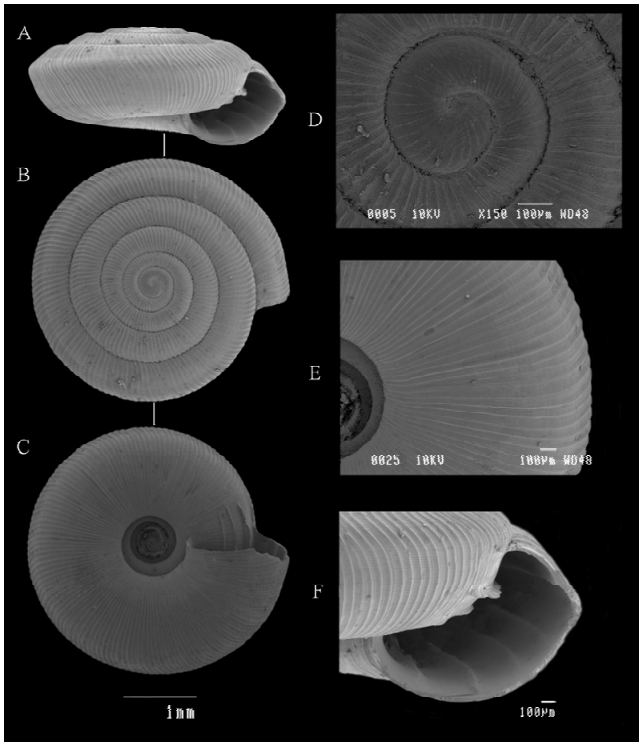

Figure 2 – *Australdonta tapina* Solem 1976, Rurutu, Archipel des Australes, Polynésie française, matériel d'étude; **A.** vue frontale ; **B.** face supérieure ; **C**. face inférieure ; **D.** détail de la protoconque ; **E.** sculpture du dernier tour, face inférieure ; **F.** détail de l'ouverture. Photographie MEB.

qu'elle n'a pas reçu de nom, et la systématique a aujourd'hui un rôle crucial à jouer dans cet inventaire.

Résumé du travail réalisé par G. Zimmermann dans le cadre de son stage de Master2 de sciences et technologies, Mention Sciences de l'univers, environnement, écologie, Spécialité Systématique, Evolution et Paléontologie.

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The First Annual Meeting of Task-Force-Limax, Bündner Naturmuseum, Chur, Switzerland 8-10 September, 2006: Presentation, Outcomes and Abstracts

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What is Task-Force-Limax?

Task-Force-Limax refers to people working on the pulmonate slug genus *Limax* and other Limacidae. It currently includes experts in field work, collecting, copulation, anatomy and molecular biology, as well as a large number of people from around the world whose contribution is to collect animals for the project. The initial focus of the project will be south-western and mid-Europe, extending to the east later on. At the recent first meeting of Task-Force-Limax, seven countries were represented (Austria, Germany, Italy, Luxemburg, Sweden, Switzerland, and Australia), and we also have members able to collect in or provide material from at least an additional ten countries.

Why *Limax*?

This is probably the only example in Europe of a group of macroscopic animals (>10cm) which still has a considerable number of undescribed species (20 or more). These slugs also have extremely unusual and varied copulation behaviour and are a serious pest in agriculture overseas.

DNA sequencing

One part of the project involves the collection of tissue for DNA extractions and the development of molecular markers to be used in identification as well as phylogenetic analysis. One marker, the mitochondrial gene cytochrome oxidase subunit I (COI), has already proven to be suitable for species identification in most cases. DNA extraction, amplification and sequencing is being carried out at the Zoologische Staatssammlung München, Germany.

Copulation and reproductive anatomy

Copulation behaviour in *Limax* is complex and bizarre, and involves several distinct stages. The slugs entwine their bodies around each other, hanging head-down from a mucus thread, and extrude and entwine their penises. Sperm mass transfer takes place at the tip of the penis. In some species, the penis length can be almost one metre! Task-Force-Limax includes several people who are studying and documenting copulation behaviour in detail. Reproductive anatomy is also very important for distinguishing species of *Limax*.

