UNIVERSIDADE DE LISBOA
FACULDADE DE CIÊNCIAS
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Pictorial Characterization of Eight Coleoptera Families with Forensic Interest

Inês de Lima e Santos Pimentel Fontes

Mestrado em Biologia Humana e Ambiente

Dissertação orientada por:
Professora Doutora Maria Teresa Rebelo

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Dissertação para a obtenção do grau de mestre em Biologia Humana e Ambiente.

Trabalho realizado no Laboratório de Entomologia (Departamento de Biologia Animal) da Faculdade de Ciências da Universidade de Lisboa.

Previous Note

The pictorial characterizations presented in this work are still under construction. Species collected in future experiments can be added to this key in order to help a better characterization of Portuguese Coleoptera fauna.

The results of the present work lead to the creation of pictorial keys and characterizations available online (www.csicoleoptera.weebly.com).

It was submitted to an online scientific journal (Ecologi@) an article with the preliminary results of this project and these same results were presented at 2015 Encontro sobre Biodiversidade e Conservação de Invertebrados in a scientific poster.

The references used in this dissertation are in accordance with the norms of the journal Forensic Science International.

One day Alice came to a fork in the road and saw a Cheshire cat in a tree.
‘Which road do I take?’ she asked.
‘Where do you want to go?’ was his response.
‘I don’t know,’ Alice answered.
‘Then,’ said the cat, ‘it doesn’t matter.’

Lewis Carroll, Alice's Adventures in Wonderland (1865)

The very basic core of a man's living spirit is his passion for adventure.
The joy of life comes from our encounters with new experiences, and hence there is no greater joy
than to have an endlessly changing horizon, for each day to have a new and different sun.
If you want to get more out of life, you must lose your inclination for monotonous security and adopt a
helter-skelter style of life that will at first appear to you to be crazy.
But once you become accustomed to such a life you will see its full meaning and its incredible beauty.

Jon Krakauer, Into the Wild (1996)

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Em memória do avô Manel que me ajudou a gostar um bocadinho mais de química e de quem eu sempre admirei a coragem para experimentar gastronomia diferente. De certeza que aprovaria a entomofagia!

Em memória da avó Ceição que foi a responsável por aguçar a minha veia forense com as histórias do Poirot e da Miss Marple. As tardes passadas no quintal da vivenda a brincar, a comer pão com manteiga sem côdea e a partir pinhões é uma das minhas memórias de infância mais querida.

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Sem a vossa força e motivação não teria conseguido!

SUMÁRIO

Desde tempos remotos, os insetos têm suscitado um interesse no ser humano, o que se pode confirmar através da observação de artefactos de civilizações antigas como os Egípcios, Chineses, Maias e Aztecas. Estas obras de arte, como pinturas e esculturas, frequentemente associavam determinados insetos não só à morte mas também à reincarnação e vida pós-morte.

A aplicação de conhecimentos entomológicos em investigações criminais tem uma história bastante extensa e bem documentada. Começando no primeiro caso registado da aplicação da entomologia na resolução de um homicídio na China Medieval, a utilização desta ciência como uma ferramenta forense tem vindo a percorrer um longo caminho repleto de contribuições científicas provenientes dos quatro cantos do mundo. No entanto, foi a investigação do homicídio de um recém-nascido em França no final do século XVII que marcou o início da entomologia forense moderna e que contribuiu para a publicação da notável obra de Mégnin "*La Faune des Cadavres: Application de l'Entomologie à la Médicine Légale*". A partir de então, esta ferramenta forense foi desenvolvida principalmente através de contribuições de investigações científicas durante os anos das grandes guerras e a sua completa aceitação só foi possível devido ao trabalho conjunto de académicos e forças de segurança.

Os insetos podem fornecer importantes informações em investigações forenses não só em casos de homicídios como também em casos de negligência tanto de seres humanos como de animais domésticos ou selvagens, por exemplo, em jardins zoológicos. Adicionalmente, numa área complementar designada entomotoxicologia, conjugam-se os conhecimentos da entomologia e da toxicologia para responder a diversas questões, como a possibilidade da presença de substâncias tóxicas no cadáver, em casos de homicídio, suicídio ou mortes acidentais. A entomologia forense pode igualmente ser utilizada em casos de investigação de pragas tanto num âmbito imobiliário como alimentar.

Contudo, a utilização dos conhecimentos entomológicos em investigações de homicídios é, possivelmente, a mais mediatizada, principalmente pelos meios de comunicação social e cinematográficos. Uma vez que contribuem para a decomposição do cadáver, os insetos constituem evidências cruciais. Assim sendo, quando é descoberto um cadáver, os insetos possibilitam o conhecimento de diversos parâmetros que, em certos casos, seriam impossíveis de descobrir utilizando outros campos do saber, nomeadamente as técnicas médico-legais, como é o caso da temperatura corporal. Os insetos que colonizam o cadáver permitem não só estimar o intervalo pós-morte (IPM) através dos seus padrões de desenvolvimento e sucessão, como também poderão auxiliar na descoberta do local do crime primário nos casos em que há a suspeita de que o cadáver foi movido *postmortem*. Contudo, a primeira grande etapa é a recolha, no local do crime, de todos os vestígios entomológicos e sua correta identificação. No entanto, este potencial forense dos insetos só pode ser totalmente reconhecido se se realizarem extensos estudos a fim de compreender melhor não só a sua biologia e funcionamento enquanto seres vivos, como também a sua bioecologia, ou seja, as interações que estes realizam com o meio à sua volta. Contudo, a aplicação de tais estudos a investigações é um processo que necessita de ser realizado com caução uma vez que, estando distribuídos globalmente, os insetos apresentam diferenças de comportamento. Adicionalmente, existem fatores que são relevantes e que necessitam de uma cuidada análise, como é o caso das condições meteorológicas. Sabe-se que a temperatura e humidade são importantes fatores que determinam a velocidade de desenvolvimento dos insetos.

O processo de decomposição, apesar de ser uma ocorrência contínua no tempo, pode ser dividido em fases que são marcadas por diferenças significativas que ocorrem no cadáver. Esta divisão, que

facilita a sua análise, é, por vezes, realizada diferentemente consoante o investigador mas, frequentemente, considera-se que todo o processo de decomposição é composto por cinco fases diferentes: fase fresca, fase de inchaço, fase de decomposição ativa, fase de decomposição avançada e, por fim, a fase seca. Estas etapas são frequentemente protagonizadas por um conjunto de fauna entomológica que auxilia a investigação e que, nos casos em que a morte ocorreu há mais de 72 horas, tornam-se evidências cruciais.

Apesar de muitos insetos visitarem cadáveres em decomposição, as ordens que mais relevância têm no âmbito da entomologia forense são a Diptera (moscas) e a Coleoptera (escaravelhos). As moscas são conhecidas por serem os primeiros colonizadores de cadáveres e são, sem dúvida, as mais utilizadas em casos em que conhecimentos de entomologia forense são requeridos.

