Characteristics for identification of larval Cholevinae (Coleoptera: Leiodidae)

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I. Abstract. Cholevinae (Kirby, 1837) is a subfamily of Leiodidae (Coleoptera). Cholevinae species live in caves or nests and tunnels of mammals and ants. These insects are non-specialised saprophagous beetles. The larvae feed from decaying animal matter, they eat the fungal spores and mycelium. The Cholevinae species have their own time schedule of when they feed on decaying matter. Some like 'fresh' decaying matter, others wait till there is hardly any matter left. These varied preferences could lead to different mouth and/or jaw developments. Other specific morphologies are due to isolation. Living in caves or animal nests causes many morphological modifications, these modifications are called troglomorphic characteristics. Possible troglomorphic characteristics are: lengthening of appendages, loss of pigment, modification of eyes, modified olfactory sensory organs, extra sensory structures and elongated legs (used as feelers). Identification of the species is not easy. The beetles and larvae are small to very small (0.8 – 9 mm), brown, grey or black. The differences between adult species are very subtle. This also applies to the larvae. The larvae have an elongated body, long legs, striking cerci and ten abdominal segments. It is known that the larvae of Cholevinae have three larval instars. The larvae of the different instars do not look the same, which makes it even harder to determine the species. The aim of this research project was to find the characteristics for the identification of the larvae for several species of Cholevinae. Based on the characteristics an identification key for the species, *Nemadus colonoides* (Kraatz, 1851), *Choleva agilis* (Illiger, 1789), *Choleva fagniezi* Jeannel (1922), *Choleva holsatica* Benick & Ihssen (1937), *Choleva oblonga* (Latreille, 1807), *Choleva spadicea* (Sturm, 1839), *Nargus velox* (Spence, 1815), *Dreposcia umbrina* (Erichson, 1837), *Sciodrepoides fumatus* (Spence, 1815), *Sciodrepoides watsoni* (Spence, 1815), *Ptomaphagus medius* (Rey, 1889), *Ptomaphagus sericatus* (Chaudoir, 1845), *Ptomaphagus subvillosus* (Goeze, 1777) and *Ptomaphagus varicornis* (Rosenhauer, 1847) is presented. This paper also contains two keys for identification to the genera, one of those keys includes the genera *Apocatops* and *Catops*. With the identification key it would be possible to use the species in forensic entomology to determine the post-mortem interval. Because the species are non-specialised they are not restricted to animals and regularly they are found on human corpses. Many species reproduce in autumn and become adults during winter. This means that the investigation can be done in the cold seasons, even if the other insects, that are used in time-of-death investigations (for example the Diptera), are absent.

Key words: larval morphology, larval identification, Cholevinae, Leiodidae, cold-season insect, troglomorphy, forensic entomology

II. Introduction

Usually, Cholevinae are hard to identify by external morphology alone. The beetles are small to very small (0.8 – 9 mm), brown, grey or black.[31] Genital morphology gives the best clue.[5] However, the genitals are not yet present in the larvae. The morphological differences between the larvae of different Cholevinae species are very subtle.[31]

Cholevinae have a one-year life cycle. After one or two months the larvae have undergone two moults. This means that the larvae of Cholevinae have three larval instars.[37] The larvae of the different instars do not look the same, which makes it even harder to determine the species.[1] For Staphylinoidea larvae most characteristics are similar for instars two and three. But they differ a lot between instars one and two. The change between instars for larval Leiodidae are growth (the body size increases) and changes in proportions and ratios. This also implies for Cholevinae larvae. (Fig.1) At the end of their third instar, the larvae go into a pupa for about ten days.[37]

The animals studied in this research are detrivores who live on the carcasses or dung of vertebrates.[22 27 33] Most Cholevinae are non-specialised scavengers. When the intestines of Cholevinae larvae were analysed by Hågvar, it contained mainly fungal hyphae and spores.[3 31] Also decaying plant material was found. Some contained nematode fragments, nematode eggs or fragments of insect larvae.[10]

The beetle family Leiodidae (Fleming, 1812) has undergone various changes in taxonomy. The family Catopidae (Thomson, 1862) was separated from the family Silphidae (Latreille, 1802) in 1936.[12] In 1979 Catopidae was changed into Cholevidae by Zwick.[7] According to M. Schilthuizen (personal communication) the former family Cholevidea is now viewed as a subfamily of Leiodidae (Cholevinae (Kirby, 1837)) by scientists from US and UK, European scientists are sometimes still prefer to treat Cholevidae as a distinct family. Leiodidae beetles are globally distributed with 3788 described species. The subfamily Cholevinae consists of 326 genera and 2411 species (plus subspecies) worldwide.[9 25 31] They live in a wide range of habitats including animal nests and tunnels, dung, corpses, leaf litter and humus.[32 34] There are three main groups of species in preference of habitat, the first group prefer the forest as their habitat, other prefer field habitats and the last group has no preferences.[20 32] Antarctica is the only continent where Cholevinea beetles do not occur.[7] According to Jeannel, the cold climate during the Pleistocene would have forced the thermophilous species to move to the warmer climates in the south, while the cold-season insect might have stayed.[12 36]

A lot of species are endemic, because they have undergone strong differentiation through isolation.[7] The isolation is due to their restrictive environment. Most cave dwelling species live in the Mediterranean basin.[20 33]

Fig. 1. *Ptomaphagus medius*; instar 1 (left), instar 2 (middle) and instar 3 (right)

Some species are highly specialized troglobitic, others are specialized hypogean/ epigean elements. Usually Cholevinae live there where there is an abundance of moisture and little or no light.[7 12] Living under the earth's surface or in caves causes many morphological modifications,[1] these modifications are called troglomorphic characteristics.[24] Possible troglomorphic characteristics are: lengthening of appendages, loss of pigment, modification of eyes, modified olfactory sensory organs, extra sensory structures and elongated legs (used as feelers).[6 21]

There are not many identification keys or descriptions of (larval) Cholevinae. But there is known that the larvae of Cholevinae have an elongated body, long legs, striking cerci and ten abdominal segments.[1] Larvae are grey, brown or white and all thorax segments have the same width. Maxilla bearing both galea and lacinia. The lacinia has four thorns and the maxillary palp has three segments. The lowest segment is short, the second is thinner and the third is pointed. The cerci are attached to the ninth abdominal segment, they are quite long, thin and exist of two segments with a setae at the end.[16 29]

Because the species are non-specialised they are not restricted to small animals and regularly they are found on human corpses.[11 31] This means that Cholevinae beetles and larvae can possibly be used in forensic entomology to determine the post-mortem interval. However, due to their small size and the difficulty in identification, they have not been a focus of forensic entomologists. In the present study, I aim to find the characteristics for the identification of the larvae of several species of Cholevinae.

Study of the biology and ecology of insects, on human corpses, is called forensic entomology (a branch in the forensic sciences). By analysing the development and succession of the arthropods the post-mortem interval (PMI) can be estimated.[17] There are many techniques that are used for estimating the PMI, for example measuring the body temperature or analysing the livor and rigor mortis. But the time since death defined by these techniques can only be accurately measured for the first two or three days after death. By determining the species present or studying the age of (immature) insect stages, post-mortem intervals from the first day to several weeks can be estimated.[2]

Several species of Cholevinae are 'cold season' insects.[10 35] Cold season beetles start to lay eggs in autumn and the eggs develop to the adult stage mainly during the winter months.[36] Even under a thick layer of snow, active beetle larvae of Cholevinae were found.[10] Cold season beetles can be active down to about -3 °C. (Aitchison, 1979, as cited in Ref. 10) The highest fitness occurred at temperatures between 5 to 10°C. The fitness decreases at higher and lower temperatures.[36] Because many species are active during the cold months, they could be a valuable tool for determining PMI in cold-season time-of-death investigations, when commonly used flies are not active or even present.[1]

Background information of the species

Nemadus colonoides (Kraatz, 1851) is a Western Palaearctic species, distributed from France and Great Britain through southern and central Europe and southern Scandinavia to Ukraine and Russia.[25 28] This species is a specialized occupant of insect nests and bird nests.[20 32] *Nemadus colonoides* lives occasionally in the nests of ants of the genus *Lasius*. But the beetles and larvae also live in the cavity nests of rodents and hornets.[12 23 28] There are reports of finding *Nemadus colonoides* in nests of sparrows and starlings and in roosting places of owls, and in the litter and between the roots of (hollow) trees.[18 30]

Choleva agilis (Illiger, 1798) prefers a wet environment. This species is common in Western Europe. The beetles live in the burrows of small animals, like moles (*Talpa europaea*), rabbits (*Oryctolagus cuniculus*) and several species of mice.[30 32] In the mountains they sometimes dwell in caves.[41] This species is adapted to a cold climate.[16]

Choleva fagniezi Jeannel (1922) lives in litter and burrows of mice and foxes.[32] It eats mainly fungi.[8] *Choleva fagniezi* is present in the Palearctic region.[13] Active specimens were collected under a permanent snow cover, which means that this is a cold season species.[10] The beetles are capable of normal reproduction and survival in different, non-cave conditions.[30 38]

Choleva holsatica Benick & Ihssen (1937) lives strictly in caves.[27] This species is endemic to the Lime Mountains of Segeberg. It eats dead Diptera, Isopoda, bats and batdung.[32] Zwick reported a short diapause of some three months old adults in small, self-made holes.[30 36]

Choleva oblonga (Latreille, 1807) is a species that is associated with the tunnels and nests of mammals, like mice, moles, rabbits and hamsters.[14 32] *Choleva oblonga* is present in open fields, sand pits and even in the city.[30]

*Choleva spadicea (*Sturm, 1839) is a rare species that lives in caves and nests of mice and mole and under deep embedded stones.[19 32] It prefers (floodplain) forests as its habitat.[14]

Nargus velox (Spence, 1815) is found largely in vegetation types like hedgerows, elm stands and poplar stands.[34] This species was found by Sokolowski at the entrance of fox and badger burrows and in the nests of rabbits, moles and crows. They are also reported in faeces and cadavers of mammals and fish.[32]

Dreposcia umbrina (Erichson, 1837) is a Palaearctic species, distributed from east to north Europe (from Romania to Denmark).[25] Like *Nemadus colonoides* this species lives in the nests of ants. It is also found in old trees, such as *Populus sp*., *Ulmus sp*. or *Aesculus hippocastanum*. It prefers forested habitats, from lowland to hills.[28] The larvae form cocoons from detritus before pupation. (J. Vávra, unpublished data, as cited in Ref. 27)