Outcomes of the meeting

1. Standardisation of terminology for discussing phases of copulation in *Limax*

Phases 1-2: Precopulation behaviour / Prelude

▪Phase 1: "Hinterher kriechen/Verfolgung" - following (Höhenprofil)

▪ Phase 2: "Kreisbildung" - formation of circle (Durchmesser, Überlappung)

Phase 3 - 9: Copulation behaviour

▪ Phase 3: "Körperumschlingung" - body entwining (freier Schwanz, Beginn Schleimsegel)

▪ Phase 4: "Abseilen" - abseiling (Länge Schleimfaden)

▪ Phase 5: "Penisausstülpung" - penis eversion (simultan?, Länge, Struktur, Verwindung)

▪ Phase 6: "Penisumschlingung" - penis entwining

▪ Phase 7: "Birnenstadium" - pear-shape - ends with sperm mass transfer

(Zurückziehen, Struktur "Löffel / Glocke")

▪ Phase 8: "Penistrennung und -retraktion" - penis separation and retraction

▪ Phase 9: "Paarungsende" - end of copulation

▪ Phase 10: Postcopulation behaviour

▪ Phase 10: "Postkopulationsverhalten" - postcopulation behaviour (z.B. Putzen, Fressen des Schleimfadens)

2. Suggestions for preservation of specimens

Good preservation of specimens for both DNA sequencing and dissection is vital. Suggested guidelines include the following steps:

▪ Photograph animal (dorsal, ventral and lateral views) and measure length of sole and length and width of mantle. Take tissue sample (small piece of tissue from left side of mantle, removed with sharp scissors) and preserve in small volume 95% ethanol.

▪ Drown animal in water mixed with a few drops supralan (for 45 minutes to 2 hours).

▪ Inject 95% ethanol into the body cavity and immerse whole animal in 95% ethanol (overnight or at least 12 hours).

▪ Inject again with 95% ethanol and place animal in fresh 70% ethanol for storage.

▪ Change 70% ethanol again in around 2-3 weeks time.

3. Plans for Task-Force-Limax – PR Journal

We plan to produce a PowerPoint file about Task-Force-Limax to be published on the internet. It will contain information about the project, including photographs of participants and logos of institutions involved. It will also contain information about the slugs and colourful photographs of animals and copulations. This PR Journal will be something we can present to potential sponsors, patrons, members and to the media.

4. Name patronships

Substantial donations to the project will be thanked by naming a new species at the suggestion of the sponsor.

5. Rules for co-operation within Task-Force-Limax

▪ Publications and funding proposals written in groups if possible.

▪ All publications written in English for world-wide accessibility; high-ranked and indexed journal should be preferred.

▪ Input of specimens warmly acknowledged; input of any form of data co-authored.

6. Next meeting of Task-Force-Limax

We will next meet on Friday 15th June to Sunday 17th June 2007, possibly near Udine, Italy (venue to be confirmed).

Abstracts of the First Annual Meeting of Task-Force-Limax

Limacidae (Gastropoda: Stylommatophora) worldwide - facts, problems, visions

Gerhard Haszprunar

The slug family Limacidae comprises more than 200 valid (and more than 600 nomimal) species. This is probably the only metazoan group, where many (>20) unknown species with body lengths more than 10 cm do exist in Europe. Up today Limacidae have gained special interest because of their unique and bizarre copulatory behaviour. In addition, introduced species have become serious pests in overseas agriculture.

Former taxonomic studies have been published in no less than 11 languages, many types and important reference material have been lost, and many species description are by far incomplete and not sufficient. Moreover, even experts can identify only sexually mature and healthy specimens on the basis of dissecting the genital anatomy. External morphology and body colours have been shown to be extremely variable in many species, whereas the radula is quite uniform throughout the family.

Based on grants by the German Science Foundation DFG molecular taxonomy (in particular a mitochondrial COI-marker) has been recently applied to overcome these difficulties. The primary aim is to infer a phylogenetic tree - the first after 300 years of limacid research. Based on that, the evolutionary and phylogeographic history of the Limacidae should be outlined. We also want to provide key sequences and standardized primers in order to make species identification fast, accurate, and repeatable for all specimens found. However, morphological or reproductive data will remain necessary to delineate species boundaries.

The "Task-Force-Limax" has been a Swiss initiative to establish a European-wide network of collectors. The declared aim of the meeting in Chur is to widen this approach and to synthesize the molecular data sets with results from field studies, morphology, and behaviour for an integrative understanding of Limacidae worldwide.

Present-day knowledge of the copulation modi within the genus *Limax*

Gerhard Falkner & Margrit Falkner

After an early start in Humanism with admirable observations on copulation of *Limax* by MARTIN LISTER (1678) and FRANCESCO REDI (1684) the knowledge and understanding of this fascinating natural spectacle remained rather poor until the first half of the 20th century. Even when H. W. KEW summarised the scattered observations in 1901, he was not yet able to recognise that he was dealing with copulation types of different species groups. A monographic study of FISCHER in 1917 on *Limax cinereoniger* was published under the name *Limax maximus* (of which the copulation was already rather well documented) and PEYER & KUHN used in 1928 the name *Limax cinereoniger* for a quite different species with an extreme penis length. Despite of this error the last two authors were nevertheless the first to remark that there existed fundamentally differing modi in copulation of *Limax*.