Ao chegarem ao cadáver, depositam os seus ovos que, após eclodirem, originam larvas que se vão alimentar dos tecidos em decomposição. Estas passam por várias fases larvares, demarcadas pelas diferenças em tamanho, e, quando prontas, pupam por um período variável de tempo. Quando completo o processo de metamorfose dentro da câmara de pupação, adultos emergem, prontos para iniciar um novo ciclo. Uma das técnicas mais utilizadas em entomologia forense é a utilização das fases de desenvolvimento larvar das moscas para a estimativa do IPM. Este método tem, no entanto, uma série de condicionantes que necessitam de cuidada atenção, como é o caso da temperatura e humidade a que as larvas estão expostas. Estas variáveis podem alterar significativamente o tempo de desenvolvimento uma vez que estes insetos são poiquilotérmicos, condicionando assim a estimativa do IPM.

Outro método utilizado para estimar o IPM é a sucessão entomológica de insetos no cadáver. Sabendo que diferentes fases de decomposição atraem diferentes espécies de insetos, a utilização deste padrão único de sucessão pode ser bastante útil, especialmente em casos em que o IPM é mais longo. O trabalho pioneiro de Mégnin, que descreveu a existência de oito ondas de colonização de cadáveres ao longo das cinco fases de decomposição, é ainda nos dias de hoje, amplamente referido quando se utiliza esta técnica. Em casos em que é necessário utilizar estes conhecimentos o cadáver já se encontra, frequentemente, em estados de decomposição mais avançados e as moscas deixaram de ser os seus principais colonizadores, passando a ser os escaravelhos os artrópodes mais relevantes. Apesar de poderem estar presentes nas fases iniciais de decomposição (frequentemente como predadores de fases larvares de outros insetos), os escaravelhos encontram-se mais associados a fases tardias deste processo e, muitas vezes, as únicas evidências entomológicas em casos nas quais a morte ocorreu há meses ou até anos.

Apesar de ser uma ferramenta válida e estabelecida no âmbito das ciências forenses, a entomologia forense é ainda pouco utilizada em Portugal. No entanto, a realização de estudos científicos cujo principal objetivo é a compreensão das interações entre insetos e cadáveres tem sido uma importante contribuição para o desenvolvimento desta ciência em Portugal.

Este estudo centra-se em oito famílias de coleópteros presentes em Portugal, fortemente relacionadas com casos de entomologia forense: Carabidae, Cleridae, Dermestidae, Histeridae, Nitidulidae, Scarabaeidae, Silphidae e Staphylinidae. O seu principal objetivo é utilizar imagens para caracterizar morfologicamente as espécies mais representativas dessas famílias. Para tal, foram utilizados espécimes previamente capturados em armadilhas com isco ou em carcaças de animais provenientes de diversas zonas de Portugal: Serra da Estrela, Sertã, Campo Grande e Aroeira. Estes espécimes foram recolhidos utilizando armadilhas do tipo "*pitfall*" e "*malaise*" e, após a sua captura., preservados em álcool 70%. Seguidamente, montaram-se os exemplares em alfinetes entomológicos e

procedeu-se à sua observação utilizando um estereomicroscópio. Com o auxílio de chaves dicotómicas e galerias fotográficas, procedeu-se à sua identificação e seleção de características diagnosticantes que foram posteriormente fotografados num segundo estereomicroscópio com melhor definição. O registo fotográfico obtido foi então utilizado para a construção de chaves pictóricas interativas e caracterizações fotográficas, disponíveis online (www.csicoleoptera.weebly.com). Esta é uma importante ferramenta de auxílio em investigações com os mais variados quesitos uma vez que permitirá uma fácil e rápida identificação de grupos relevantes por técnicos forenses sem experiência em entomologia.

Palavras-chave: Entomologia forense, Coleoptera, características diagnosticantes, chaves de identificação, microscopia ótica.

ABSTRACT

Since ancient times, humans have shown a unique interest in insects, which can be seen in artworks of ancient civilizations like the Egyptians, Chinese, Mayans and Aztecs. These works of art, like paintings and sculptures, often associate certain insects not only with death but also with the reincarnation and afterlife.

Despite of the fact that criminal investigations arouse the most curiosity within the scope of forensic entomology, this science can be used to deal with a wide range of other investigations. Neglect cases in civil investigations are also known to use this tool to answer several questions. Another known and well developed area is the entomology of stored products. In the last years, the union of toxicology and entomology created another field of work within forensic entomology: entomotoxicology. This recent tool is very useful to know if, at the time of death, toxic substances were present in the body.

Insects can provide significant information in death investigations because of their contribution to corpse decomposition. As so, if a dead body is discovered, insects can help in the estimation of the *postmortem* interval (PMI). However, to apply entomological knowledge to criminal investigations is essential not only to have information regarding insects' bioecology that is valid for the geographic place where the body was found but also to accomplish an accurate identification of the species found on the corpse.

The focus of this study lies in eight Coleoptera families present in Portugal, strongly related to forensic entomology cases: Carabidae, Cleridae, Dermestidae, Histeridae, Nitidulidae, Scarabaeidae, Silphidae and Staphylinidae. Its main goal is to use pictures to characterize morphologically the most representative genus and species belonging to these families using specimens previously caught in baited traps or on animal carcasses. To proceed with this analysis, the diagnostic structures of the specimens are described and photographed using optical microscopy and the data obtained was used to build pictorial interactive keys and characterizations, available online (www.csicoleoptera.weebly.com). This will allow an easy identification of relevant groups for forensic technicians without expertise in entomology.

Keywords: Forensic entomology, Coleoptera, diagnostic structures, identification keys, microscopy.

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PMI – *postmortem* interval

EBCI – Encontro sobre Biodiversidade e Conservação de Invertebrados

EFD – Experimental Field Devices

mag – magnification

ns – notopleural suture

mtc – *metacoxae*

S1 – first abdominal *sternite*

S2 – second abdominal *sternite*

nsa – notopleural suture absent

cl – *clypeus*

el – *elytron*

S1-S5 – first through fifth visible abdominal *tergites*

an – *antenna*

s – abdominal *tergites*

pc – *procoxa*

tca – *trochantin* absent

prp – *propygidium*

pyg – *pygidium*

eli – *elytron striae*

pr – *pronotum*

mxp – terminal palpomere of the maxillary palps

sos – supraorbital *setae*

fr – *frons*

wh – white hairs

sc – *scutellum*

gla – gular lobe absent

mn – mandibles

ey – eye

bd – border

sp – spur

epi – epipleura

I. State of the Art

I. State of the Art

1. Forensic Entomology

1.1. Definition and main areas

Forensic entomology is the result of the synergy between two important fields of knowledge, forensic sciences and entomology, and therefore is where arthropod science and the legal system cooperate [1,2]. Although most of the events where forensic entomology assists are related to cases of homicide detected within a short period of time [1], the insects used as evidences reach far beyond just those that exhibit necrophilic feeding habits on human cadavers [3].