Sciodrepoides fumatus (Spence, 1837) prefers wet wooded sites as its habitat. It appears to be more restricted to low altitudes (below 500m). The most important environmental factors are moisture of the soil, extent of soil temperature variation and detritus availability.[33] *Sciodrepoides fumatus* is most active during spring.[14] Specimens of this species showed a preference for forest habitat.[15] It is found in hornbeam oak wood forests, floodplain forests and shrubby ecotope of a lowland forests.[14] It is found in the nests of rabbits, hamsters, badger, magpie, heron and raptorial birds.[30]

Sciodrepoides watsoni (Spence, 1815) is the most common representative of family Leiodidae (Fleming, 1812). It is active throughout spring to late autumn.[14] It often occurs in open landscapes,[11] like meadow habitats,[15] but is also present both in caves and pits as well as in dens (and burrows) of mammals and other animals (like birds),[32] in forest detritus, etc.[12 19] Besides open field habitats there are records of finds in hornbeam oak wood forest, floodplain forest and shrubby ecotope of a lowland forest.[14] The beetles are necrophagous.[30]

Ptomaphagus medius (Rey, 1889) beetles prefer dry sites. It is a common species of the woodland-floor litter layer.[33] They live in ants, badger, hamster, mice and mole nests. It has also collected between the roots of trees. *Ptomaphagus medius* eats from the cadavers of small mammals and residuals of foxes and birds.[30]

Ptomaphagus sericatus (Chaudoir, 1845) is active throughout spring to late autumn.[14] It prefers a meadow habitat,[15] but they have also been found in hornbeam oak wood forests, floodplain forests and shrubby ecotope of lowland forests.[14]

Ptomaphagus subvillosus (Goeze, 1777) prefers a forest habitat.[15] It is found in nests of rabbits and wasps and the burrows of mice. It is also found in decaying grass and cadavers of mammals and birds.[30]

Ptomaphagus varicornis (Rosen hauer, 1847) is active throughout spring to late autumn. There are records of finds in hornbeam oak wood forests, floodplain forests and shrubby ecotope of a lowland forests.[14] Mostly found in nests and burrows of mice, sometimes in nests of moles or rabbits.[30]

III. Material and methods

Microscope slides

Larvae and beetles were collected and cultured by Peter Zwick (Germany, 1960s) and Menno Schilthuizen (The Netherlands, 1980s). They were found in mole nests or by trapping them (or the adults) with smelly cheese. The larvae were preserved in 70% alcohol or put in Euparal (or Kanada balsam) as microscope slides. Details of the microscope slides are in the appendices. (Table 3 and 4)

Available species are: 8x *Nemadus colonoides* (Kraatz, 1851), 6x *Choleva agilis* (Illiger, 1789), 16x *Choleva fagniezi* Jeannel (1922), 12x *Choleva holsatica* Benick & Ihssen (1937), 8x *Choleva oblonga* (Latreille, 1807), 1x *Choleva spadicea* (Sturm, 1839), 1x *Nargus velox* (Spence, 1815), 16x *Dreposcia umbrina* (Erichson, 1837), 9x *Sciodrepoides fumatus* (Spence, 1815), 16x *Sciodrepoides watsoni* (Spence, 1815), 15x *Ptomaphagus medius* (Rey, 1889), 6x *Ptomaphagus sericatus* (Chaudoir, 1845), 4x *Ptomaphagus subvillosus* (Goeze, 1777) and 3x *Ptomaphagus varicornis* (Rosenhauer, 1847).

Thanks to Kim Renkens I also included in this research: 2x *Apocatops nigrita* (Erichson, 1837), 2x *Catops coracinus* (Kellner, 1846), 3x *Catops fuliginosus* (Erichson, 1837), 3x *Catops grandicollis* (Erichson, 1837), 2x *Catops kirbyi* (Spence, 1815), 3x *Catops morio* (Fabricius, 1787), 3x *Catops nigricans* (Spence, 1815), 4x *Catops nigriclavis* (Gerhardt,1900), 3x *Catops picipes* (Fabricius, 1787), 3x *Catops subfuscus* (Kellner, 1846) and 2x *Catops tristis* (Panzer, 1794).

Preparations

The larvae, which were preserved in 70% ethanol, are used to make new microscopic slides. The larvae were macerated for about two hours in warm (70 °C) 10% KOH (for chemical cremation).[1] After washing in distilled water a small cut between two abdominal segments was made. Than the intestines were removed with a thin needle (with at the end a hook). The chitin was coloured with Phenosaphranine 1% (between 3-5 minutes). After removing the water with different concentrations (30%, 50%, 70% and 96%) of alcohol, the larvae were placed in Euparal green. (Fig. 2) Complete protocol is added in the appendices.

Measurements of external morphological characteristics

Two microscopes with camera were available for my research. A binocular/stereomicroscope (Olympus SZX10) with a Colorview IIIu camera (brand: Soft imaging system, software: Cell^D) and a compound/light microscope (Axioimager.M2) with an axioCam MRc 5 camera (software: Axiovision SE64). The measurements were done with the software Axiovision SE64.

The following characteristics were measured. The **bold** characteristics were used to make the identification key that includes genera *Apocatops* and *Catops*.

Body length without head (µm), Body width without head (µm), Body length with head (µm), Body width with head (µm), **Antenna segm. 1 length (µm)**, Antenna segm. 1 width (µm), **Antenna segm. 2 length (µm)**, Antenna segm. 2 width (µm), **Antenna segm. 3 length (µm)**, Antenna segm. 3 width (µm), **Head length (µm)**, **Head width (µm)**, Mandible perimeter (µm2), Mandibular base width (µm), Lacinia (# thorns), Maxillary palp segm. 1 length (µm), Maxillary palp segm. 1 width (µm), Maxillary palp segm. 2 length (µm), Maxillary palp segm. 2 width (µm), Maxillary palp segm. 3 length (µm), Maxillary palp segm. 3 width (µm), Ant. leg coxa length (µm), Ant. leg coxa width (µm), Ant. leg trochanter length (µm), Ant. leg trochanter width (µm), Ant. leg femur length (µm), Ant. leg femur width (µm), Ant. leg tibia length (µm), Ant. leg tibia width (µm), Ant. leg tasungulus length (µm), Ant. leg tarsungulus width (µm), Pos. leg coxa length (µm), Pos. leg coxa width (µm), Pos. leg trochanter length (µm), Pos. leg trochanter width (µm), Pos. leg femur length (µm), Pos. leg femur width (µm), Pos. leg tibia length (µm), Pos. leg tibia width (µm), Pos. leg tasungulus length (µm), Pos. leg tarsungulus width (µm), **Prothorax length (µm)**, Prothorax width (µm), Mesothorax length (µm), Mesothorax width (µm), **Metathorax length (µm)**, Metathorax width (µm), **Abd. segm. 1 length (µm)**, **Abd. segm. 1 width (µm)**, Abd. segm. 2 length (µm), Abd. segm. 2 width (µm), Abd. segm. 3 length (µm), Abd. segm. 3 width (µm), Abd. segm. 4 length (µm), Abd. segm. 4 width (µm), Abd. segm. 5 length (µm), Abd. segm. 5 width (µm), Abd. segm. 6 length (µm), Abd. segm. 6 width (µm), Abd. segm. 7 length (µm), Abd. segm. 7 width (µm), Abd. segm. 8 length (µm), Abd. segm. 8 width (µm), Abd. segm. 9 length (µm), Abd. segm. 9 width (µm), Abd. segm. 10 length (µm), Abd. segm. 10 width (µm), **Cerci segm. 1 length (µm)**, Cerci segm. 1 width (µm), **Cerci segm. 2 length (µm)**, Cerci segm. 2 width (µm), **Cerci terminal seta length (µm)**.

Identification keys

Pictures of the mandibles were taken with the compound/ light microscope (Axioimager.M2) with an axioCam MRc 5 camera (software: Axiovision SE64). With these pictures an identification key was made.

The other identification keys were constructed with ctree (package party), with RStudio (version 0.98.1091 – 2009-2014). For one identification key to the genera, the descriptions (by Kim Renkens) of the species from the genera *Apocatops* and *Catops* were included. For that key the characteristics and specimens with too many gaps were deleted. The second key of the genera and the identification key to the species do not include *Apocatops* and *Catops*. Because some specimens were dissected not all characteristics could be measured I used multiple imputation (package Amelia) to fill in the gaps.

Also two overviews, one of the mandible characteristics and one of the siginificant measured morphological characteristics were made. (Table 1 and 2) For the measured characteristics the smallest and largest measurement were taken. The smallest are rounded down and the largest are rounded up. *Apocatops* and *Catops* are not in this overview, as well as the measured characteristics for *Choleva spadicea* and *Nargus velox* because there were not enough specimens to include those species. The habitat preferences of the species is also included in the first overview, because morphological characteristics can be due through their environment.[7]

IV. Results

The search to the characteristics for identification of larval Cholevinae resulted in four identification keys.

The first key identifies the species by their mandible characteristics. The Cholevinae species have their own time schedule of when they feed on decaying matter. Some like 'fresh' decaying matter, others wait till there is hardly any matter left. These varied preferences lead to different mouth and/or jaw developments.[3] I looked at the size and shape of the mandibles. If the mandible width is <200 µm than the mandibles are called 'small', if not than they are 'stout'. And if the right and left mandible look the same they are 'symmetric', if not they are 'asymmetric'. Other used characteristics are: prostheca, incisor, apical teeth and molar area. (Fig. 3)

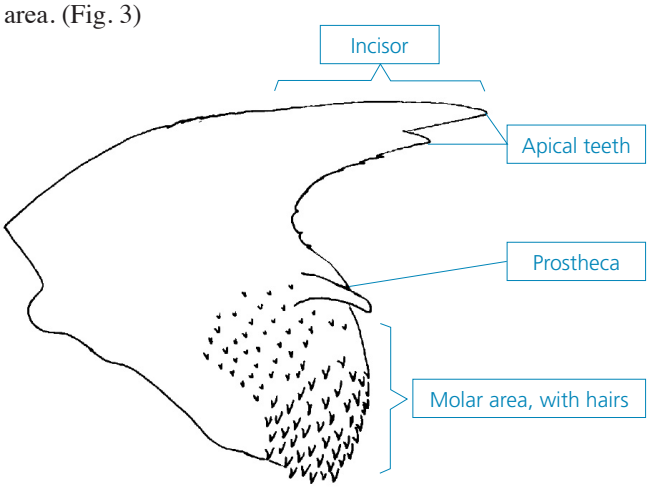

Fig. 3. Mandible [37]

The other three keys are made with the measurements. Before those keys were made I did a Principle Component Analysis with the data to see if there was a relation between the measured characteristics and the identification of larval Cholevinae. The plot of the PCA shows that there is a diagonal and vertical distribution between the genera due to the characteristics. 54% is explained by the first component. (Fig. 19) Also a second PCA is presented in this paper. (Fig. 23) In this PCA the maker of the microscopic slides is coloured to see if the maker of the slide influenced the results.

