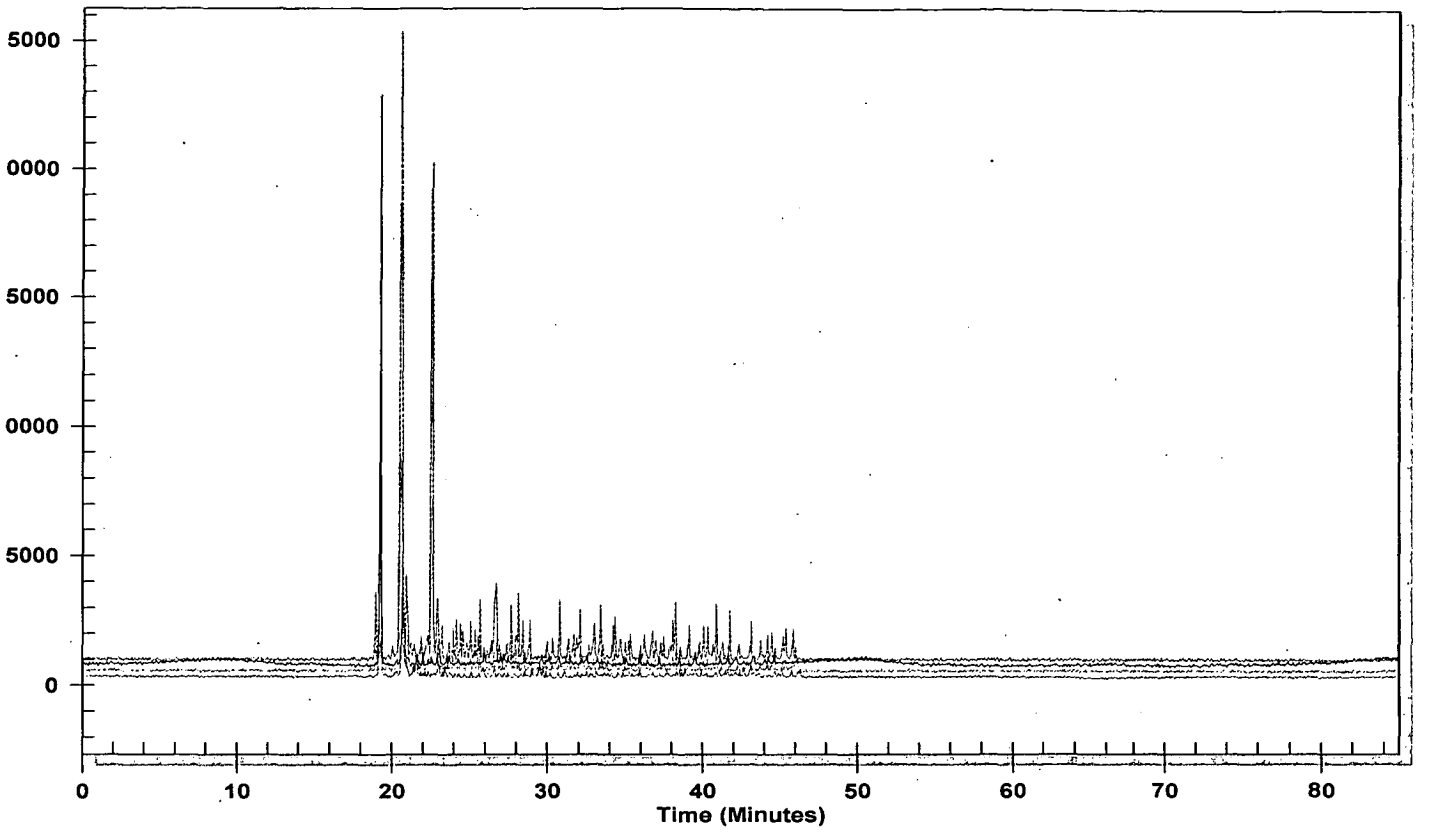

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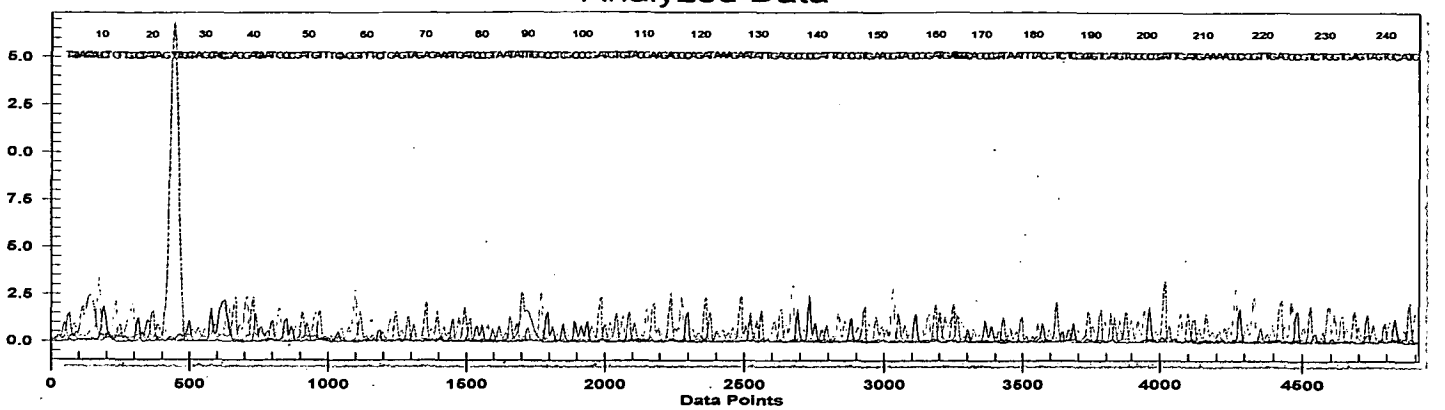

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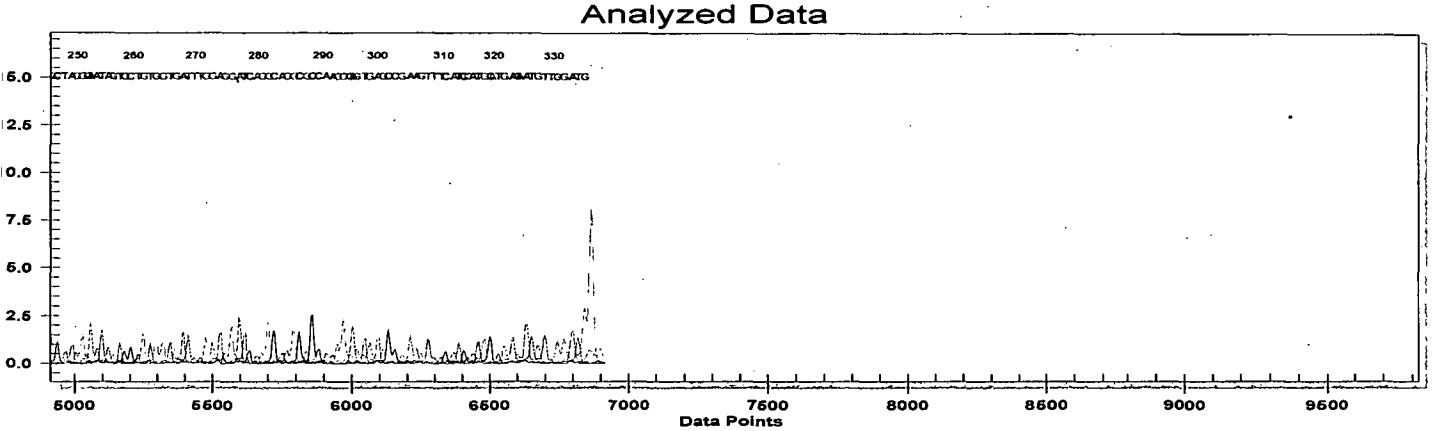

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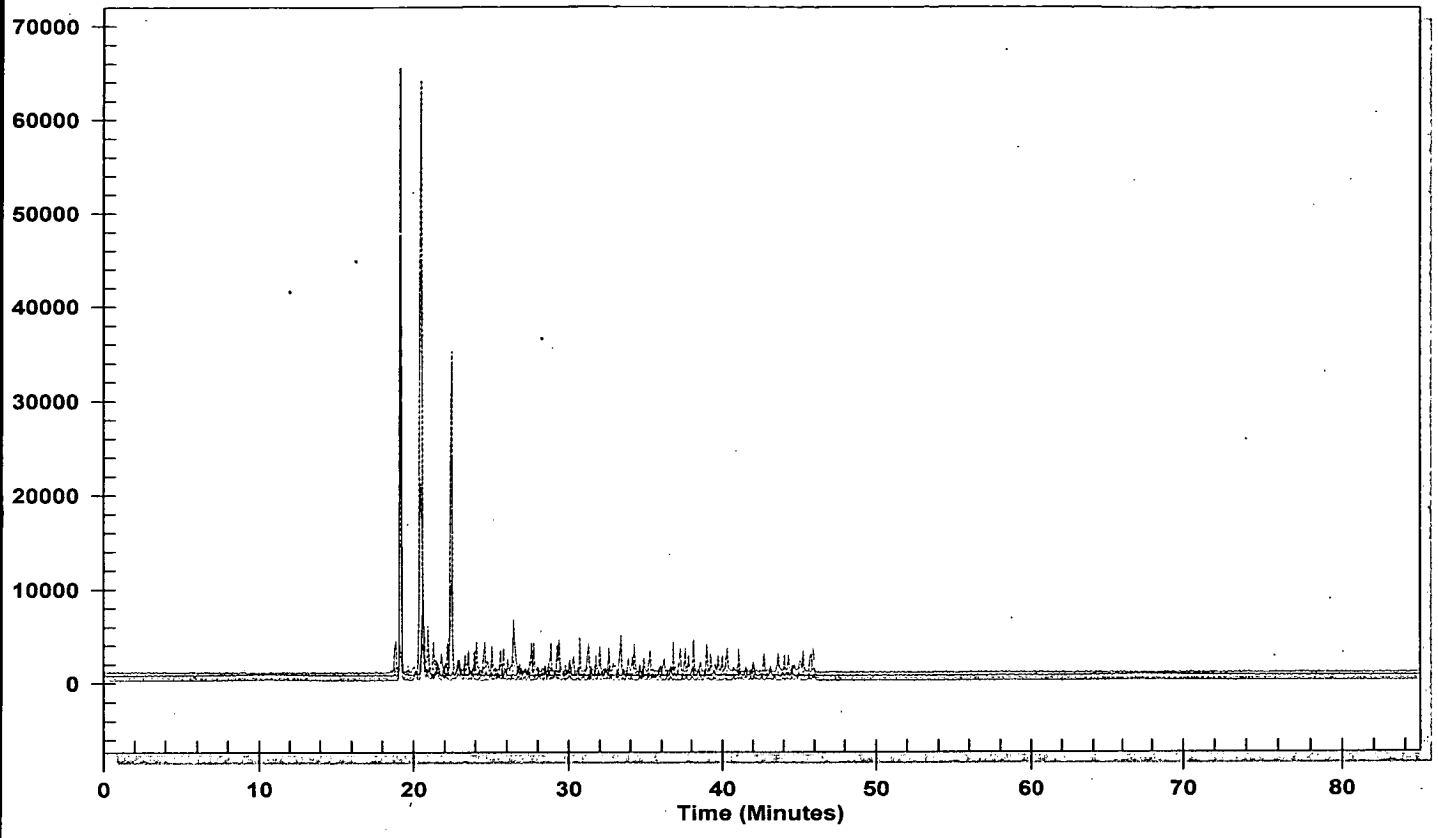
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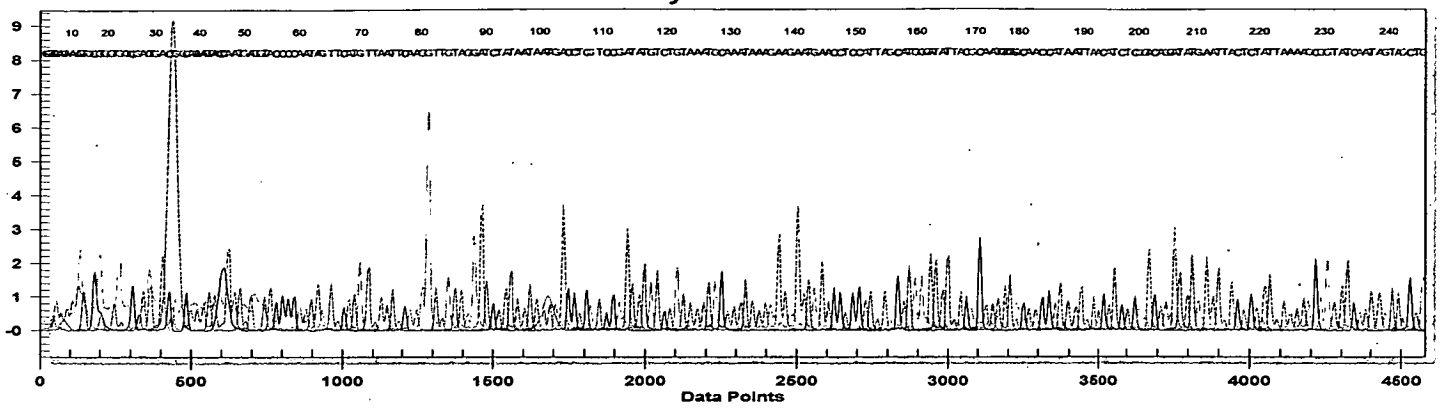
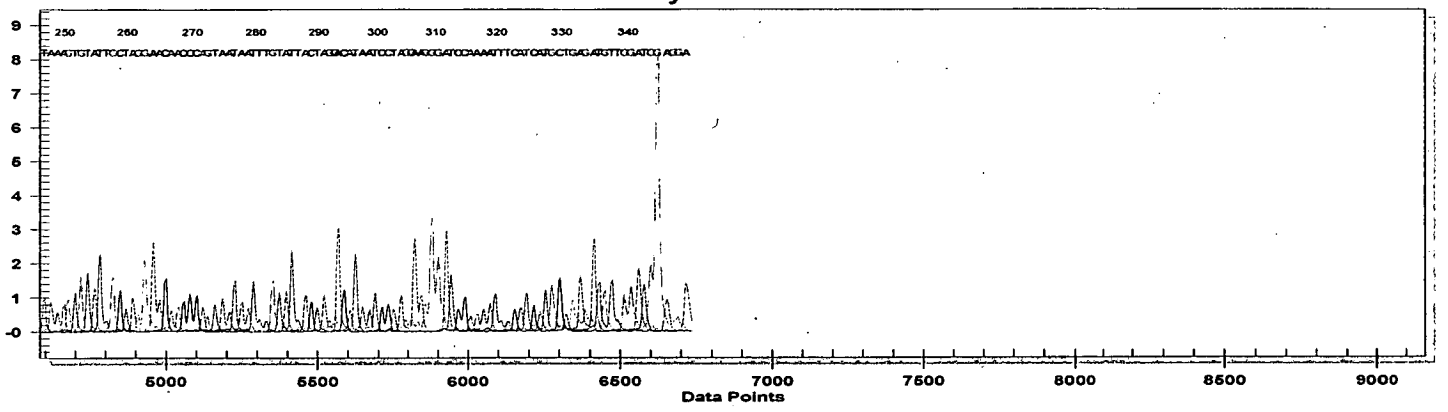