Following the publication of PEYER & KUHN it was ULRICH GERHARDT who by his thorough experimental studies and thoughtful theoretical fundamentation became the founder of modern sexual biology of slugs. As a professor in Halle he published his results from 1933 to 1944. Thereafter there was for the genus *Limax* no further progress in knowledge for many decades and the results of GERHARDT remained nearly forgotten. This may be illustrated by the fact, that in 1989 the leading specialist, A. WIKTOR, copied drawings of PEYER from 1928 and considered them still to be representative for *Limax cinereoniger*.

Summing up his results after studying 8 taxonomic units (species/subspecies) of *Limax*, GERHARDT had defined four copulation modi corresponding to the species *Limax cinereoniger*, *L. redii*, *L. gerhardti*, and *L. maximus*. Our own studies on the group of *Limax corsicus* on Corsica and in Italy revealed that the last-defined copulation modus of *Limax gerhardti* is that which in its basic traits is characteristic for this group. Two further copulation types are imperfectly known from the French Alps and the Lake Lugano region. In both cases the animals copulate hanging at the end of a mucus thread like *L. maximus*, but the very specialised penial morphology suggests that they may constitute real functional and behavioral modi in the sense of GERHARDT.

Important additions to our knowledge are to be expected from G. WEITMANN on the *L. maximus*-group and from R. HEIM on alpine *Limax*-species.

For the systematics of *Limax* the final conclusion of GERHARDT remains valid: "Das sicherste Artkriterium ist aber die Biologie der Kopulation, die keine Täuschung zuläßt."

How to obtain living Limacidae from all over the world? "Task–Force–Limax", a first idea, its progress and dynamic

Ulrich E. Schneppat

One of the problems we face in studying widely distributed groups such as Limacidae is how to get slugs from all over Europe and other continents. One has to be aware that there is still no true understanding of the identity of *Limax sensu stricto* or many of the other limacoid groups we are working on. Species borders and exact species numbers are unknown. We have to deal with at least 450 nominal taxa today and more new species are discovered with nearly every collection trip.

Our investigation area stretches from the British Isles at least to the Caucasus and even the Himalayas, and from Scandinavia to northern Africa. At least three species have been introduced worldwide. To research Limacidae successfully we need to sample as many populations as possible. We cannot travel to all necessary localities, so good local collectors are vital in providing material.

Finding such collectors can be difficult due to ethical and cultural differences. Other problems arise due to the difficulty of providing reimbursement for travel and postage, and restrictions in the transportation of living animals.

At least 200 people have been contacted to date, resulting in about 60 who are currently actively collecting for the project. This provides us with material from many places we would otherwise not be able to sample, including many parts of the western Palaearctic and also Canada, the USA and New Zealand. Groups who have responded extremely well to requests for living material include: horticulturalists and gardeners, taxidermists and people who love nature. Some collectors were also found among malacologists, biologists, naturalists, hunters, game wardens, national park rangers and foresters.

Photographic documentation of the copulation of *Limax* spp., demonstrated using *Limax* cf. *alpinus* A. Férussac 1821

René Heim

The extraordinary copulation behaviour seen in slugs from the genus *Limax* is one of the most important characters used in distinguishing species. For the comparison of species and identification of differences in copulation modes, it is necessary to record each stage of the copulation in detail. This requires good photography and detailed notes of timing and the appearance and behaviour of the animals at each stage. To illustrate this, a complete photo documentation of a copulation of *Limax* cf. *alpinus* is presented.

(Note: discussion following this talk resulted in the standardisation of terminology used in describing *Limax* copulations.)

Friuli Venezia Giulia, a small but significant region for species of the genus *Limax* in northeast Italy

Manuela Giovanelli

As far as their terrestrial malacofauna is concerned, the south-eastern Alps probably represent the richest Italian region as to species number. In their chorological composition, the SE Alps appear to be rather heterogeneous: in fact, in the Julian Alps and in the Carso Triestino the more xerothermophilic Dinaric and Balkan components are prevailing. Some of these species often reach the northern and western edges of their range corresponding with the Isonzo or Tagliamento rivers. Moreover, in the SE Alps, the fauna is enriched by the presence of several endemic species, whose presence is probably related to the glacial periods by the occurrence of refuge centres and several cave habitats. In the Italian region Friuli Venezia Giulia we can find three species ascribed to the genus *Limax*: *L.* cf. *maximus* with a more broad distribution, *L.* cf. *bielzi* with an alpine distribution and *L. dacampoi* living near the Tagliamento mouth. The species have been identified with "cf." due to a different anatomy. The study will probably clarify the taxonomic status of these taxa.