In order to assist legal inquiries, it is very important to know the distribution, bioecology and behaviour of insects found in a cadaver. This knowledge can provide relevant information to answer the classic forensic questions about a crime: when?, where? and how? [4,5].

Although publicity surrounding forensic entomology comes from criminal cases, this branch of forensic sciences can also assist civil inquiries [6]. In 1986, Lord and Stevenson were responsible for the separation of forensic entomology in three principal areas [2,7]:

- Urban entomology includes all cases in which insects, most commonly termites and cockroaches, cause problems in human environments. Furthermore, cases of myiasis (infestation caused by fly larvae) due to neglect of human and animals leading to civil claims are also under this area [2,8];
- Entomology of stored products investigates mostly contaminated food by insects or insect parts;
- Medico-legal entomology uses arthropod evidence in the investigation of crimes or in cases of sudden and unexplained death.

1.2. History

Despite the fact that forensic entomology was only recognized as a branch of forensic sciences in the late 18th century, the knowledge that insects and some other arthropods actively contribute to the decay of cadavers and possibly help solve forensic investigations is known for many centuries.

1.2.1. Historical records of early human civilizations

Long before the 13th century famous case occurred in China (to be discussed later on this chapter) but not very mentioned in the texts about entomology history, the Christian Bible appears to be one of the earliest written documents that provides rough descriptions about insect scavenger activity on decomposing flesh [3,9]. The Old Testament, Job 7:5, also mentions the activity of fly larvae present on a man’s infected wound, also known as, myiasis [10].

Ancient civilizations like Egyptians and Mayans, and also Chinese and Aztecs, have records, mostly through art work like paintings and sculptures, associating insects (cicadas, butterflies and beetles) with death and the rebirth or reincarnation followed by the afterlife [11–14]. The logical reasoning behind this association is related with a variety of key features of insect’s life cycles associated to the stages of metamorphosis and feeding habits, like necrophilic activity. Additionally, in ancient Egypt, scarab beetles were worshiped because their habit of forming dung balls and rolling them from one place to another were an explanation of the sun's movements [11,15].

1.2.2. Medieval China, cultural entomology and early influences

The first forensic report mentioning the use of entomology to solve a murder was written by chinese lawyer and death investigator Sung Tz'u and dates back to the 13th century medieval China. Entitled 洗去冤屈, "The Washing Away of Wrongs", this document reports a homicide investigation that took place near a rice field in which the victim had been stabbed with a sickle. On the day after the body's discovery, the lead investigator ordered all farmers to place their working tools on the ground and, after a while, it was possible to observe a concentration of flies around just one. These flies were not there by chance since they were able to detect imperceptible odours released by organic substances present in invisible blood traces, and doing so they helped in the identification of the murder weapon [16,17].

Apart from medico-legal experts, there were other parts interested in the insect-mediated pattern of body mass reduction: painters, sculptors and poets also devoted some of their works to this theme; the study of insect's influence in these artworks is called cultural entomology [14]. Dating back to the 15th century, there are interesting artworks like oil paintings and the woodcut "Dances of the Dead"; from the 16th century, the ivory carving, "Skeleton in the Tumba" is a curious work of art; most recent are the tombstones engravings like the one of Robert Touse grave, in the 19th century; written in the same century, the poem "*Une Charogne*" by the French poet Charles Baudelaire (1821-1867) contains observations on the decomposition of human cadavers [9].

The next important historical mark is the experiment leaded by the Italian physician and biologist Francesco Redi in 1667 which tested the theory of spontaneous generation. Using the flesh of different animal species, Redi verified that flies were not originated from decomposing meat like was widely accepted but rather the adults were attracted to the substances released from the decayed meat and then placed their eggs on or near it [18]. Redi's experiments brought back the knowledge from ancient China: since adult flies are attracted to decaying flesh, the reproduction of flies relies on the utilization of carrion [1].

Certainly known to all entomology community, Swedish botanist, physician, and zoologist Carolus Linnaeus was the author of the binomial nomenclature system for the classification of zoological and botanical specimens. Linnaeus was also responsible for the first identification, description and naming of over 2.000 insect species, some of them with forensic interest. Additionally, Linnaeus made an observation that "three flies would destroy a horse as fast as a lion", referring to the fact that they would produce a large mass of maggots [3].

1.2.3. Casework, research, war and public policy that made the groundwork of forensic entomology

During mass exhumations that took place in France and Germany between the 18th and 19th centuries, medico-legal doctors were able to observe that various kinds of arthropods colonized buried bodies and were responsible for their breakdown [9].

Although, undoubtedly, all the mentioned works have been important to the foundation of forensic entomology, the first case that marks the beginning of modern forensic entomology and helps in its establishment as a recognized tool for investigating crime scenes was the investigation of a murdered newborn baby in France. This was the first report to mention the use of forensic entomology in the estimation of the PMI. His mummified body was discovered inside a chimney behind a mantelpiece in a guesthouse when renovation works began, in 1850. The medical doctor responsible for the autopsy (dated March 28 of the same year) was Dr. Marcel Bergeret. He found immature stages of fleshfly *Sarcophaga carnaria* (Linnaeus, 1758) and several moths and in his report gives a brief description on the life cycle of insects in general. Bergeret's observations enabled him to reach the conclusion that the newborn baby had been put there two years prior to its discovery. However he wrongfully assumes that the process of metamorphosis (i.e. between the stage of larvae to the pupae hatch) would require a full year [1,3,9]. With this investigation, the house occupants prior to 1848 were accused and the present occupiers exonerated [1].

Also in France, January 15, 1878, took place the autopsy of another newborn child. The pathologist responsible for the autopsy was the president of the French Society of Forensic Medicine, Paul Camille Hippolyte Brouardel. After mentioning the work of Dr. Bergeret, Brouardel reports the presence of numerous arthropods inhabiting the cadaver, mostly butterfly larvae and mites. To proceed with the investigation, Brouardel requested assistance of the professor Edmond Perrier (left with the study of butterfly larvae) and army veterinarian Jean Pierre Mégnin (in charge of the mite's research). Perrier came to the conclusion that the butterfly larvae belonged to the genus *Aglossa* (family *Pyralidae*) and with his observations concluded that the baby probably born and died in the summer of the previous year (i.e. six to seven months before its discovery). Additionally, Mégnin's observations and calculations about the number of mites present on the body corroborated Perrier conclusions [9]. This case was also mentioned in the remarkable Mégnin's work "*La Faune des Cadavres: Application de l'Entomologie à la Médicine Légale*" [19] and led to the publication of several articles on this topic [9].

In his two books (the one previous mentioned and "*Faune des Tombeaux*" published in 1887), Mégnin states the existence of a correlation between the stages of human body decomposition and the insects' succession pattern in the cadaver colonization: eight waves of insects on bodies found in terrestrial environments and two waves observed in buried bodies. Also, he described the adult and larval morphology of several families of flies and identified new species of necrophagous flies with the binomial system created by Linnaeus [3,9]. But Mégnin took his observations further: he correctly claimed that the length of decomposition stages would depend upon weather conditions (like the temperature or the presence of rain) and other variables (for example, whether the corpse was clothed or not). This new insights became the groundwork to the PMI estimation [1].