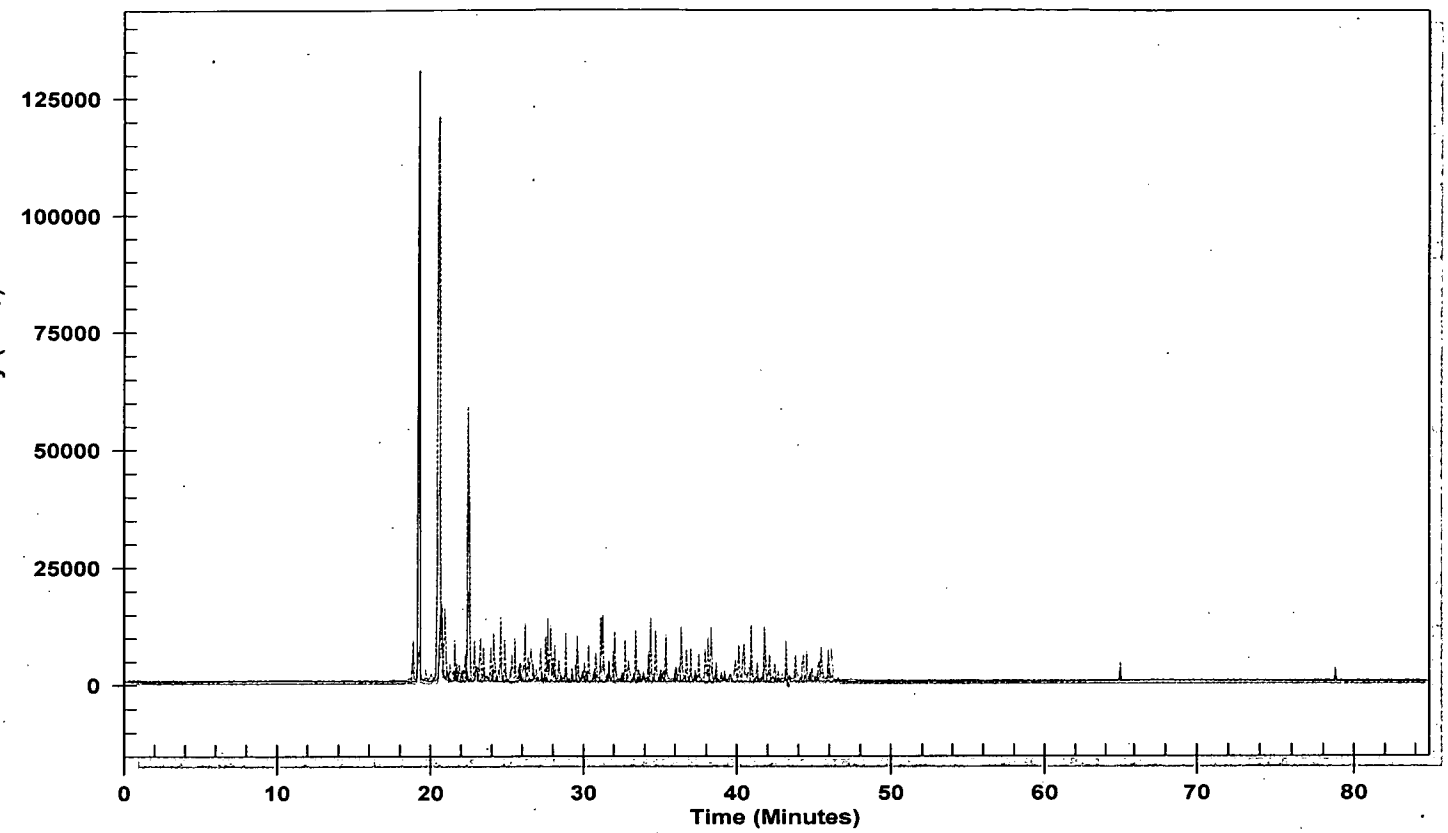


Analyzed Data



Raw Data


125000
100000
75000
50000
25000
0
0
10
20
30
40
50
60
70
80
Time (Minutes)

¡quence Results: KF3.C11_060520017Q

ed:	05/20/06 02:50:20	Project: System:	Default System 1 [Rev 0]
ed:	05/20/06 02:50:20	Project: System:	Default System 1 [Rev 0]
le Name:		KF3.C11_060520017Q	
le Plate:		Unknown	
le Subject ID:			
le Position:		Unknown	
d:		Unknown	
ment:		Unknown	