The second key is a key to the genera: *Nemadus, Choleva*, *Nargus*, *Dreposcia*, *Sciodrepoides*, *Ptomaphagus*, *Apocatops* and *Catops*. The third identification key is also a key to the genera, but this key does not include *Apocatops* and *Catops*. This is not the only difference between those keys. The second key (the one that includes *Apocatops* and *Catops*) is made with less characteristics. Also multiple imputation was not used for this key. I did use multiple imputation for the third key to fill in the gaps.

The last identification key is a key made with the same dataset as the third key. This key does not include *Apocatop* and *Catops*. The fouth identification key is a key of the species: *Nemadus colonoides* (Kraatz, 1851), *Choleva agilis* (Illiger, 1789), *Choleva fagniezi* Jeannel (1922), *Choleva holsatica* Benick & Ihssen (1937), *Choleva oblonga* (Latreille, 1807), *Choleva spadicea* (Sturm, 1839), *Nargus velox* (Spence, 1815), *Dreposcia umbrina* (Erichson, 1837), *Sciodrepoides fumatus* (Spence, 1815), *Sciodrepoides watsoni* (Spence, 1815), *Ptomaphagus medius* (Rey, 1889), *Ptomaphagus sericatus* (Chaudoir, 1845), *Ptomaphagus subvillosus* (Goeze, 1777) and *Ptomaphagus varicornis* (Rosenhauer, 1847).

The keys two, three and four are presented as tekst and as figures. (Fig. 20-22) The figures show the characteristics and a choice between bigger or smaller. At the roots of the tree are boxes. The y-axis is the number of specimens. The x-axis are the genera or species. The order of the genera or species on the x-axis is noted in the subtitle of the figures.

At the end of the results paragraph overviews are presented. (Tables 1 and 2) These tables show as well as the mandible characteristics as the measured characteristics for the species. *Nemadus colonoides* (Kraatz, 1851), *Choleva agilis* (Illiger, 1789), *Choleva fagniezi* Jeannel (1922), *Choleva holsatica* Benick & Ihssen (1937), *Choleva oblonga* (Latreille, 1807), *Choleva spadicea* (Sturm, 1839), *Nargus velox* (Spence, 1815), *Dreposcia umbrina* (Erichson, 1837), *Sciodrepoides fumatus* (Spence, 1815), *Sciodrepoides watsoni* (Spence, 1815), *Ptomaphagus medius* (Rey, 1889), *Ptomaphagus sericatus* (Chaudoir, 1845), *Ptomaphagus subvillosus* (Goeze, 1777) and *Ptomaphagus varicornis* (Rosenhauer, 1847). Table 2 does not include *Choleva spadicea* (Sturm, 1839) and *Nargus velox* (Spence, 1815) because there were not enough specimens.

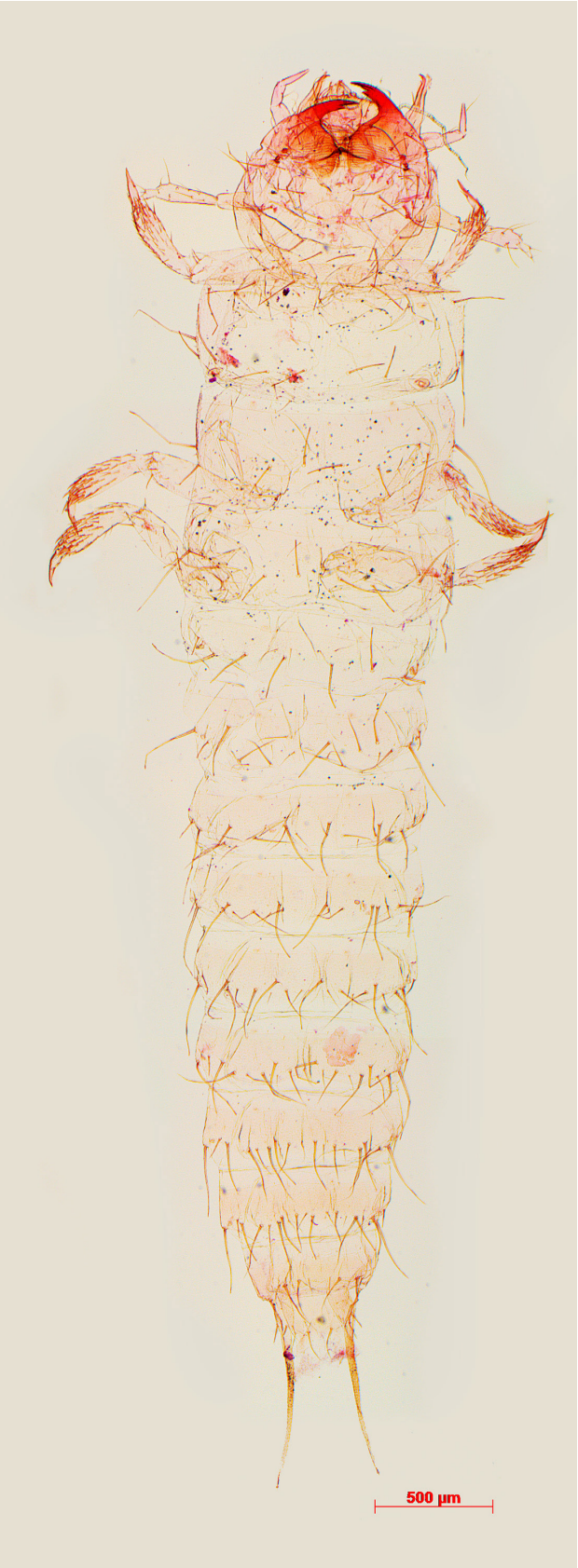

Fig. 2. Microscope slide made by Susanne Pinto; *Ptomaphagus varicornis (dorsal)*

Mandible characteristics (Fig. 2)

Nemadus colonoides (Kraatz, 1851);
Mandibles small (<200 µm), symmetric; prostheca present; incisor acute, short; apex bidentate, small dens; mola not developed, without teeth (Fig. 4)

Choleva agilis (Illiger, 1798);
Mandibles stout (>200 µm), asymmetric; prostheca present; incisor acuminate, long ; apex tetradentate, right dens smaller than left ; mola not developed, without teeth (Fig. 5)

Choleva fagniezi Jeannel (1922);
Mandibles stout (>200 µm), asymmetric; prostheca present, pointed; incisor acuminate, long; apex bidentate (left), tetradentate (right), with larger dens left than right; mola distinct, with teeth (Fig. 6)

Choleva holsatica Benick & Ihssen (1937;
Mandibles stout (>200 µm), symmetric; prostheca present; incisor acuminate, long; apex bidentate; mola not developed, without teeth (Fig. 7)

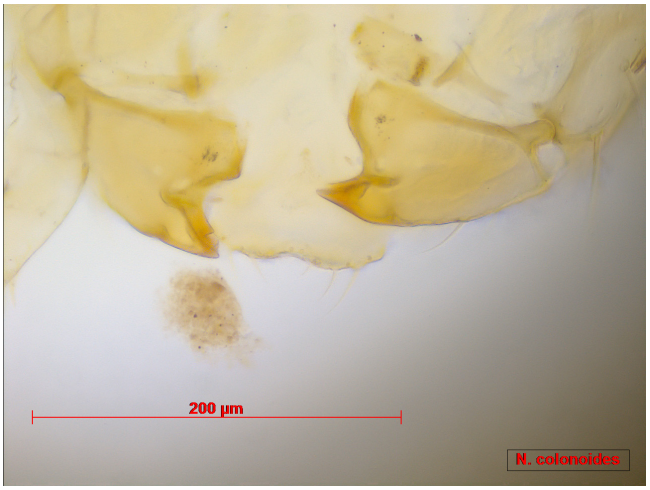

Fig. 4. Mandibles *Nemadus colonoides*

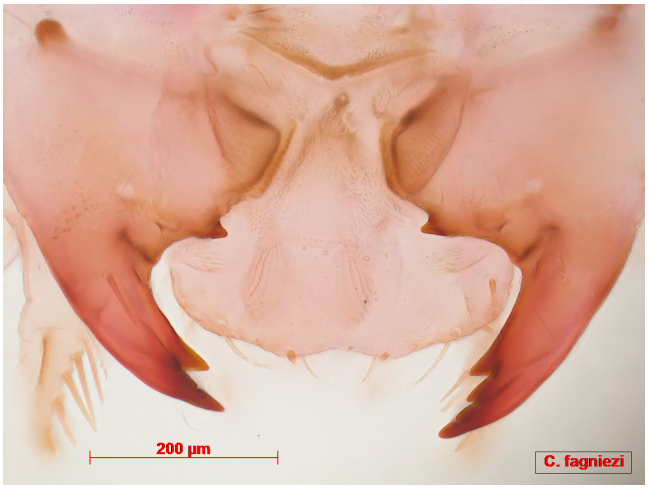

Fig. 6. Mandibles *Choleva fagniezi*

Fig. 5. Mandibles *Choleva agilis*

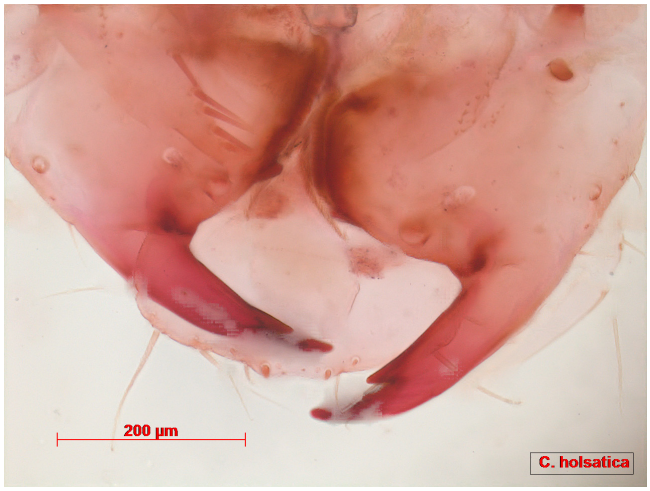

Fig. 7. Mandibles *Choleva holsatica*

Choleva oblonga (Latreille, 1807);
Mandibles stout (>200 µm), asymmetric; prostheca present, pointed; incisor acute, long; apex tridentate (left), tetradentate (right); mola distinct, with teeth (Fig. 8)

Choleva spadicea (Sturm, 1839);
Mandibles stout (>200 µm), symmetric; prostheca present; incisor acute, long; apex monodentate (maybe bidentate but worn); mola not developed, without teeth (Fig. 9 and Fig. 10)

Nargus velox (Spence, 1815);
Mandibles small (<200 µm), asymmetric; prostheca absent; incisor acuminate, long; apex bidentate (right), tetradentate (left), with larger dens right than left; mola not developed, without teeth (Fig. 11)