Mégnin's work inspired the Canadian researchers W. Johnston and G. Villeneuve to begin several systematic entomological studies on human cadavers, around 1897. They came to the conclusion that if Mégnin research was to be used, it should be adapted to the conditions (i.e. fauna and clima conditions) of the place where the body was found [9].

The next significant point in the history of forensic entomology resulted from research made by the German physician and entomologist Hermann Reinhard, in the late 19th century. Reinhard dedicated his work in the observation of insect succession on buried bodies which led to the discovery of species of flies belonging to the *Phoridae* family, identified by the entomologist Brauer; furthermore, he observed beetles in graves older than 15 years. In addition, he made the distinction between insects that were truly necrophagous and the ones that were merely related with the burial site feeding on plant roots, for instance [3,9].

Also related to buried cadavers was the work of Murray Galt Motter for the US Bureau for Animal Industry, in 1898: he related the burial conditions (like the depth of the grave and the soil types) and the insect fauna found in 150 exhumed remains [3,9]. Going back a little back in time, the Civil War that took place in the United States between 1861 and 1865 brought a new perspective: eyewitness accounts recognized that necrophagous and carnivorous activity carried out by carrion flies was beneficial to victims of tissue necrosis [3]. In spite of the fact that this was the first officially documented use of larval therapy, it is known that in Napoleon's army, French surgeon Baron Dominique-Jean Larrey observed that "blue fly" maggots removed only the dead flesh from the wounds which had a positive effect on the rest of the healthy tissue [10].

1.2.4. The twentieth century: baby deaths investigations contributed to new advances in understanding necrophagous insects

In the turn of the 20th century, case reports from the German physicians Klingelhöffer and Horoszkiewicz and Austrian medical examiner Maschka document the observations of cadavers distortions made by insect feeding or bite marks (mostly cockroaches and ants). Their research was very important on the distinction between *antemortem* and *postmortem* wounds and these cases occurred frequently because typically, during this period, human bodies were not buried immediately after death [3]. In 1898, Klingelhöffer made a case report of a sickly infant, who died on May 26, 1889 and was autopsied three days later. During the autopsy, the local doctor found patches on the baby's face, mostly in the nose and lips area, which led to the arrest of the father, whose police suspected of forcing the child drink sulphuric acid. However, Klingelhöffer findings helped to prove parents innocence: he found no evidences of poisoning inside the baby's throat and stomach, and concluded that the patterns found on the face were caused probably by cockroaches [3,9].

Another baby death was presented in a case report made by Horoszkiewicz in 1902: a child was autopsied in April, 1899, during which no internal signs of a violent death were found; however, like the previous case investigated by Klingelhöffer, several abrasions could be seen not only on the face of the child (nose, cheeks, lips and chin) but also on the surface of the neck and backside of the left hand, fingers, genitals and the inner thighs. After receiving the statement of the baby's mother that had found the baby covered with a black shroud of cockroaches but without any skin lesions, Horoszkiewicz made an experiment. After placing pieces of fresh tissue from human bodies in glasses with cockroaches, at first there were no evidences of cockroach feeding but when the skin dried out the reported abrasions became visible [3,9].

The Austrian medical examiner Maschka also wrote several case reports in which ants and some other arthropods were most likely the responsible for skin lesions observed on deceased children's bodies [9]. In the end, Maschka concluded that the abrasions were made after the child's death [3,9].

The beginning of the 20th century was also characterized by advances in general entomology made mostly by Jean Henri Casimir Fabre's descriptive works. Fabre was known for his excellent teaching and research skills with delicate attention to detail and exactness. The academic work of Fabre is collectively referred as the "Souvenirs of Insect Life" and many entomologists think of him as the father of modern entomology [3].

The first report of the 20th century that includes the presence of beetles on carrion belongs to the E. Ritter von Niezabitowski's experiments. From May 1899, to September 1900, Niezabitowski used aborted human fetuses and cat, fox, rat, mole and calf cadavers. Although his first observations were about flies (mostly *Lucilia caesar* and *Sarcophaga carnaria*), he also mentioned the presence of beetles belonging to the *Silpha, Nicrophorus* and *Dermestes* genera. With his research, Niezabitowski proved that animal carrion and human cadavers share the same decomposing fauna. Also related to beetles, C. Morley published an article in 1907 about the classification of carrion beetles. His collection of specimens during ten years showed that carrion beetles were mostly found during the winter and they are not carnivorous but "act as final dissolvers to the ancient carcasses." [9].

The progress of forensic entomology was also being done in South America. In 1908, scientists Roquette-Pinto and Oscar Freire came to an important conclusion: despite the high diversity of the Brazilian native fauna of necrophagous insects it was impossible a direct application of the methods developed in Europe because clima and weather conditions were so different. With his investigations using human corpses and small animals, Oscar was the first to present a collection of insects associated with decomposing bodies. As for Herman Lüderwaldt, he was in charge of revising the collection of beetles of the Museu Paulista and published in 1911, a list of carrion associated Coleoptera. In this article, Lüderwaldt not only addressed the systematics of this group but also made significant notes about the ecology of carrion beetles. Also regarding forensically important Coleoptera, Samuel Pessôa and Frederick Lane published, in 1941, a paper about Scarabaeidae of medico-legal importance [20,21].

1.2.5. Forensic entomology during the World Wars

The period of time between the two great wars (1918-1939) sparked the interest in entomology applied to other fields such as pest control and archaeoentomology. New insights related to the insect succession on carrion were presented by Karl Meixner and Hermann Merkel: the age of the deceased and the circumstances around the death bring differences in the decomposition of the cadaver [3]. In 1922, Meixner made a series of case reports mentioning the fast decomposition of corpses stored into the basement of the Institute for Legal Medicine in Vienna, his workplace at that time. He also mentioned that the decay happened more quickly on juvenile cadavers [9].

A case report made by German forensic scientist Kurt Walcher in the early 1930's, described an alleged suicide in which the cadaver was found outside and with an estimated PMI of 100 days. Although the skeleton was intact, Walcher found maggots entering the spongiosa of the long bones, almost reaching the bone marrow. This suggests the insect's ability of entering through small gaps present in the bones, the *foramina nutritiva* [9].

Just before the beginning of World War II, the work of medical examiner Josef Holzer (Institute for Legal Medicine in Innsbruck, Austria) was important in investigations involving feeding activity of caddisfly (Order Trichoptera) larvae on corpses submerged in freshwater. In a case report from April 1937, Holzer made exhaustive descriptions of skin aberrations: during feeding activity, caddisflies had destroyed parts of facial skin and all skin layers of the thighs [9]; these skin lesions were very similar to those made by cockroaches and ants on land [3]. Since Holzer never had seen similar lesions, he collected caddisflies from the crime scene and put them in three separate aquariums containing an aborted fetus, a rat and a guinea pig; with this experiment, he was able to prove that these insects were responsible for the observed lesions on the child's skin [9].