sis Parameters:		Unknown	
		Loaded from SCF File: KF3.C11_060520017Q.scf	
rties:			
ary Array Serial Number:			
ary Array Part Number:			
Length of Capillary:		0.0 cm	
h of Capillary to Detector:		0.0 cm	
al Diameter of Capillary:		0.0 µm	
er of Runs:		0	
on Instrument:		0.0 days	
art Number:			
t Number:			
lgorithm Type:			
on Instrument:		0 hours	
Part Number:			
Lot Number:			

se Sequence: KF3.C11_060520017Q

```
1   TTCCAAGGTTATAGGACCCATAAATATTTTTTACCTCGGCCAATGTGAATATAAATACAAATGAAGGAAGAACGAAGCTC
1   CATTGGCATGAATAGTACGTAGTATGGGGAGACCATACTTGACATCTCGACAAATATGGACTACCTCTATTAAAAGCTGT
1   TTCAATAATTGCTGTAAAGTGTATTGCTAAAAATAATCCAGCTGATGATTGTGTAATAATTAACACATAAATCCTAAGAG
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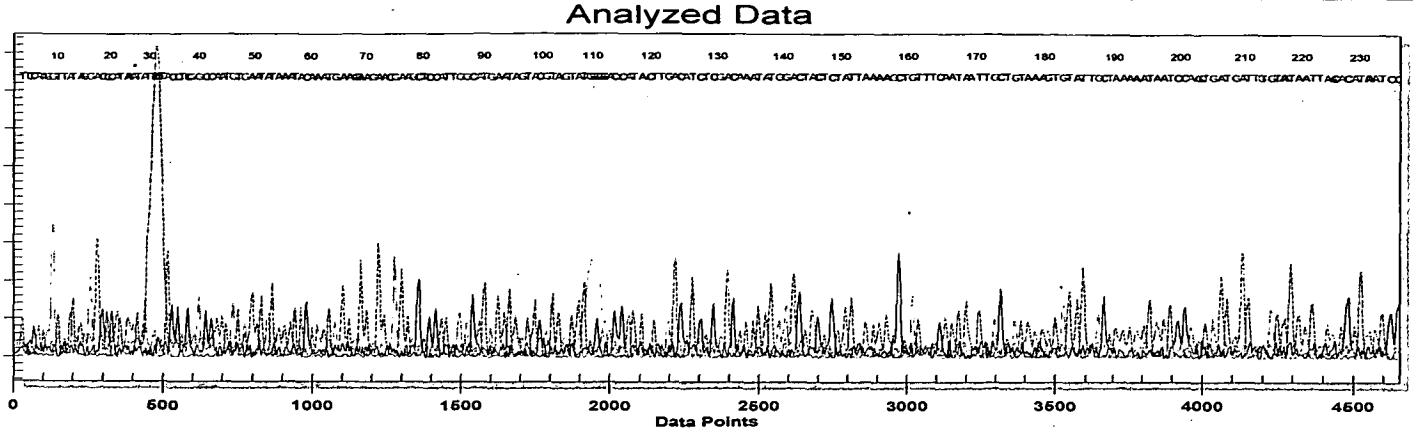
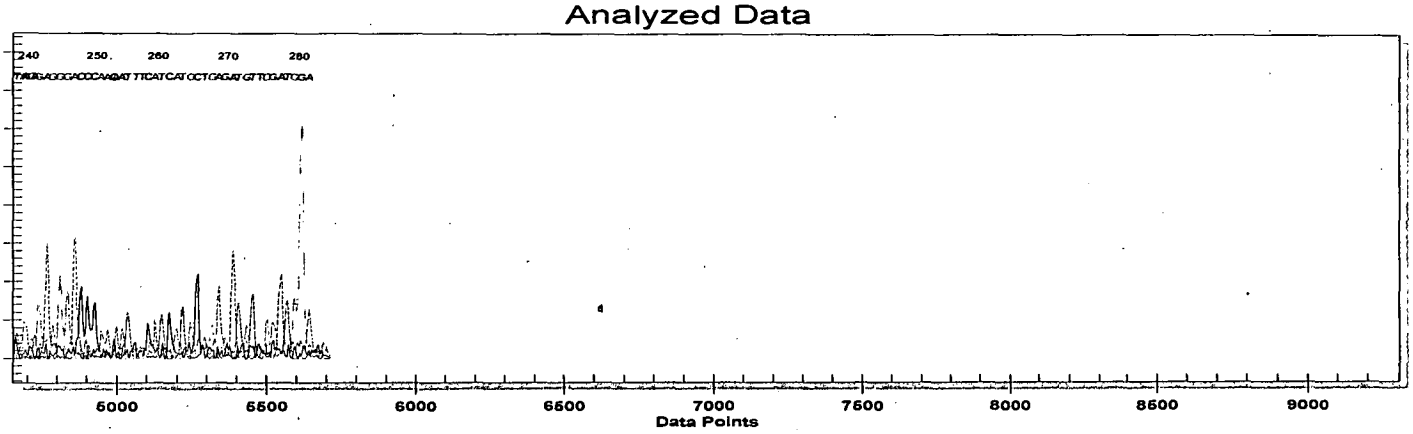
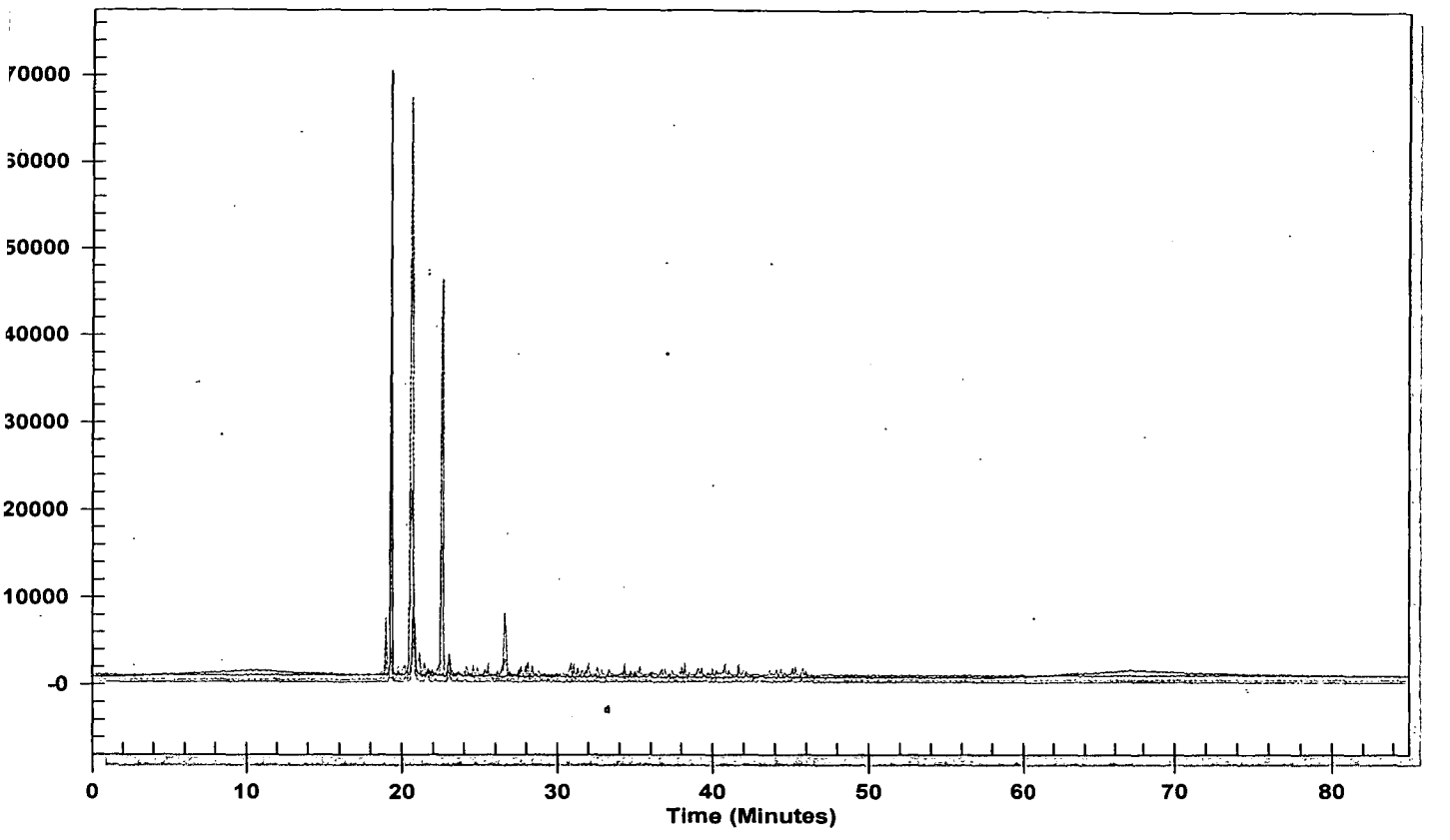
Analyzed Data



Analyzed Data



Raw Data


70000
60000