How slugs are hiding, how can slugs be found and transported

Clemens M. Brandstetter & Wolfgang Brandstetter

Slugs are known to avoid daylight. Their typical biotope includes crevices of old trees (e.g. beech and chestnut) or old man-made structures. Rocks and gorges are other good places to look. To find *Limax*, it is best to look during the day for mucus trails on trees, and then return to the same area at night to search. Mushrooms should always be examined closely. Recommended equipment for night-time excursions includes: a torch, a head-lamp, a backpack and small plastic containers for keeping the slugs in. Once slugs have been collected, they need to be kept alive until the end of the collecting trip. This is best done by taking a cool box containing frozen bottles of water to store the live animals in. The captive slugs can be fed on mushroom.

Preliminary mitochondrial phylogeny of the genus *Limax*

Barbara Klee

Species in the genus *Limax* have always been defined based on morphological characters. A new dataset has now been generated from 613 nucleotides of the mitochondrial gene cytochrome oxidase subunit I (COI). The preliminary phylogenetic results give a first impression of relationships in this genus. To date, 229 specimens of *Limax* and other Limacidae are included in the molecular dataset, with a geographic range covering central Europe.

Initial tree reconstructions show that the genus *Limax* seems to be a monophlyletic clade. *Limax* itself splits into multiple groups. Each of these groups contains at least one species, often several species, already defined by morphological characters. The represented groups include species that are widely distributed in Europe such as *Limax maximus* and *Limax cinereoniger*, each of which is part of a monophyletic group of possibly more than one species. Alternately, the tree includes radiations of rare species, such as a clade of 6–8 species that is endemic to Corsica. Many of these species are not yet formally described.

These initial results indicate the need for wider biogeographic and taxonomic sampling. Species of *Limax* from eastern Europe are currently poorly represented in the tree, but appear to diverge basally within the genus. The inclusion of more of these species may give additional insights into the phylogeography of *Limax*. Furthermore, it would be beneficial to increase the number of other limacid genera in the analysis. Finally, future work will confirm and refine the preliminary results on the basis of sequencing further genes (for example ITS1 and 2, 16S rRNA and 28S rRNA) and AFLP-fingerprinting.

Researching *Limax maximus*: Old literature and new sequences

Isabel Hyman & Barbara Klee

Limax maximus is an important species in the study of *Limax* and Limacidae. It is one of the most widespread and morphologically variable species in the genus. In addition, this species has been introduced throughout the world. Before any biological control can be considered, *Limax maximus* needs to be well understood in its natural environment. We are currently working on a revision of this species using morphological and molecular data from native and introduced populations around the world.

Limax maximus was formally described by Linnaeus (1758). His sources were Lister (1678), who gave a detailed description of *Limax cinereus maximus maculatus & striatus* from England, and two of his own publications mentioning *Limax cinereus maculatus* from Sweden (Linnaeus, 1745, 1746). The identity of these animals is not yet clear. One of the problems with the interpretation of early literature is the lack of figures. Another problem is the misidentification of other species as *Limax maximus*. It can be time-consuming and difficult to find, translate, read and correctly interpret old literature, but in many cases it is vital to the understanding of the identity of *L. maximus*.

Our preliminary molecular data is based on 613 nucleotides of the mitochondrial gene cytochrome *c* oxidase subunit I (COI), with specimens from Germany, Austria, Italy and France. Current work involves sequencing additional specimens from these countries and from England, Scotland, Spain, Andorra, Sweden, Switzerland, and Australia. In addition, more samples are being sourced through

museums and a network of collectors in Europe, America, Hawaii and Africa. A nuclear gene, the internal transcribed spacer 2 (ITS2) from the ribosomal gene cluster, will also be sequenced.

Observations of mating behaviour and copulation modi in different morphs of *Limax* cf. *maximus* Linnaeus 1758

Gerhard Weitmann

No abstract available.

Betaversion of an Access-based database for molecular collection management

Barbara Klee & Isabel Hyman

During our work with sequence data and associated voucher specimens, we have encountered problems in the management of the large datasets generated. This is further complicated by the need to repeat procedures during molecular work, resulting in multiple datasets belonging to one voucher specimen. Datasets such as these cannot be managed easily using spreadsheets and are not catered for by existing collection management databases, so we have developed a Microsoft Access–based database designed for this purpose.