1.2.6. Developments after the “great” Wars

The end of World War II was marked with the entomological research progressing towards the development of insecticides in order to suppress pest populations. Also worth mentioning was the work of D.G. Hall, “Blowflies of North America”, and the fly bionomics research leaded by A.S. Kamal. Finally, J. Payne presented also important experiments about ecological succession on baby pig carcasses [3].

Following Holzer’s work involving caddisflies as a tool for forensic investigation is the research of Hubert Casper from the Zoological Institute and Museum of the State Hamburg. Casper described a case of a dead woman’s body discovered in 1948 in a moat of a windmill. The body was found inside a sack completely naked with the exception of a pair of red socks. The investigators wanted Casper to answer a question: was the body dumped there right after the crime or was it stored elsewhere before ending inside the moat? The entomological evidence collected from the crime scene was a caddisfly casing (probably *Limnephilus flavicornis*) found on one sock. The casing had fibers of the red socks but only at the very top and very bottom suggesting that it had been built before entering the sack; the caddisfly “then finished the casing (fibers on top) and attached it to the sock (fibers on bottom). Since the attachment procedure lasts at least some days, it was estimated that the body was lying in the water for at least one week” [9]. Also, further evidences pointed to the conclusion that the woman’s body had been stored elsewhere for one week before it was put where it was found [3,9].

1.2.7. Recent history and the growth of forensic entomology

As seen before, the acceptance of forensic entomology not only by the legal system but also by the general public has depended upon both academics and practitioners that had worked side by side with law enforcement agencies all over the world [1]. With their pioneering researches and experiments, forensic entomology became a recognized and legitimate subdiscipline of entomology and forensic sciences [3].

After the 1960’s, scientists kept doing research and maintained forensic entomology as an active subdiscipline: the pioneering work of Belgium doctor Marcel LeClerq and collaborators related to entomology and forensic medicine; case works of Finnish biology professor Nuorteva and his collaborators about the use of blowflies (Diptera: Calliphoridae) as medico legal indicators; German medico legal doctor Reiter was the author of a series of scientific articles, most of them applying the study of blowflies to forensic investigations [9]; in 1986, entomologist Kenneth Smith (head of the fly collection in the British Museum, London) published the famous “*Manual of Forensic Entomology*” [22] that, in spite of holding mostly historical value, it’s still used in some laboratories; across the Atlantic, the researches of Lord and collaborators largely about flies with forensic interest are also important references.

With the turn of the millennium came Goff’s book “A Fly for the Prosecution: How Insect Evidence Helps Solve Crimes” [16] which approaches the basic fundaments of forensic entomology. Regarding the Diptera order is the book “Entomology and the Law: Flies as Forensic Indicators” [17], written by professors Bernard Greenberg and John Kunich, addresses both biology and use of forensic important flies and also the legal aspects of the forensic entomology evidences. In a broader scope of forensic entomology, Dorothy Gennard’s “Forensic Entomology: An Introduction” [1], Byrd and Castner’s “Forensic Entomology: The Utility of Arthropods in Legal Investigations” [23] and Rivers and Dahlem’s “The Science of Forensic Entomology” [3] are also well accomplished books. In 2003 was published the first forensic entomology book in Portuguese: “Entomologia Forense - Quando Os Insectos São Vestígios” [24]. With the scope turned to the Coleoptera order, Leonardo Gomes’s book is also an important reference in the Brazilian forensic entomology: “Entomologia Forense: Novas Tendências e Tecnologias nas Ciências Criminais” [25].

Since basic research and advanced application of forensic entomology opened the way to routine casework, the emergence of the first organizations dedicated to this subject became a necessity. In the USA, the American Board of Forensic Entomologists and the North American Forensic Entomology Association; in Europe, the European Association for Forensic Entomology [3,26].

In spite of all progress made in the last decades, there is still plenty of research and improvement in need to be done not only because of the constant technological advances but also due to the global climatic changes that affect the bioecology of insect communities [9]. Furthermore, as it can be seen throughout this chapter, the use of beetles as entomological clues is still very recent and more research about their bioecology is needed in order to understand their full potential as forensic evidences.

2. Medico-Legal Entomology

2.1. *Postmortem* changes

2.1.1. First *postmortem* changes

Right after death, a body undergoes a process of transformation which can be viewed through a series of decomposition stages. This cycle ends with the reintegration of the body’s organic matter to the ecosystem through its metabolic transformation into simple organic and inorganic compounds [27]. Throughout the decomposition stages, several biochemical transformative phenomena occur, such as the release of enzymes which cause tissue autolysis and the chemical breakdown of the cells leading to its death [3]. But not all the transformations are caused by chemical changes; there are also external processes that contribute to the decomposition, like the activities of fungi, bacteria and arthropods [28,29]. Both of these two types of transformations are highly dependent on climatic and seasonal variations [3,30].

In the first 72-96 hours after death, there is a series of body changes that allows the forensic pathologist not only to estimate the PMI as well as the manner and the cause of death [31,32]. The first *postmortem* change is called lividity (also known as *livor mortis*) which is characterized by the red discoloration of the skin. After death, the circulation stops and by the action of gravidity red marks are formed when the blood settles in the lowest parts of the body, leaving the rest of the body pale [33].

In these first hours after death there is also a second process which helps the forensic pathologist to estimate the time since death: the *algor mortis*. Since the body lost the ability to self regulate, its internal temperature begins to approximate to the ambient temperature. There are, however, some factors to be taken into account in the PMI estimation using the *algor mortis*: the body’s size and the exposure to sunlight or other types of temperature changes [34]. In order to do a more accurate estimation, the liver temperature can also be taken into account [35,36].

A third *postmortem* change important to mention is chemical: the stiffening of the body muscles, most commonly known as *rigor mortis*. This process is caused by the breakdown of glycogen and the accumulation of lactic acid [37] and its duration depends on various factors like the metabolic state of the body and its temperature; if the individual was involved in intense exercise immediately before death or if the corpse was exposed to lower temperatures, the *rigor mortis* will develop faster; also, the lower temperatures tend to prolong the *rigor mortis* duration [38].

Other body transformations can also be visible after death [36,38]:

- Skin slippage – the production of hydrolytic enzymes from cells at the junction between the epidermis and the underlying dermis causes the separation of these two skin layers; this occur mostly in moist or wet habitats;
- Greenish discoloration – the most common gas produced in the decomposition process, hydrogen sulfide (H_2S), reacts with the blood's hemoglobin and forms a greenish pigment (sulfhemoglobin) visible where the lividity has settled;
- *Tache noir* – characterized by the appearance of a re-orange to black discoloration caused by the loss of the cornea's water;
- Marbling – caused by the spreading of anaerobic bacteria through the blood vessels, this process leads to a purple to greenish discoloration of the subcutaneous vessels.