50000
40000
30000
20000
10000
-0
0
10
20
30
40
50
60
70
80
Time (Minutes)

Sequence Results: KF7.G11_060523104K

ated:	05/23/06 11:45:27	Project:	Default
		System:	System 1 [Rev 0]
dified:	05/23/06 11:45:27	Project:	Default
		System:	System 1 [Rev 0]
mple Name:		KF7.G11_060523104K	
mple Plate:		Unknown	
mple Subject ID:			
mple Position:		Unknown	
thod:		Unknown	
trument:		Unknown	
alysis Parameters:		Unknown	
te:		Loaded from SCF File: KF7.G11_060523104K.scf	
perties:			
pillary Array Serial Number:			
pillary Array Part Number:			
tal Length of Capillary:		0.0 cm	
ngth of Capillary to Detector:		0.0 cm	
ernal Diameter of Capillary:		0.0 µm	
mber of Runs:		0	
ys on Instrument:		0.0 days	
l Part Number:			
l Lot Number:			
l Algorithm Type:			
urs on Instrument:		0 hours	
ffer Part Number:			
ffer Lot Number:			

Base Sequence: KF7.G11_060523104K

```
  1   AAGGAAGACGACGAGGGTGCGGAGGAGTGCAGGATCTGGAGAAGGACCGAATGGAGAGAATTCCGAATATAGATTCCAGG
 81   GTTAATTCGAAGGGTTAATAGTGGATCCCATTAGTTATTATATCCCTCCGGACCCAATAATGGAATTATTAAATAACGCA
161   AATAGGAAGGAAGATAAGGGAGGCCTCCCATTGAGCCGTGGGATGGGTGCCGCGAATATGGCCAACCCATTAGTTAAACC
241   ATCATCGGAAAAATTTGGAACTACATCTATTAAAGGCCTGGTTTCAATAGTTGCTGTAAAGATGTATTGCTAAAAATAAC
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401   TGTTTGGGATGGGAAAA
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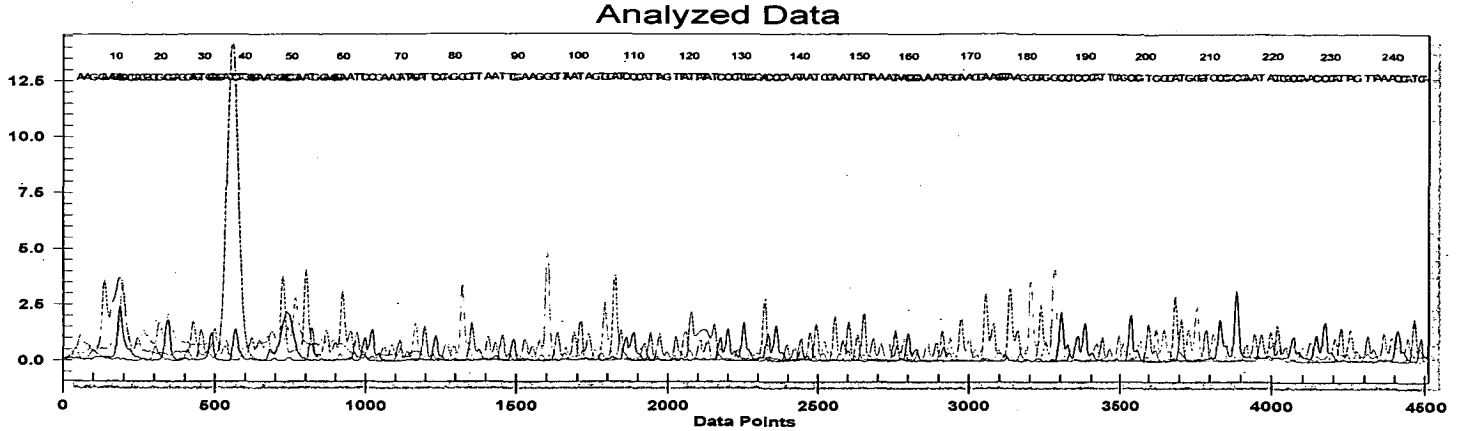