One major benefit of a database is that entries are linked to reduce the need for repeated data entry. Data are stored in tables and can be entered easily using forms. The report function can be used to produce user–specific output, as Word or Excel files. This can be used, for example, in making labels and producing mapping data. Other files or internet–based information can be linked to each entry (for example, links to sequences deposited in GenBank). Another benefit is that it is possible to track the history of each specimen, concerning preservation, molecular work, dissection, determination and any other procedure.

The database is still under construction, so we have new features to implement and new data to add. In the future, we aim to make parts of the database available online. Members of Task–Force–Limax would have password–protected access for data entry and retrieval of unpublished work.

Polychromatism in *Limax* cf. *alpinus*

René Heim

Colouration has always been considered to be very important in the identification of species of *Limax*, despite common knowledge of the variation existing within species. Within a population there can be several different colour morphs, and differences between juveniles and adults have been observed. However, little is known about how colouration changes during ontogeny.

In this study, individual specimens from a single population of *Limax* cf. *alpinus* in Glaubenberg, Switzerland were photographed at ages ranging from newly hatched to fully grown. The most commonly observed adult colouration consisted of a light brown body with a dorsolateral row of black spots, and a unicolour mantle. The tripartite sole was dark with distinct flecks of pigment on the outer fields, gradually fading towards the pale inner field, with no sharp margin between the regions. Variation in the body colour of the adults ranged from purely white to nearly black and the dorsolateral spots varied in size and degree of fusion. There were considerable changes in the colouration of animals during the growth of a juvenile to adulthood.

This example demonstrates the importance of sampling more widely in a population to include a variety of colour morphs and stages of development. This should allow better understanding of species boundaries, allowing more accurate identification.

Wishes, dreams, fancies and realities in preservation techniques of slugs for scientific collections

Ulrich E. Schneppat

In the current project we have a huge amount of material coming in from all over the world. If this is to be useful in the years to come, it needs to be preserved with optimal colouration, shape and anatomical structures. In my role as a taxidermist and preservation technician with 40 years experience, I would like to point out some major errors in what most malacologists believe are good preservation techniques.

Old and widely accepted rules should be examined from the perspective of modern chemistry. One aim of preservation is to stop the action of the enzymes pepsine and kathepsine, which have an autolytic function and are responsible for the destruction of collagen, the main protein of skin and many internal organs. This autolytic function is stopped with a fixation chemical such as ethyl alcohol, which gives good results, providing a high concentration is used. During fixation, the preservation fluid is diluted. If 70% ethyl alcohol is used, the actual concentration is reduced to below the critical level of 60%, where tissue maceration starts. A better solution is to use 95% ethyl alcohol for fixation, and to counteract the slow speed of diffusion of ethyl alcohol by injecting it into the body cavity.

For the preservation of colour, it is important to know that the pigmentation is situated in the outermost cell structures of the skin and/or the mucus. These layers are very fragile, and can be easily destroyed if tissue autolysis has started before preservation of the already dead animal. This often happens when animals are killed by drowning.

Many of the common problems in preservation, such as gradual fading or brittle internal organs, are greatly reduced if the initial preservation techniques are good. Storage conditions should also be optimised by storing animals in 75% ethyl alcohol, filling jars to the top with no air bubble (to keep the pH stable) and reducing excessive vibration and exposure to heat and UV radiation.

Collecting and transporting living slugs (Pulmonata: Limacidae)

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Note de la redaction : La traduction française de cet article est disponible sur le site www.journal-malaco.fr à la rubrique Actualités.

Distribution

Slugs of the genus *Limax* and other Limacidae are common in forests that are not strongly influenced by man, for example in beech– and oak–forests, in natural woods of mountain ranges and in alpine forests. Occasionally *Limax cinereoniger*, *Malacolimax* and *Lehmannia* can be found in old spruce plantations (*Picea abies*). In southern Europe, they can additionally be found in chestnut and cork oak (*Quercus suber*) forests, in Macchia and in old olive tree plantations. In these areas you can be successful in little valleys facing to the north with creeks up to rocky fields (even above the timberline), where the evening dewondenses. *Limax maximus* and *Limacus flavus* can appear in gardens and parks near cities.

Collecting

Turning old wood and stones during the day can be successful sometimes, but the more efficient way is collecting at night after the beginning of complete darkness (normally after 9:30 p.m.) with a headlight or torch. Limax is nearly exclusively active at night. You