As said before, the time frame for the appearance of these first *postmortem* changes is 72 to 96 hours after death. Beyond that, the PMI estimation provided by the medical examiner becomes less reliable or even impossible when we're facing years between the death and the discovery of the body. To overcome the difficulties in these cases, the investigation can rely on entomology, since the insects with forensic interest are able to provide relevant evidences [35].

2.1.2. Stages of decomposition

The decomposition process begins right after death with the early *postmortem* changes described before. At the same time, the progression of the body's decay can be separated into three recognizable processes: autolysis, when the body's cells are digested by enzymes (mostly lipases, proteases and carbohydrases); putrefaction, when the breakdown of tissues by bacteria and anaerobic fermentation takes place, resulting in the release of gases responsible for the putrid smell; and diagenesis, which is the process of the bone decomposition where the organic and inorganic remains are broken down and reduced to soil components [1,39,40].

The whole process of decomposition is highly dependent on climate and environmental conditions [41]. Also, the circumstances in which the body is present are very important to this process. For instance, bodies exposed to the elements tend to decompose faster than buried ones. Furthermore, the decomposition process is also different if the cadaver is submerged in water. Even though the whole process of corpse decomposition is a continuous process, it can be viewed as a series of discrete stages [42,43]:

- Stage 1: also known as fresh stage, it starts 4 minutes after death [39] and ends with the first signs of bloating, usually 2 days after death. Externally, the remains show no signs of decay but the activity of bacteria, protozoa and nematodes present inside the corpse begins the decomposition process from within. There are four important phases beginning in this stage: (1) exposure phase, (2) detection phase, (3) acceptance phase and (4) consumption phase [22,44] – Figure 1. Blowflies belonging to the Calliphoridae family are the first organisms to arrive and the most common species are *Calliphora vicina* and *C. vomitoria* [1,16,45] Depending on the season, Silphidae beetles will arrive at any time during this stage [3];

- Stage 2: during the bloated stage (2 to 12 days after death), the bacterial activity is responsible for the continuous body's decay process which leads to the release of gases causing the corpse to bloat [22]. The smell of the breakdown gases attracts more and more blowflies ready to oviposit and their eggs and maggots will serve as "ready meals" to predators such as the rove beetles (Staphylinidae) [1];
- Stage 3: during this stage (12 to 20 days after death), the flesh acquires a creamy consistency and the exposed parts become black, giving this stage the name of black putrefaction stage (or active decay stage). The previous accumulated gases begin to escape and the inflation slowly fades. The odour of decay becomes very strong and the putrefaction fermentation generates butyric and caseic acids [1,36,43]. Later on, the advanced putrefaction leads to an ammoniacal fermentation of the body which attracts a new series of insects, mostly beetles: *Nicrophorus humator* (Coleoptera: Silphidae), *Hister cadaverinus* (Coleoptera: Histeridae) and *Saprinus rotundatus* (Coleoptera: Histeridae) [1,45]. Carrion and hide beetles will feed on the fly larvae, while checkered beetles (Coleoptera: Cleridae) feed directly on the decomposing flesh [46,47];
- Stage 4: known as post-decay stage or advanced decay stage (20 to 40 days after death), is characterized by the presence of only skin, cartilage and bones (sometimes, with a few remnants of dry flesh). The increase in the presence of beetles and the reduction of flies is an indicator of the beginning of this stage [43,48]. Also, it is when begins the dispersal stage of the necrophagous insects [44] – Figure 1. In this stage, predaceous beetle species include staphylinids, silphids, histerids and clerids [49]. Dermestidae beetles and Piophilidae flies are also very abundant [36];
- Stage 5: the skeletonization or dry stage occurs 40 to 50 days after death. At this point, the carcasse is almost dry with only hair and bones visible. The rate of decay now is very slow and the most common fauna associated are beetles belonging to the Nitidulidae family [1].

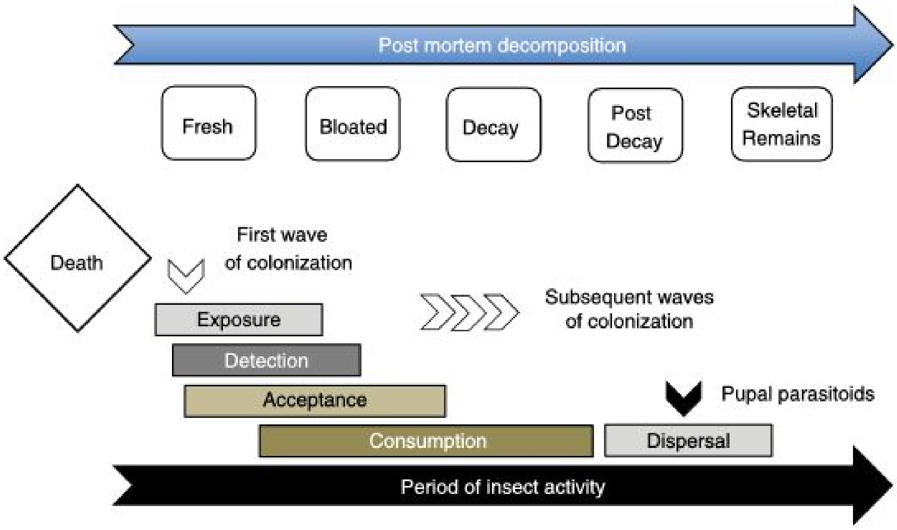

Figure 1. Decomposition stages and their relation with insect activity [3].

Since the rate of decay is dependent on both intrinsic factors (age, body's constitution and cause of death) and extrinsic factors (geographical region, temperature, seasonality, wind, rainfall and humidity), the duration of each stage is variable; nevertheless, there are a particular set of insects that can be linked to every stage, depending on the geographical site and climatic conditions [1,50,51]. The temperature is the main factor which affects the insects' growth and development rate [27,51–53].

Regarding the decomposition process of the bodies submerged in water, all these five stages still occur along with an additional one: the floating decay stage, in which the body rises to the water surface by the action of the accumulated gases. However, there is a difference in the decomposition speed. Giertsen's research came to the conclusion that a body submerged in water takes twice as long to decompose compared to a body found on land (above ground) and citing Casper's Dictum he explains why: "The reason for this difference in decomposition is that the speed at which the body loses heat in water is twice the speed at which the body will lose heat in land." [1].

2.2. Insects with forensic importance

2.2.1. Classification

Insects are among the first and therefore most important invertebrates colonizing decaying bodies. Their activity accelerate the decay and disintegration of organic matter since these insects use it as protein source in order to stimulate oviposition or to develop their immature stages [35,54]. Given the fact that these insects need carrion in their life cycle, they can be used as evidences in legal investigations. So, it is imperative to know the specimens' taxonomic identification and their bioecology [7].