Fig. 8. Mandibles *Choleva oblonga*

Fig. 11. Mandibles *Nargus veloc*

Fig. 9. Mandible *Choleva spadicea*

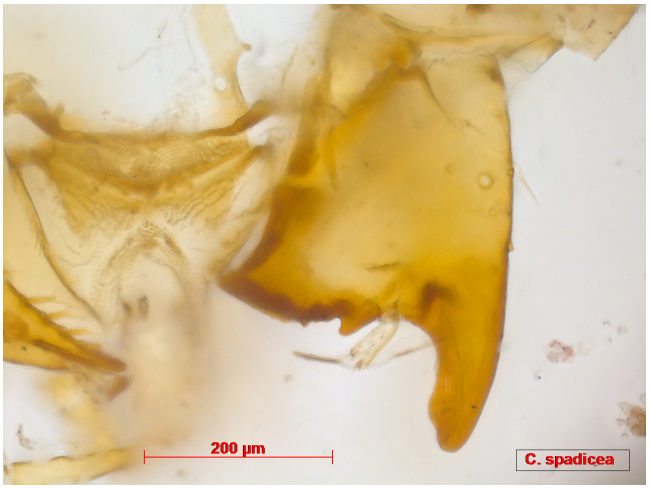

Fig. 10. Mandible *Choleva spadicea*

Dreposcia umbrina (Erichson, 1837);
Mandibles small (<200 µm), asymmetric; prostheca present, pointed; incisor acute, short; apex monodentate (left), bidentate (right); mola not developed, without teeth (Fig. 12)

Sciodrepoides fumatus (Spence, 1837);
Mandibles small (<200 µm), symmetric; prostheca present; incisor acute, short; apex monodentate, no dens; mola not developed, without teeth (Fig. 13)

Sciodrepoides watsoni (Spence, 1815);
Mandibles small (<200 µm), asymmetric; prostheca present; incisor acute, short; apex monodentate (left), bidentate (right), small dens; mola not developed, without teeth (Fig. 14)

Ptomaphagus medius (Rey, 1889);
Mandibles stout (>200 µm), symmetric; prostheca present; incisor acuminate, long; apex bidentate; mola distinct, with teeth (Fig. 15)

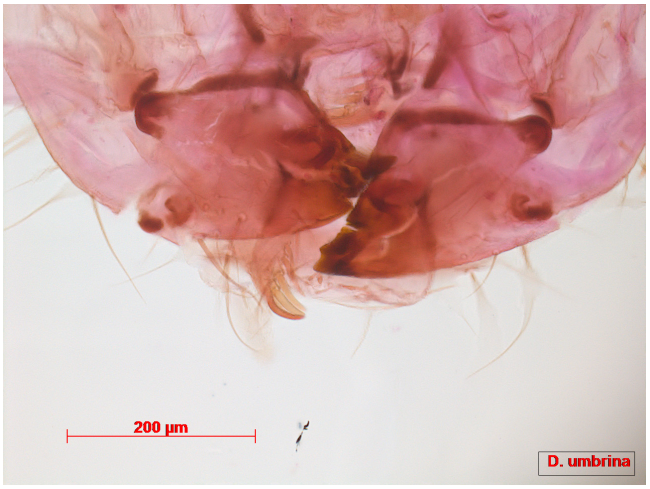

Fig. 12. Mandibles *Dreposcia umbrina*

Fig. 14. Mandibles *Sciodrepoides watsoni*

Fig. 13. Mandibles *Sciodrepoides fumatus*

Fig. 15. Mandibles *Ptomaphagus medius*

Ptomaphagus sericatus (Chaudoir, 1845);
Mandibles stout (>200 µm), asymmetric; prostheca present; incisor acuminate, long; apex monodentate (right), bidentate (left); mola distinct, with teeth (Fig. 16)

Ptomaphagus subvillosus (Goeze, 1777);
Mandibles stout (>200 µm), asymmetric; prostheca present; incisor acuminate, long; apex monodentate (right), bidentate (left); mola not developed, without teeth (Fig. 17)

Ptomaphagus varicornis (Rosen hauer, 1847);
Mandibles stout (>200 µm), symmetric; prostheca present; incisor acuminate, long; apex bidentate; mola distinct, with teeth (Fig. 18)

Fig. 16. Mandibles *Ptomaphagus sericatus*

Fig. 17. Mandibles *Ptomaphagus subvillosus*

Fig. 18. Mandibles *Ptomaphagus varicornis*

Identification key made with the mandible characteristics

1a Incisor acute	2
1b Insicor acuminate	7
2a Mandibles small (<200 µm)	3
2b Mandibles stout (>200 µm)	5
3a Mandibles symmetric	4
3b Mandibles asymmetric	*Sciodrepoides watsoni* (Spence, 1815)
4a Apex bidentate, small dens	*Nemadus colonoides* (Kraatz, 1851)
4b Apex monodenatate, no dens	*Sciodrepoides fumatus* (Spence, 1837)
5a Mandibles symmetric	*Choleva spadicea* (Sturm, 1839)
5b Mandibles asymmetric	6
6a Apex monodentate (left), bidentate (right)	*Dreposcia umbrina* (Erichson, 1837)
6b Apex tridentate (left), tetradentate (right)	*Choleva oblonga* (Latreille, 1807)
7a Mola distinct, with teeth	8
7b Mola not developed, without teeth	11
8a Mandibles symmetric	9
8b Mandibles asymmetric	10
9a Mandibles about 300 µm	*Ptomaphagus medius* (Rey, 1889)
9b Mandibles about 400 µm	*Ptomaphagus varicornis* (Rosen hauer, 1847)
10a Apex monodentate (right), bidentate (left)	*Ptomaphagus sericatus* (Chaudoir, 1845)
10b Apex bidentate (left), tetradentate (right), with larger dens left than right	*Choleva fagniezi* Jeannel (1922)
11a Mandibles small (<200 µm)	*Nargus velox* (Spence, 1815)
11b Mandibles stout (>200 µm)	12
12a Mandibles symmetric	*Choleva holsatica* Benick & Ihssen (1937)
12b Mandibles asymmetric	13
13a Apex monodentate (right), bidentate (left)	*Ptomaphagus subvillosus* (Goeze, 1777)
13b Apex tertadentate, right smaller dens as left	*Choleva agilis* (Illiger, 1798)

Statistics of measurements

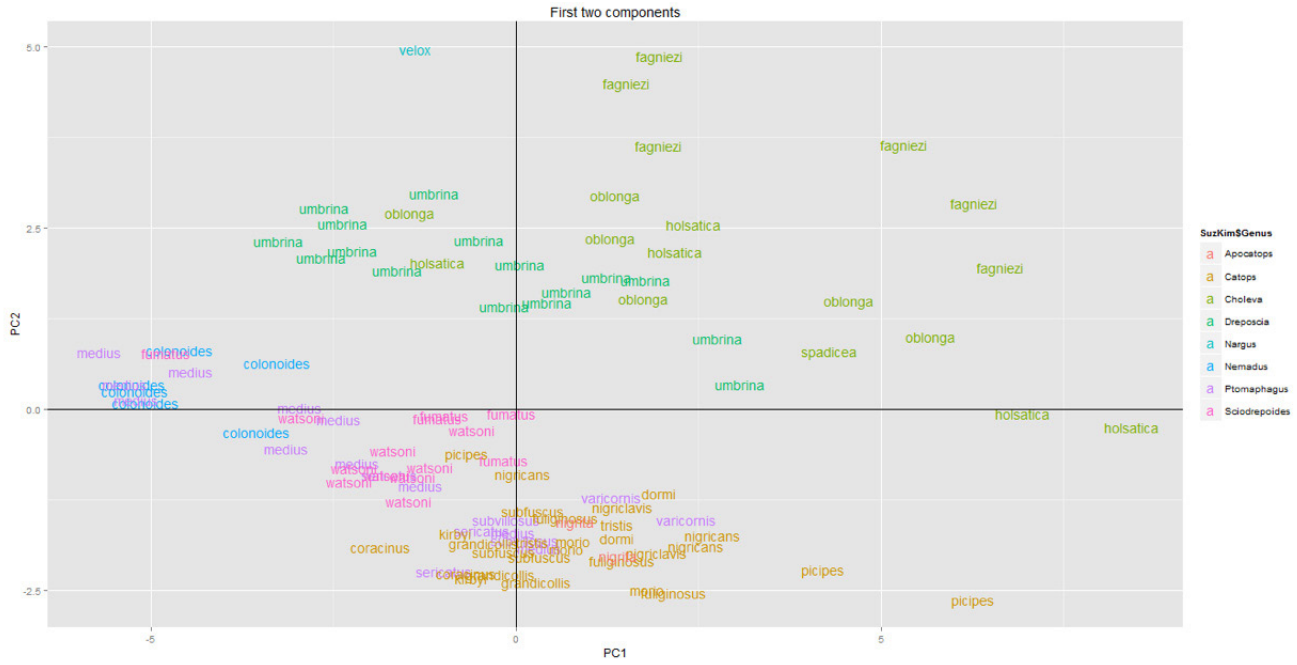

Fig. 19. PCA (including *Apocatops* and *Catops*); genera are coloured and species are marked in the names

Identification key for genera (inclusive *Apocatops* and *Catops*) (Fig. 20)

1a Length antenna segment 2 ≤ 105.37 (p<0.001)	2
1b Length antenna segment 2 > 105.37 (p<0.001; n=23)	*Choleva*, *Dreposcia*, *Nargus*, *Sciodrepoides*
2a Length cerci segment 1 ≤ 315.87 (p<0.001)	3
2b Length cerci segment 1 > 315.87 (p<0.001; n=14)	*Choleva*, *Dreposcia*
3a Length abdominal segment 1 ≤ 328.83 (p<0.001)	4
3b Length abdominal segment 1 > 328.83 (p<0.001; n=30)	*Apocatops*, *Catops*, *Ptomaphagus*
4a Length cerci segment 1 ≤ 132.77 (p=0.008)	5
4b Length cerci segment 1 > 132.77 (p=0.008; n=14)	*Catops*, *Ptomaphagus*, *Sciodrepoides*
5a Length antenna segment 2 ≤ 138.45 (p=0.019; n=10)	*Nemadus*, *Ptomaphagus*, *Sciodrepoides*
5b Length antenna segment 2 > 138.45 (p=0.019; n=12)	Cato*ps*, *Ptomaphagus*