Analyzed Data



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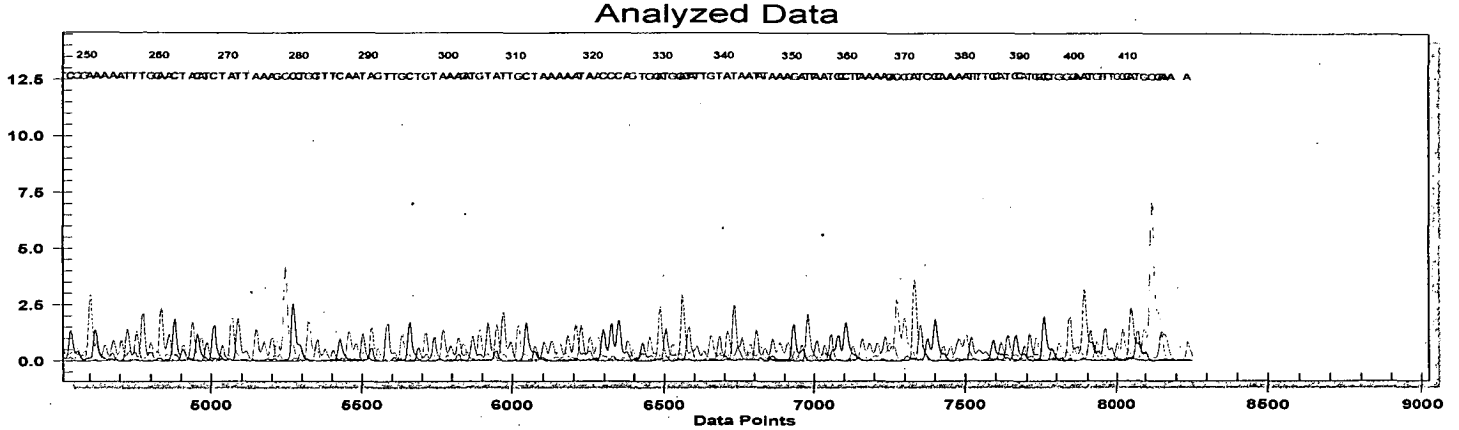
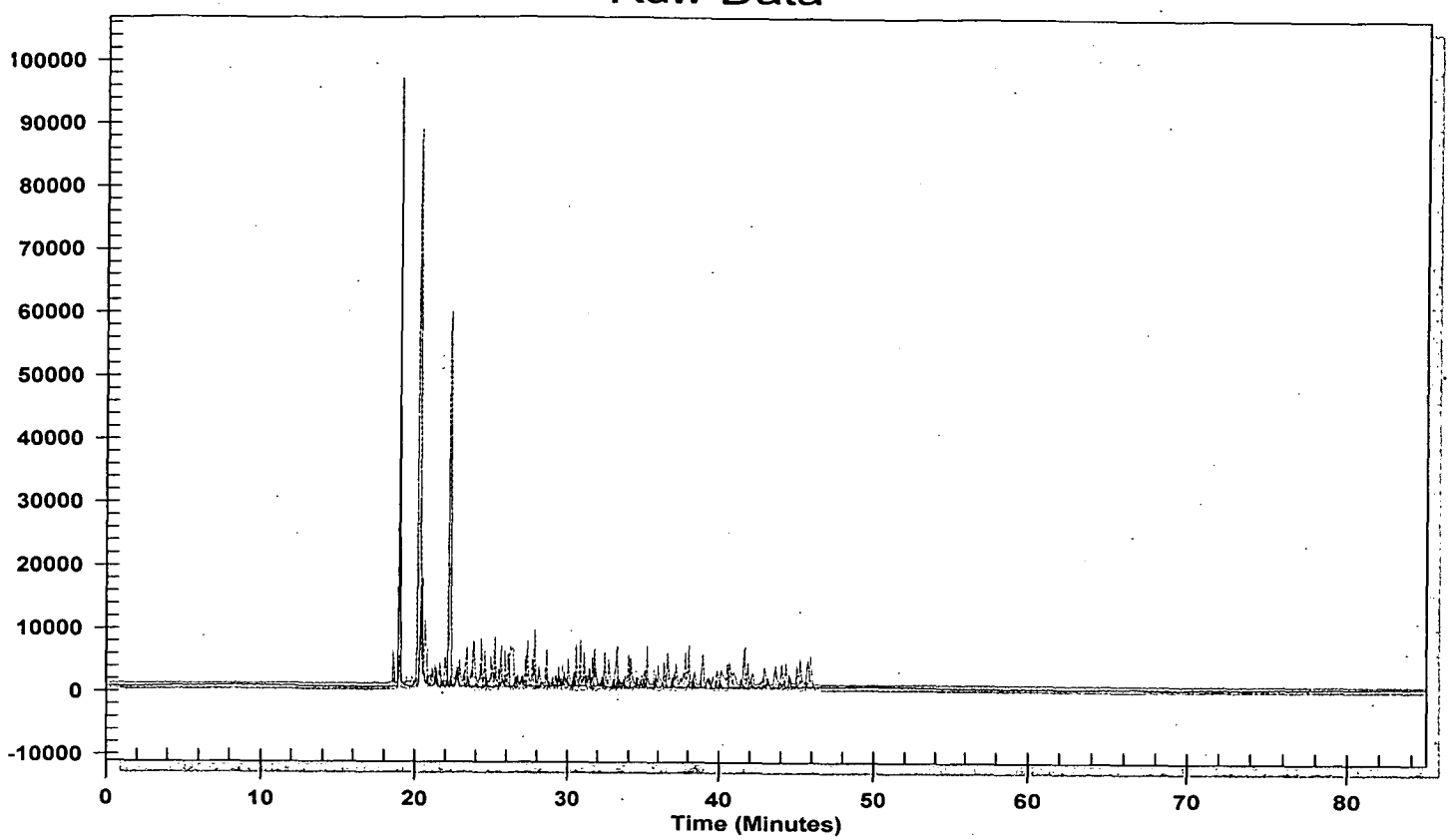


Raw Data


100000
90000
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70000
60000
50000
40000
30000
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10000
0
-10000
0
10
20
30
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50
60
70
80
Time (Minutes)