The insects associated with a decomposing body belong to a broad range of *taxa*, like Lepidoptera, Hymenoptera and Acari, but the two central orders which are responsible for answering most of the forensic questions are the Diptera (flies) and Coleoptera (beetles) [51,55–57]. The flies, which are attracted to the smell of decomposing flesh, are the first ones to colonize the corpse and arrive in large numbers just few minutes after death. They start to oviposit in humid tissue at body's natural orifices or inside open wounds [55,58,59]; later on, the eggs will hatch and the first instar larvae will start to feed on the decomposing flesh [58]. This first wave of flies is composed mostly by the families Calliphoridae, Sarcophagidae and Muscidae [60].

As the majority of forensic entomology research is focused on flies, beetles have been at best under-emphasized [61]. The Coleoptera order is the one with more species recorded and, worldwide, there are at least as many species of Coleoptera that may visit a particular carcass as Diptera. Since beetles form a taxonomically and ecologically diverse part of the carrion insect community, they are able to provide a wide spectrum of potential evidence [61–70]. Despite this, little knowledge is available about this group [57,60].

Although also present in the first stages of decomposition, the beetles are more numerous and diverse during advanced and dry stages of decomposition [46,71]. Therefore, when corpses are found with only bones and dry skin left, these arthropods are the main entomological evidence for the investigation [59,60,72]. This work focus on beetles belonging to eight families with forensic interest: Carabidae, Cleridae, Dermestidae, Histeridae, Nitidulidae, Scarabaeidae, Silphidae and Staphylinidae. The works of Kulshrestha & Satpathy [60], Almeida and Mise [71] and Anton et al. [73] state that these eight families are the ones with greater forensic interest.

Payne [74] was amongst the first scientists to design an experiment to relate the stages of body decomposition to insect succession. Using carcasses of *Sus Scrofa*, he was able to characterize the feeding styles of the carrion species [1]. According to Smith [22], the insects present in a decomposing corpse may belong to a series of groups, according to their food preferences and behaviour:

- Scavengers or necrophagous species are those who feed only on the decomposing tissues. Since they are the first arriving to the cadaver, they constitute the most important group to estimate the PMI [46]. The most relevant are Diptera families like Sarcophagidae, Muscidae and Calliphoridae; some Coleoptera families are also part of this group, like Scarabaeidae, Silphidae and Dermestidae [55]. Less known for this feeding habit are the Lepidoptera families Tineidae and Pyralidae.
- Omnivorous insects are those that feed both of the corpse as of the associated fauna. Some ants and wasps (order Hymenoptera) and several beetles (order Coleoptera) display this feeding behaviour [58,75]. The presence of a large number of these insects can delay the decomposition process since they feed of the scavengers or necrophagous species [55].
- Parasites and predators; the first ones are those who, through the parasitation of the body colonizers, use their reserves for their own development; on the contrary, predators are those that only feed upon carrion insects. These groups include some Silphidae, Staphylinidae and Histeridae species (order Coleoptera) and the Diptera genera *Chrysomya* (Calliphoridae) and *Hydrotaea* (Muscidae). Also some dust mites (Macrochelidae, Parasitidae, and Parholapidae) and Dermaptera species fall under this category. Finally, Hymenoptera species can also be predators or parasites of immature stages of Diptera [1,35,58].
- Accidental insects are those who use the corpse as an extension of their usual habitat or as a refuge, like small arthropods belonging to the Collembola order, spiders and centipedes [36,75].

Most recently, Goff's work gives another classification of organisms according to their role in carrion community [36,76] as it can be seen on Table 1.1:

Table 1. Biotic components of carrion organisms [3].

Classification	Organisms	Role in carrion community
Accidentals	Non-predictable insect species	No real role on carrion but for whatever reason are found on corpse
Adventive species	Some non-necrophagous insects and arthropods	Species that use corpse as habitat or dwelling
Decomposers	Microorganisms (e.g. bacteria, fungi and protozoa)	Responsible for micro-decomposition and putrefaction
	Necrophagous insects	Responsible for macro-decomposition of carrion
	Saprophagous insects	Responsible for macro-decomposition of cadaver decomposition island
Omnivores	Ants, wasps, beetles and some non-insect arthropods	Feed on a wide range of organisms on and around body
Predators/parasites	Ants, wasps, beetles and some non-insect arthropods	Consume early stages of necrophagous insects, especially fly eggs and larvae
Scavengers	Mostly vertebrate species (e.g. birds, rodents, fox and raccoons)	Feed on corpse tissues and carrion insects

The body's decomposition causes gradual physical and chemical changes and sets in motion a predictable pattern of insects' succession that will not only witness but also take part on the decomposition process. Because this succession is predictable, scientists can do research to find out the changes that occur in this pattern according with variations in the climatic conditions, like temperature and humidity [7,77].

Often underestimated, the forensic importance of some Coleoptera families represents much more than those that occur only at late stages of the decomposition process. The families with predatory and necrophagous feeding habits, which appear in the first stages of decomposition, also have the potential for forensic importance [22,23]. Midgley and Villet's [78] work came to the conclusion that *Thanatophilus micans* (Coleoptera: Silphidae) is able to locate corpses and start breeding within 24 hours of death. To prove their role and fully understand their importance, it's necessary to do more biology, taxonomy and behaviour studies [20].

2.2.2. Collection of entomological evidence

In order to help in forensic investigations as entomological evidences, insects present at the crime scene must be properly documented, collected, labelled and preserved. The collection of entomological evidences should be done at the scene and at the morgue (in cases of death), prior to or during the autopsy or necropsy procedure. It is essential collections of live and preserved samples. The live samples will later aid in species identification by allowing the insects to develop to a more advanced life stage with more readily identifiable characters. The preserved samples are also essential in order to demonstrate the stage of insect development in the crime scene [79].

To ensure that all relevant evidences are examined and the sampling is properly done, the investigator should collect samples of insects of all stages from different areas of the body, clothing and, ideally, from the surroundings. If the investigation involves a dead body, it is important to know that insects will often form masses in open wounds and in and around natural orifices [79].

As said before, the two main insect orders present on corpses are flies and beetles. The investigator responsible for processing the crime scene and gathering the evidences must be familiar with the ecology and morphology of these two types of insects because both look very different at different stages of their life cycles. As for the flies, they can be found as eggs, larvae, pupae and/or empty pupal cases and adults [1]:

- **Eggs:** although usually laid in clumps or masses, the collection of flies' eggs must be done thoroughly since they are very small. The collection is usually done with a small paint brush dipped in water or with forceps. Half of the collected eggs should be preserved in 75% alcohol or 50% isopropyl alcohol and the rest should be kept alive in a vial with a little damp tissue paper to prevent dehydration.
- **Maggots:** found crawling on the body or in the vicinities, the maggots can be at different instars of development. Usually they form large masses which lead to an increase of the temperature, speeding their development and the decomposing process. After its collection using forceps, approximately half of the larvae should be preserved in 75-90% alcohol or 50% isopropyl alcohol. The other half should be kept alive in several vials, with air and food (preferably beef liver).
- **Pupae:** these are usually found under clothing, hair or soil near the body. After collection, all the pupae must be kept alive because it is almost impossible to identify a pupa until it emerges as an adult.