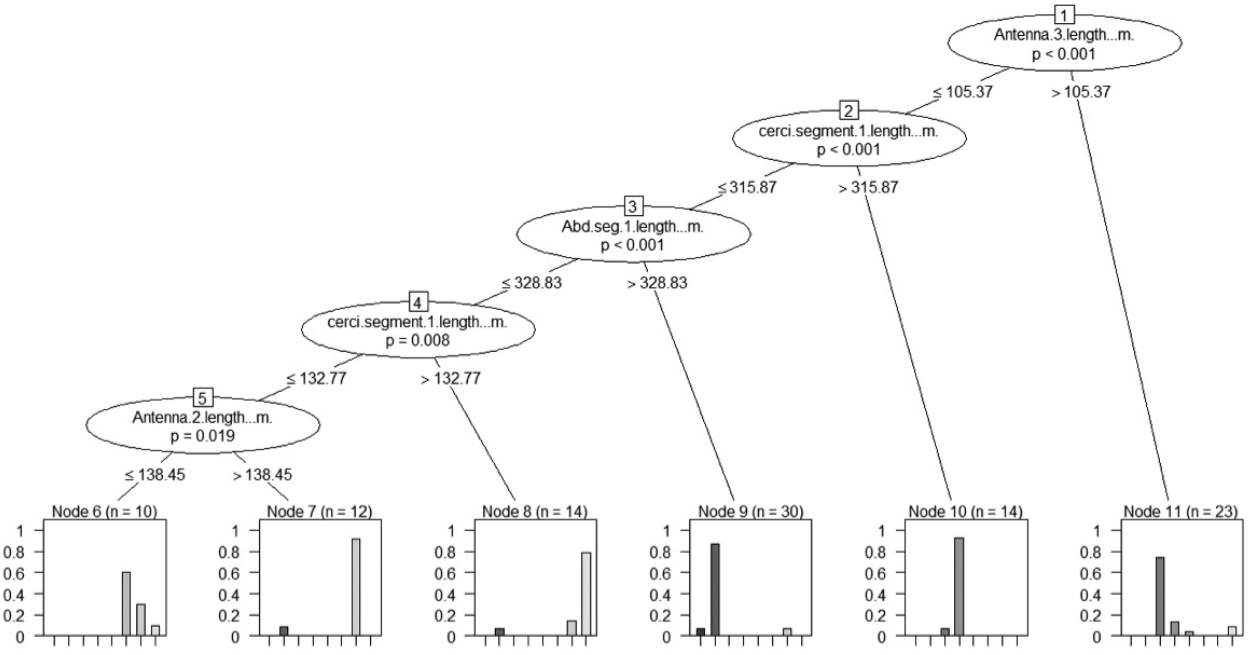

Fig. 20. Identification key; dots on x-axis are *Apocatops, Catops, Choleva, Dreposcia, Nargus, Nemadus, Ptomaphagus and Sciodrepoides*

Identification key for genera (exclusive *Apocatops* and *Catops*) (Fig. 21)

1a Length maxillary palp segment 3 ≤ 131.41 (p<0.001)	2
1b Length maxillary palp segment 3 > 131.41 (p<0.001)	6
2a Length head ≤ 580.06 (p<0.001)	3
2b Length head > 580.06 (p<0.001)	7
3a Length cerci segment 1 ≤ 266.94 (p<0.001)	4
3b Length cerci segment 1 > 266.94 (p<0.001; n=8)	*Dreposcia*
4a Length cerci segment 1 ≤ 131.58(p<0.001)	5
4b Length cerci segment 1 > 131.58 (p<0.001; n=22)	8
5a Width tarsungulus posterior leg ≤ 15.45 (p=0.006; n=10)	*Nemadus*, *Sciodrepoides*
5b Width tarsungulus posterior leg > 15.45 (p=0.006; n=13)	*Ptomaphagus*
6a Width tarsungulus posterior leg ≤ 27.87 (p=0.005; n=36)	*Choleva*
6b Width tarsungulus posterior leg > 27.87 (p=0.005; n=7)	*Choleva*, *Dreposcia*
7a Length head ≤ 690.21 (p=0.004; n=18)	*Choleva*, *Dreposcia*, *Ptomaphagus*
7b Length head > 690.21 (p=0.004; n=7)	*Dreposcia*, *Nargus*, *Ptomaphagus*
8a Width mandibular base ≤ 160.62 (p=0.029; n=7)	*Nemadus*, *Sciodrepoides*
8b Width mandibular base > 160.62 (p=0.029; n=16)	*Sciodrepoides*

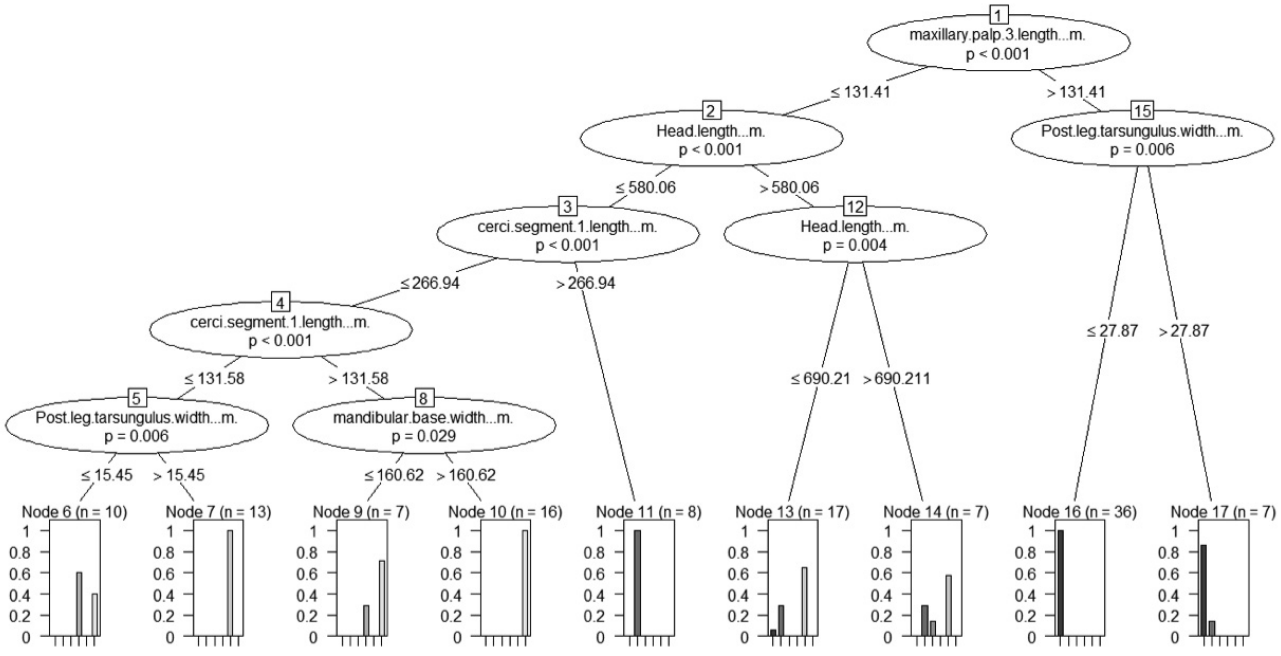

Fig. 21. Identification key; dots on x-axis are *Choleva, Dreposcia, Nargus, Nemadus, Ptomaphagus and Sciodrepoides*

Identification key for species (exclusive *Apocatops* and *Catops*) (Fig 22)

1a Length maxillary palp segment 3 ≤ 52.42 (p<0.001; n=10)	*Nemadus colonoides*, *Sciodrepoides watsoni*
1b Length maxillary palp segment 3 > 52.42 (p<0.001)	2
2a Length maxillary palp segment 3 ≤ 181.92 (p<0.001)	3
2b Length maxillary palp segment 3 > 181.92 (p<0.001; n=22)	*Choleva fagniezi*, *Choleva holsatica*, *Choleva oblonga*, *Choleva spadicea*
3a Length maxillary palp segment 3 ≤ 131.41 (p<0.001)	4
3b Length maxillary palp segment 3 > 131.41 (p<0.001; n=21)	*Choleva agilis*, *Choleva fagniezi*, *Choleva holsatica*, *Choleva oblonga*, *Dreposcia umbrina*
4a Length head ≤ 690.211 (p<0.001)	5
4b Length head > 690.211 (p<0.001; n=7)	*Ptomaphagus medius*, *Dreposcia umbrina*, *Ptomaphagus varicornis*, *Nargus velox*
5a Length cerci segment 1 ≤ 304.46 (p<0.001)	6
5b Length cerci segment 1 > 304.46 (p<0.001; n=14)	*Ptomaphagus medius*, *Dreposcia umbrina*
6a Length cerci terminal setae ≤ 65.315 (p<0.001)	7
6b Length cerci terminal setae > 65.315 (p<0.001; n=9)	*Choleva agilis*, *Sciodrepoides fumatus*, *Sciodrepoides watsoni*
7a Width maxillary palp segment 1 ≤ 42.12 (p=0.001; n=16	*Nemadus colonoides*, *Sciodrepoides fumatus*, *Ptomaphagus medius*, *Sciodrepoides watsoni*
7b Width maxillary palp segment 1 > 42.12 (p=0.001; n=22)	8
8a Width antenna segment 1 ≤ 48.616 (p=0.024; n=15)	*Ptomaphagus medius*, *Ptomaphagus sericatus*
8b Width antenna segment 1 > 48.616 (p=0.024; n=7)	*Sciodrepoides fumatus*, *Ptomaphagus medius*, *Ptomaphagus sericatus*, *Ptomaphagus subvillosus*

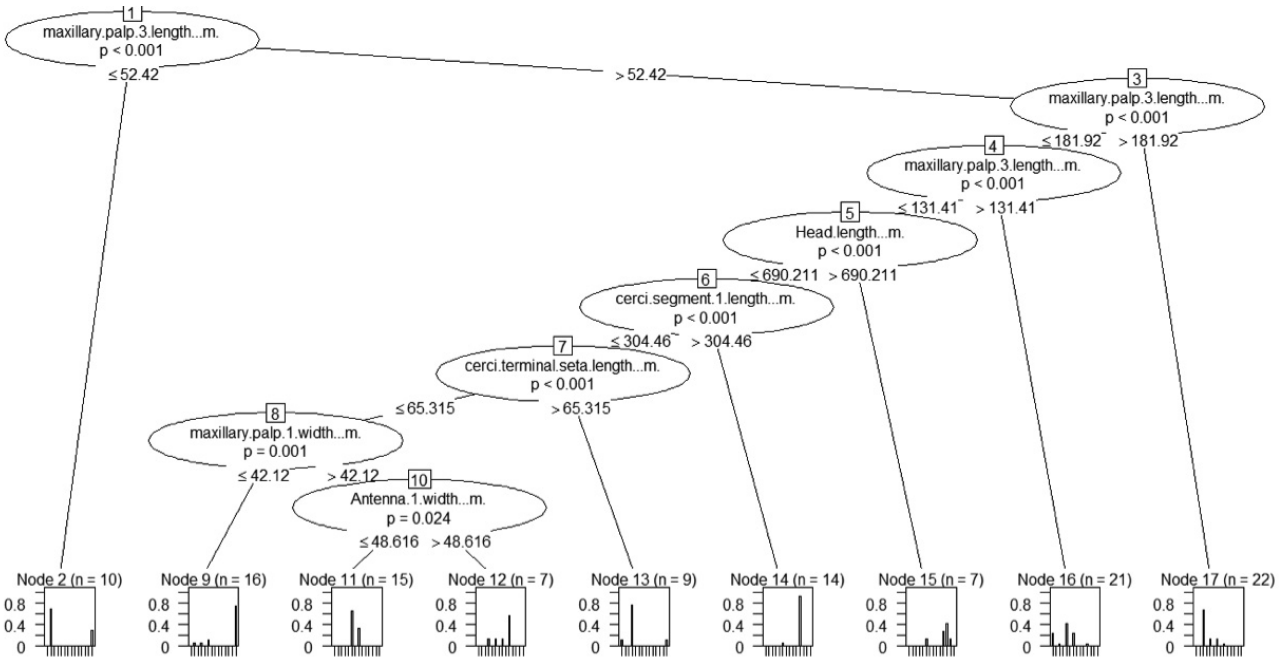

Fig. 22. Identification key; dots on x-axis are *Choleva oblonga, Ptomaphagus sericatus, Choleva spadicea, Choleva subvillosus, Dreposcia umbrina, Ptomaphagus varivornis, Nargus velox and Sciodrepoides watsoni*

Table 1. Habitat preferences and mandible characteristics

Species	Habitat	Mandibles small (<200 µm)/stout (>200 µm)	Mandibles (a)symmetric	Prostheca	Incisor shape	Incisor	Apex	Molar area
Nemadus colonoides (Kraatz, 1851)	Nest of animals (insects and birds)	*small*	symmetric	present	acute	short	bidentate, small dens	not developed, without teeth
Choleva agilis (Illiger,1798)	burrows of animals (f.e. moles and rabbits), prefers a wet environment, it is a cold-climate species	*stout*	asymmetric	present	acuminate	long	tertadentate, right smaller dens as left	not developed, without teeth
Choleva fagniezi Jeannel (1922)	Litter and burrows of animals, it is a cold season insect	*stout*	asymmetric	present	acuminate	long	bidentate (left), tetradentate (right), with larger dens left than right	distinct, with teeth
Choleva holsatica Benick & Ihssen (1937)	Caves	*stout*	symmetric	present	Acuminate	long	bidentate	not developed, without teeth
Choleva oblonga (Latreille, 1807)	Tunnels and nests of mammals, present in open fields, sand pits and in the city	*stout*	asymmetric	present	acute	long	tridentate (left), tetradentate (right)	distinct, with teeth
Choleva spadicea (Sturm, 1839)	Caves/mole and mice nests, present in forests	*stout*	symmetric	present	acute	long	monodentate (maybe bidentate but worn)	not developed, without teeth
Nargus velox (Spence, 1815)	Vegetation, tunnels and nests of animals	*small*	asymmetric	absent	acuminate	long	bidentate (right), tetradentate (left), with larger dens right than left	not developed, without teeth
Dreposcia umbrina (Erichson, 1837)	Nests of ants and old trees, it prefers forested habitats	*stout*	asymmetric	present	acute	short	monodentate (left), bidentate (right)	not developed, without teeth
Sciodrepoides fumatus (Spence, 1837)	Prefers wet wooded sites, forests, animal nests, more restricted to low altitudes, most active during spring	*small*	symmetric	present	acute	short	monodentate, no dens	not developed, without teeth
Sciodrepoides watsoni (Spence, 1815)	Caves, nests of animals, open landscapes and forests, active throughout spring to late autumn	*small*	asymmetric	present	acute	short	apex monodentate (left), bidentate (right), small dens	not developed, without teeth
Ptomaphagus medius (Rey, 1889)	Litter on woodland floor, animal nests, between the roots of trees	*stout*	symmetric	present	acuminate	long	bidentate	distinct, with teeth
Ptomaphagus sericatus (Chaudoir, 1845)	(Meadow) forests, active throughout spring till late autumn	*stout*	asymmetric	present	acuminate	long	monodentate (right), bidentate (left)	distinct, with teeth
Ptomaphagus subvillosus (Goeze, 1777)	Forests and animal nests and tunnels	*stout*	asymmetric	present	acuminate	long	monodentate (right), bidentate (left)	not developed, without teeth
Ptomaphagus varicornis (Rosen hauer, 1847)	Forest, animal nests, forested habitats, active throughout spring till late autumn	*stout*	symmetric	present	acuminate	long	bidentate	distinct, with teeth

Table 2. Smallest and largest measurements of external morphological characteristics

Species	Length cerci segm.1 (µm)	Length maxillary palp segm. 3 (µm)	Length head (µm)	Width tarsungulus posterior leg (µm)	Width mandiblular base (µm)	Length antenna segm. 2 (µm)	Length abdominal segm. 1 (µm)	width maxillary palp segm. 1 (µm)	Length cerci terminal seta (µm)	Width antenna segm. 1 (µm)
Nemadus colonoides (Kraatz, 1851)	78-126	38-53	273-344	10-15	89-136	77-139	53-190	26-31	27-90	30-45
Choleva agilis (Illiger,1798)	287-396	120-151	735-867	14-31	222-276	280-385	165-217	32-61	84-109	44-81
Choleva fagniezi Jeannel (1922)	365-739	177-242	551-1043	16-30	188-397	493-826	254-491	44-73	91-252	66-114
Choleva holsatica Benick & Ihssen (1937)	299-782	135-203	588-1037	22-33	261-418	363-656	176-550	48-85	33-123	63-111
Choleva oblonga (Latreille, 1807)	259-505	145-205	539-999	19-29	215-394	320-549	250-570	50-70	86-161	65-98
Dreposcia umbrina (Erichson, 1837)	382-783	57-88	418- 756	25-44	137-218	284-441	125-451	28-66	67-151	57-82
Sciodrepoides fumatus (Spence, 1837)	114-267	71-90	340-581	14-25	141-186	126-259	156-323	33-45	59-81	48-67
Sciodrepoides watsoni (Spence, 1815)	93-200	44-87	285-558	14-24	68-226	97-216	104-325	15-43	60-62	30-55
Ptomaphagus medius (Rey, 1889)	66-174	56-98	321-696	16-29	147-272	126-215	99-364	40-55	18-50	39-54
Ptomaphagus sericatus (Chaudoir, 1845)	11-114	69-86	533-639	20-28	186-272	168-195	209-337	45-52	15-35	42-46
Ptomaphagus subvillosus (Goeze, 1777)	68-102	73-87	638-659	22-30	154-256	123-174	306-327	55-67	32-42	57-68
Ptomaphagus varicornis (Rosen hauer, 1847)	128-207	119-120	809-857	34-40	215-385	263-280	305-323	71-74	28-37	73-76

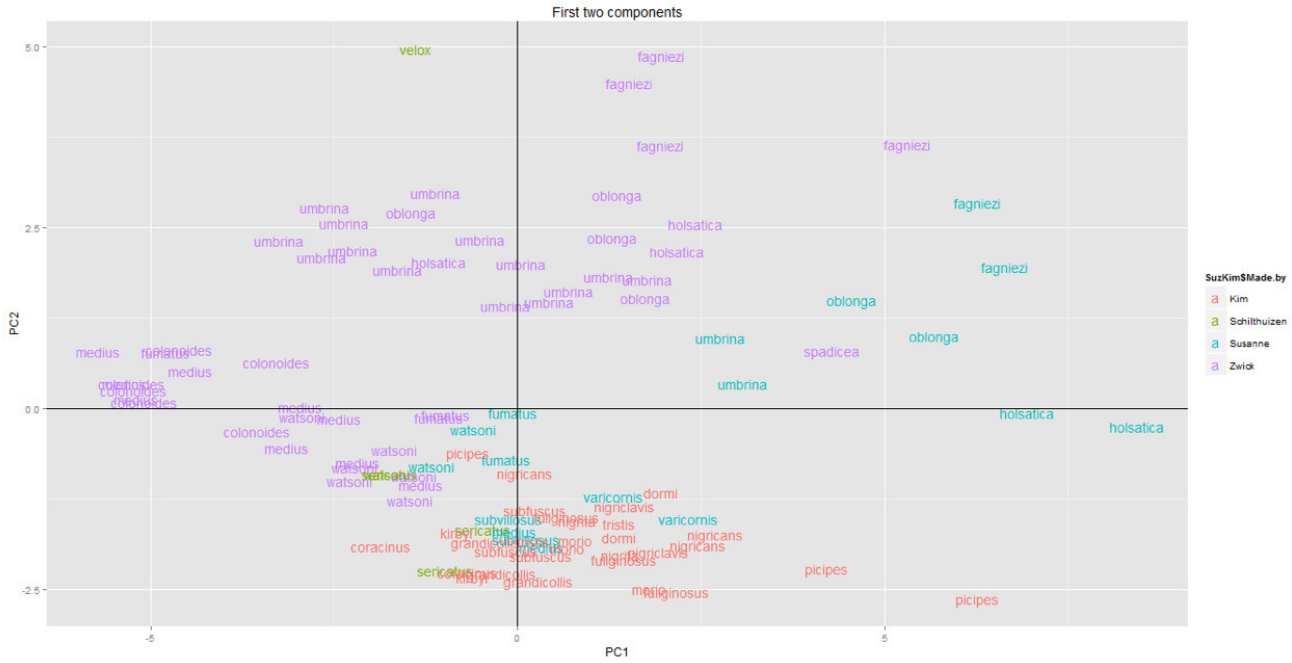

Fig. 23. PCA (including *Apocatops* and *Catops*); Maker of microscopic slides is coloured and species are marked in the name

V. Discussion

For this research microscopic slides were used from P. Zwick (1960s), M. Schilthuizen (1980), K. Renkens (2015) and S. Pinto (2015). There is a difference between the slides made by Zwick and the slides made by Renkens and Pinto (Fig. 23). This difference is probably due to non-random choosing of specimens by Renkens and Pinto. We chose the biggest samples to make the microscopic slides, because the characteristics differ between the different instars.[1] But for Staphylinoidea larvae most characteristics are similar for instars two and three.[37] Although it is plausible that those larvae are close to maturing, the instar of these specimens is not known. Zwick also choose larval instar one and two for his microscopic slides.

The instars for most specimens from P. Zwick are known therefor it was possible to do a test to see which characteristics are significant different between the instars. Only body width is dependent of the instar. The younger the larvae are, the smaller the body width. (Fig. 24) The microscopic slides made by K. Renkens and S. Pinto were not included in this test, because the instar of those specimens is unknown.

Other differences between the microscopic slides from Zwick and Renkens and Pinto are: not coloured with Phenosaphranine 1% / coloured with Phenosaphranine 1%, embedded in Euparal for about 55 years / preserved in ethanol 70% for about 55 years and more, sometimes more dissected animals on slide / always one complete animal on slide.

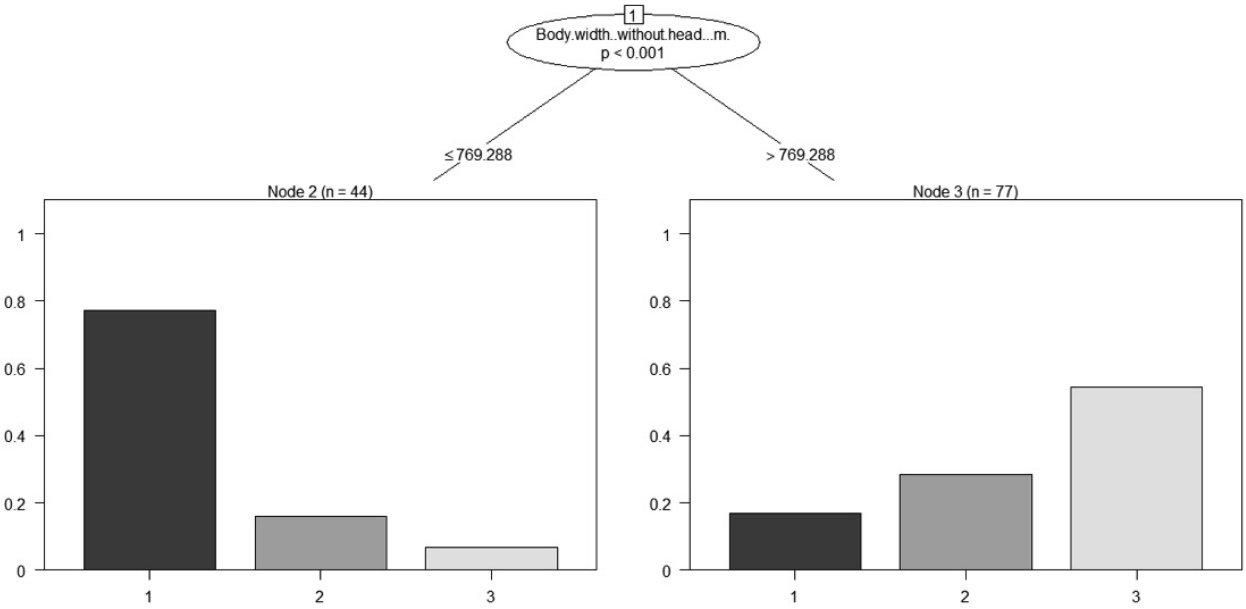

Fig. 24. Determination key; dots are instar one, instar two and instar three

One identification key of the genera includes *Apocatops* and *Catops* while the other key does not. Both keys have advantages and disadvantages. The key without the genera *Apocatops* and *Catops* includes a lot more characteristics, but also a lot more gaps. Because P. Zwick dissected some animals, not all characteristics were available. With rfImpute these gaps were filled for the statistical analysis, the influence of this imputation is unknown. For the key that includes the genera *Apocatops* and *Catops* the characteristics and specimens with too many gaps were deleted. This resulted in fewer imputations, but also in fewer characteristics. It is not yet possible to say which one is the best.

The characteristic 'length cerci segment 1' is present in the three identification keys made with the measurements. The characteristics 'length maxillary palp segment 3' and 'length head' can be used in the identification of the genera as well as the species. Other important characteristics for identifying the genera are: 'width tarsungulus posterior leg', 'width mandibular base', 'length antenna segment 2' and 'length abdominal segment 1'. For identification of the species the characteristics: 'width maxillary palp segment 1', 'length cerci terminal seta' and 'width antenna segment 1' are used.

The correlation between characteristics is subject for further research. It is plausible, for example, that a larger femur also means a larger tibia, because they were measured on one leg. In this research all characteristics are treated as individual, uncorrelated characteristics.

Altough the identification keys (exept for the key with mandible characteristics) presented in this research paper do not give clear outputs I sincerely believe that it is possible to make an identification key for larval Cholevinae. For example with an other (statistical) method (like cforest) or software (like DELTA). In the futere Cholevinae larvae will and can be used in forensic entomology to determine the post-mortem interval.

VI. Acknowledgements

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VIII. Appendices

Method for making microscopic slides of Cholevinae larva
With Kim Renkens

Thanks to Kees van den Berg

Goal: Making microscopic slides of Cholevinae larvae, which can be used for morphological research.

Material:
- Cholevinae Larva
- Insect needle (Stainless steel 38)
- Embryo glass
- Binocular (SteREO, Zeiss, Discovery.V8)
- KOH 10%
- Alcohol tube
- Label stickers
- Water bath (Köttermann)
- paintbrush 5/0
- Demi water
- Photoflo (Kodak)
- Paper
- Gloves
- Pipette
- Wooden skewer and insect needle (stainless steel 0.1)
  - One sharp, one with a hook
- Paper with template microscope slide (Fig. 25)

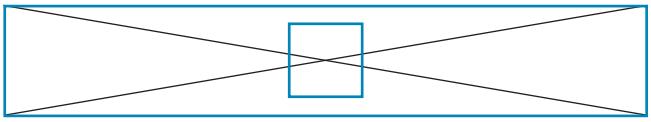

Fig. 25. Template

- 3-Well concavity slide (1.4-1.6 mm thick, Cat#71878-04)
- Phenosaphranine 1%
- Alcohol (30%, 50%, 70%, 96%)
- Microscopic slide (RD France, 76mm x 26mm, 1.1 mm thick, ISO 8037)
- Cover glass (Thermo scientific, 10 mm x 10 mm, 0 mm thick)
- Pincer
- Glass stick
- Euparal green
- Glascribe (Bel-art, F44150)
- Slide mailer
- Stove (Termaks)

Method/protocol

Removing the intestines

1- Make holes in the larva with the insect needle, ventral between the legs and between 7th and 8th abdominal segment.
2- Put larva in 1 cm KOH 10% (in alcohol tube).
3- Leave it till the skin of the larva isn't brown anymore.
- Overnight at room temperature
- Or about two hours in the water bath (70°C)
  - (possibly the duration of time is due through freshness of material, the time increases when the larvae are older)
4- Put larva in demi water with a few drops of Photoflo, shake tube.
5- Put larva in embryo glass (filled with demiwater with a few drops of Photoflo), make a cut between two abdominal segments with insect needles and remove the intestiness and other inside stuff with the insect needles and a paintbrush.
6- Leave larva overnight in the alcohol tube with 1 cm demiwater with a few drops of Photoflo.

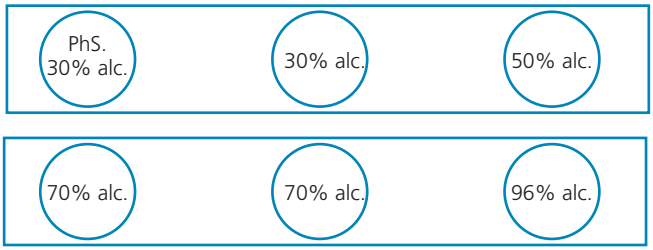

Fig. 26. Colouring with Phenosaphranine

Colouring with Phenosaphranine (Fig. 26)

7- Larva in Phenosaphranine 1% with 30% alcohol (between 3-5 minutes)
8- Larva in next hole with 30% alcohol, remove the pigment with the paintbrush, put the larva in the right form
9- Larva in next hole with 50% alcohol, remove the pigment with the paintbrush and insect needle, put the larva in the right form, make a cut ventral between head and prothorax and move head up.
10- Larva in next hole with 70% alcohol, remove the pigment with the paintbrush and insect needle, put the larva in the right form.
11- If necessary: larva in next hole with 70% alcohol, remove the pigment with the paintbrush and insect needle, put the larva in the right form.
12- If necessary: repeat step 7-11 for more colour.
13- larva in next hole with 96% alcohol, make sure the larva lies in the right form.

Making the microscopic slide

14- Clean microscopic slide (not with alcohol!).
15- Put a stripe, as long and wide as the larva, of Euparal (green) in the middle of the microscopic slide with a stick made of glass.
16- Put the larva on the stripe of Euparal. Lay the larva down as preferred.
17- Leave the slide to dry for a few minutes, this could be at room temperature or in the stove, put something over the slide to protect it from dust.
18- Put more Euparal round and on the larva.
19- Place the cover glass with a pincer.
20- Put some pressure on it (till air is gone).
21- If necessary: put some drops of Euparal round the cover glass to fill it up.
22- Label the slide (with a sticker and by writing the number on the slide with a glass pen).
23- Let it dry for a while in the slide mailer.
24- Leave the microscopic slide for 2 or 3 weeks in the stove at 40°C.
25- If necessary: remove superfluous Euparal with alcohol 96%.

Table 3. Microscopic slides made by P. Zwick and M. Schilthuizen

box nr.	slot nr.	genus	species	life stage	prep.	medium	label	condition
I	4	*Choleva*	*fagniezi*	L1	whole animal	Kanadabalsam	Zucht, [female] aus Berlin-Gruenewald, Jagen 86 [?], okt. 1963	reasonable (contracted)
I	5	*Choleva*	*fagniezi*	exuviae L1/2 L3	whole exuviae and dissected	euparal	P-generation: Berlin-Grunewald, okt. 1963; Zucht	poor (dried in)
I	6	*Choleva*	*fagniezi*	exuviae L2	whole exuviae	euparal	Zucht; P-Generation aus Berlin-Grunewald, Jagen 86; Okt. 1963	reasonable
I	7	*Choleva*	*fagniezi*	exuviae L2	whole exuviae and dissected	Kanadabalsam	Zucht; P-Generation aus Berlin-Grunewald, Okt. 1963; Jagen	reasonable
I	8	*Choleva*	*fagniezi*	exuvia L3, pupa (female)	whole exuvia, whole (damaged) pupa	Kanadabalsam	Zucht; P-Generation aus Berlin-Grunewald; Okt. 1963	reasonable
I	9	*Choleva*	*fagniezi*	exuviae L2	whole exuviae	Kanadabalsam	Zucht; P-Generation aus Berlin-Grunewald; Jagen 86; Okt. 1963	good
I	10	*Choleva*	*fagniezi*	exuviae L1	whole exuviae and dissected	Kanadabalsam	Zucht; P-Gener.: P. Zwick, Okt. 63; Berlin-Grunewald; Jagen 86	good
I	11	*Choleva*	*holsatica*	L1	whole larva	clove oil/ Kanadabalsam	Zucht; Juni 1963; P-Generation: Leg. Zwick; Febr. 1963; Hoehle in Bad Segeberg, Holstein [note: something about part of the antenna being 2-Spitzig]	good
I	12	*Choleva*	*fagniezi*	exuviae L1	whole exuviae	Kanadabalsam	Zucht; leg. Zwick; Okt. 1963, Berlin-Grunewald; Jagen 86; Kaesekoeder	good
I	13	*Choleva*	*fagniezi*	L3	whole larva	clove oil/ Kanadabalsam	Zucht; P-Generation aus Berlin-Grunewald; Okt. 1963	good
I	14	*Choleva*	*holsatica*	L3	mouthparts only	Glycerin / Kanadab.	[none]	reasonable (dried in?)
I	15	*Choleva*	*holsatica*	L2	whole larva	clove oil/ Kanadabalsam	Zucht; P-Generation: Leg. Zwick, Hoehle in Bad Segeberg / Holstein	good
I	16	*Choleva*	*holsatica*	exuviae L1	whole exuviae and mouthparts dissected	Kanadabalsam	F2 der Tiere aus der Segeberger Hoehle, Holsetin, Leg. Zwick; Febr. 1963	good
I	17	*Choleva*	*holsatica*	L3	mouthparts only	Euparal	Zucht! F1! Bd. Segeberg/Holstein	good
I	18	*Choleva*	*holsatica*	exuviae L2	exuviae and mouthparts	Kanadabalsam	Exuviae der F2! F2 der im Feb. 1963 gesammelten Tiere Leg. Zwick Segeberger Hoehle, Holstein	good
I	19	*Choleva*	*fagniezi*	exuviae L3 + pupa-exuvia (male)	exuviae and mouthparts	Kanadabalsam	Zucht, P-Generation aus Berlin-Grunewald Feb. 1963	reasonable
I	28	*Choleva*	*oblonga*	exuvia L1	exuvia with mouthparts separate	euparal	Zucht; Schlitz / Hessen; Februar 1969	reasonable (very low contrast)
I	29	*Choleva*	*oblonga*	exuvia L1	whole exuvia	euparal	Zucht; Schlitz 1969; Februar/Maerz	good
I	30	*Choleva*	*oblonga*	exuviae L2	exuviae with mouthparts dissected	euparal	Zucht Schlitz Fruehl. 1969	good
I	31	*Choleva*	*spadicea*	L3	whole larva; head dissected	Eukitt	Zucht! Schlitz, Eisenberg; April/Mai 1971; Zwick	reasonable (somewhat damaged)
I	32	*Choleva*	*oblonga*	exuvia L2 and L3	exuviae with mouthparts dissected	euparal	Schlitz; 2.3.1969; Zucht 69/8	reasonable
I	33	*Nemadus*	*colonoides*	exuviae L1	dissected exuviae	euparal	Zucht; Material aus Berlin-Zoo; Okt.1968; Leg. Zimmermann	reasonable
I	34	*Nemadus*	*colonoides*	L1, L2, L3	whole larvae and dissected heads	euparal	Zucht-Material aus Berlin Zoo von ZIMMERMANN 1968; Zwick	good (but L1 lateral view)

I	35	*Nemadus*	*colonoides*	larva (unknown stage)	larva with dissected head	euparal	Zucht Material aus Berlin Zoo; leg. Zimmermann; Okt. 1968	good
I	36	*Choleva*	*agilis*	L1 and L2	dissected larvae or exuviae	euparal	Zucht! P.-Generation 19.10.1964 Berlin-Tiefw… (unreadable)	reasonable
I	37	*Choleva*	*agilis*	exuvia L1	exuvia with head dissected	Kanadabalsam	Zucht! Leg. Zwick; 19.10.1964 Berlin-Tiefw… (unreadable)	good
I	43	*Ptomaphagus*	*subvillosus*	L1 and L2	dissected larvae	euparal	Zucht; Schlitz 1965	reasonable
I	44	*Ptomaphagus*	*variicornis*	larva (unknown stage)	dissected head	euparal	23.7.65; Zucht! Ploen, VII.65	good
I	45	*Ptomaphagus*	*medius*	L0, L1 and L2 exuviae	exuviae, dissected	euparal	P-Generation Mai 1964; Leg. Zwick; Berlin, Boettekerberg	poor
I	46	*Ptomaphagus*	*medius*	L1 exuviae	exuviae, dissected	Kanadabalsam	Zucht; P-Generation: v.64; Boettekerberg, Berlin; kaesekoeder	reasonable
I	47	*Ptomaphagus*	*medius*	L1, L2, L3	whole larvae	Kanadabalsam	Zucht; P-Generation: V.64; Berlin, Boettekerberg; kaesekoeder	good
I	48	*Ptomaphagus*	*medius*	L1, L2, L3	whole larvae	Kanadabalsam	P-Generation; Leg. Zwick; Mai 1964; Berlin-Boettekerberg	good
II	2	*Sciodrepoides*	*watsoni*	larva (unknown stage)	3 whole larvae	?	"Sc. Watsoni"	good
II	3	*Sciodrepoides*	*watsoni*	larva (unknown stage)	dissected larvae	?	Aaskoeder; Juli 1960; Oberhausen, Rhoen 4 Larven	reasonable
II	4	*Sciodrepoides*	*watsoni*	larva (unknown stage)	6 whole larvae	?	Larven KOH-Preparat; Gezuechtet; imagine aus Berlin-Tiefwerder	good
II	6	*Dreposcia*	*umbrina*	L1, L2, L3	8 dissected and whole larvae	euparal	Zucht paar aus Berlin-Grunewald 1966 Zwick	good
II	21	*Sciodrepoides*	*fumatus*	L1, L2, L3	whole and dissected larvae	euparal	Zucht; Schlitz, 1966	good (L3 slightly dried in)
II	46	*Dreposcia*	*umbrina*	L1	4 exuviae; 2 whole; 2 dissected	euparal	Zucht; Schlitz; (Mater. Berlin)	reasonable
II	48	*Dreposcia*	*umbrina*	L2	4 exuviae; 1 whole, 3 dissected	euparal	Zucht Schlitz; (Material aus 1966 Berlin)	good
II	49	*Dreposcia*	*umbrina*	L1	1 larva, mouthparts dissected	euparal	Zucht Berlin 1966	good
II	59	*Sciodrepoides*	*watsoni*	L1, L2, L3 (?)	11 whole larvae (3 separate batches)	?	Zucht Schlitz 1966	poor (occluded; L3 dried in)
III	7	*Ptomaphagus*	*sericatus*	larva (unknown stage)	whole larva	Berlese	Schiedam kweek	poor (dried in)
III	10	*Sciodrepoides*	*watsoni*	L2 (?)	whole larva	Berlese	Katwijk: Panbos; x.1987; kweek	reasonable/poor
III	11	*Nargus*	*velox*	L1 (?)	whole larva	Berlese	Leiden: Cronesteijn; kweek; leg. M. Schilthuizen	good
III	25	*Ptomaphagus*	*sericatus*	larva (unknown stage)	whole larvae (2x)	euparal	cultured; P.-Gen.: Holland: Schiedam (1980s); Leg. M. Schilthuizen; prep. D. v.d. Horst	good
III	26	*Ptomaphagus*	*sericatus*	larva (unknown stage)	whole larvae (2x)	euparal	cultured; P.-Gen.: Holland: Schiedam (1980s); Leg. M. Schilthuizen; prep. D. v.d. Horst	good
III	27	*Ptomaphagus*	*sericatus*	larva (unknown stage)	whole larva	euparal	cultured; P.-Gen.: Holland: Schiedam (1980s); Leg. M. Schilthuizen; prep. D. v.d. Horst	good

Table 4. Microscopic slides made by S. Pinto

box nr.	slot nr.	genus	species	life stage	locality	date	collector	condition
IV	1	*Dreposcia*	*umbrina*	larvae (unknown stage)	Cultured (P.-Gen. From Berlin-Grunewald)	1960s	P. Zwick	reasonable (contracted)
IV	2	*Dreposcia*	*umbrina*	larvae (unknown stage)	Cultured (P.-Gen. From Berlin-Grunewald)	1960s	P. Zwick	poor (dried in)
IV	3	*Sciodrepoides*	*watsoni*	?	Culture; P.-Gen. From Berlin-Tichnerder	1963/1964	P. Zwick	reasonable
IV	4	*Ptomaphagus*	*subvillosus*	larvae (unknown stage)	Culture; P.-Gen.: Ploen	1960s	P. Zwick	reasonable
IV	5	*Ptomaphagus*	*subvillosus*	larvae (unknown stage)	Culture; P.-Gen.: Ploen	1960s	P. Zwick	reasonable
IV	6	*Sciodrepoides*	*watsoni*	?	Enschede	1-5-2012	T. Hoogenboom	good
IV	7	*Sciodrepoides*	*fumatus*	larvae (unknown stage)	Culture Schiltz	1960s	P. Zwick	good
IV	8	*Sciodrepoides*	*fumatus*	larvae (unknown stage)	Culture Schiltz	1960s	P. Zwick	good
IV	9	*Ptomaphagus*	*varicornis*	larvae (unknown stage)	Zucht/Ploh	1965/1966	P. Zwick	good
IV	10	*Ptomaphagus*	*varicornis*	larvae (unknown stage)	Zucht/Ploh	1965/1966	P. Zwick	good
IV	11	*Ptomaphagus*	*medius*	larvae (unknown stage)	Culture	?	P. Zwick	reasonable (dried in?)
IV	12	*Ptomaphagus*	*medius*	larvae (unknown stage)	Culture	?	P. Zwick	good
IV	13	*Choleva*	*oblonga*	larvae L, L2, L3	Culture Schiltz/ Hesseni	1969	P. Zwick	good
IV	14	*Choleva*	*oblonga*	larvae L, L2, L3	Culture Schiltz/ Hesseni	1969	P. Zwick	good
IV	15	*Choleva*	*lederiana/ septenrionis holsatica*	larvae and pupae	P-gen from Fetz, F1 from Segeberg	1963	P. Zwick	good
IV	16	*Choleva*	*lederiana/ septenrionis holsatica*	larvae and pupae	P-gen from Fetz, F1 from Segeberg	1963	P. Zwick	reasonable
IV	17	*Choleva*	*fagniezi*	larvae L1, L2, L3, and pupae	Culture; P.-Gen: Berlin-Grunewald	okt. 1963	P. Zwick	reasonable (very low contrast)
IV	18	*Choleva*	*fagniezi*	larvae L1, L2, L3, and pupae	Culture; P.-Gen: Berlin-Grunewald	okt. 1963	P. Zwick	good