- **Adults:** these are less important as it's impossible to determine whether an adult has developed on the corpse or has just arrived there to oviposit. Its collection can be done using a net or an inverted vial. It's important to notice if there are empty pupal cases at the scene as they indicate that an insect has developed on the corpse which indicates that at least one generation of flies has completed its life cycle on the corpse.

Regarding the collection of beetles, they also can be found in different stages of development: larvae, pupae (and cast skins when the adult emerge from the pupae) and adults. Its collection can be difficult as they move fast. Since, some species are cannibals, each specimen should be placed separately in vials with some air or just preserved in alcohol [1].

As for the labelling process, each vial should contain the information of the body region or soil area where it was collected, the date and time of the collection, the name of the collector and the stage of development in which the specimen was collected. Additionally, it's important to take several notes regarding the scene and the remains: the presence and type of vegetation, the soil type, the weather, temperature, elevation and exact location (map coordinates), the presence of clothing, if the body was found buried or covered, the most likely cause of death, the presence of any body fluids and drugs, the position, direction and state of decomposition in which the body was found, the presence of maggot masses and, if so, the temperature of its centre [79].

In conclusion, insects are a very important piece of evidence, especially if the death has occurred for at least 72 hours and its use may be the only method available to determine elapsed time since death. And so, it is essential a properly collection of the insects for them to serve its purpose as evidences in the investigation.

2.3. Estimating the *postmortem* interval

After the occurrence of the first post-mortem changes, the medical examiner's PMI estimation becomes an impossible task to perform and is only possible through the study of arthropod species found on the corpse [35,58].

The accurate PMI estimation is essential in homicide investigations as it helps in the victim's identification, reconstruction of the events that lead to the death and its circumstances, verification of witnesses' statements and suspects' alibis or even to identify the actual criminal. On the other hand, if the cause of death has been ruled as natural or accidental, the determination of the time of death can be relevant for legal purposes like inheritance allocations or insurance questions [4,58,80].

Since the determination of the exact time of death is not possible using entomological evidences, the PMI estimation based on insects' activity [45] will help setting a minimal and maximal probable time, creating a time frame within which the death occurred. For this reason, insect samples must be properly collected and processed [4,55]. The PMI estimation can be done using two main approaches according to the state of decomposition. When the body is still fresh and full of immature specimens of necrophagous insects like blowflies, the PMI determination is based upon a direct age estimation of these first colonizers [1,4,81] using size as a surrogate for age or using physiological age by identifying developmental landmarks [61]. This method can only infer of the minimum PMI since it is impossible to determine the exact time of the oviposition [80,82]. To use it, it's essential to do an accurate species identification and have reliable temperature information, because insects are poikilotherms [1,53,58]. There are, however, some latter models that are less biased and more precise

[83] since they measure not the size but the actual age, which can be affected by a variety of factors other than age [84]. There are several techniques used to estimate the rate of development of insects but the most used is the "accumulated degrees hours" which is obtained by the sum of temperature (in °C) multiplied by time [4]. Due to their fast development, this technique is mostly used using Diptera specimens over short periods of time.

Most development studies were made using flies, creating accurate models for various species [81,85–88]. Since coleopteran development models are not as common, further research is required to develop statistically robust models [61]. As said before, *Thanatophilus micans* is a species which is found in fresh corpses and its development can be a useful tool for forensic entomologists. The particular development of *T. micans* has been meticulously modelled [78,89] and not only meet the minimum statistical requirements for regression modelling [90], but have coefficients of determination greater than 0.98 for all post-hatching stages. Another advantage of using beetle larvae to estimate the minimum PMI is that they are solitary and furtive which simplifies the application of thermal accumulation models of development because they experience temperatures close to ambient [61].

In case of longer PMI, the first colonizers have already left the body and Coleoptera species become the main tool in the estimation of the time frame. In these cases, the PMI determination is based on a predictable pattern of insect succession. This method provides an estimation of both minimum and maximum PMI limits [35,80] based on the community of animals present on a corpse, mostly dominated by beetles [22,62,64,67,82]. In order to do an accurate estimation of the minimum PMI it is imperative to understand their bioecology and geographic distribution. So, it is essential not to blindly follow the results of previous studies and, ideally, proceed with experimentations that will help to know which insects are present or absent locally and in what season. This data will also be useful to know if the body has been moved: "if an unexpected species is present, which is more characteristic of a different habitat or geographic region, then the body may have been moved" [1].

Mégnin's work [19] in France describes a series of changes carried out by eight waves of arthropod colonization during the five stages of decomposition (as it can be seen on Table 1.2). Since each stage attracts a different group of insects, the arthropod colonization creates a succession pattern [1,51,55]. Mégnin recorded several Coleoptera species colonizing the cadavers spanning from 3rd to 8th wave (stage 3 to 5 of the decomposition stages). The genus *Dermestes* (Coleoptera: Dermestidae) appears within the 3rd wave and is followed by specimens of *Necrobia* (Coleoptera: Cleridae) in the end of 4th wave. The 5th wave is the one with most beetles' diversity: Silphidae genera *Nicrophorus* and *Silpha* appear in the middle of the wave and Histeridae genera *Hister* and *Saprinus* show in the end. When the body is already dry, Dermestidae is once again present with *Attagenus*, *Anthrenus* and *Dermestes* specimens (7th wave) and two new families appear: Ptinidae and Tenebrionidae (8th wave).

Table 2. Mégnin's records of principal members of the faunal succession on human cadavers [19,22].

Fauna		State of corpse	Approx. age of corpse
Exposed corpses			
1st wave	*Calliphora vicina* (Diptera: Calliphoridae) *C. vomitoria* (Diptera: Calliphoridae) *Lucilia* spp. (Diptera: Calliphoridae) *Musca domestica* (Diptera: Muscidae) *M. autumnalis* (Diptera: Muscidae) *Muscina stabulans* (Diptera: Muscidae)	"Fresh" (variable with season)	First 3 months

2nd wave	*Sarcophaga* spp. (Diptera: Sarcophagidae) – may occur in 1st wave	Odour developed	
	Cynomya spp. (Diptera: Calliphoridae)		
3rd wave	*Dermestes* (Coleoptera: Dermestidae)	Fats rancid	
	Aglossa (Lepidoptera: Pyralidae)		
4th wave	*Piophila casei* (Diptera: Piophilidae)	After butyric fermentation; Protein of "caseic" fermentation	3 to 6 months
	Madiza glabra (Diptera: Piophilidae)		
	Fannia spp. (Diptera: Fanniidae)		
	Drosophilidae (Diptera)		
	Sepsidae (Diptera)		
	Sphaeroceridae (Diptera)		
	Eristalis spp. (Diptera: Syrphidae)		
	Teichomyza fusca (Diptera: Ephydridae)		
	Necrobia spp. (Coleoptera: Cleridae)		
5th wave	*Ophyra* (Diptera: Muscidae)	Ammoniacal fermentation; Evaporation of sanious fluids	4 to 8 months
	Phoridae (Diptera)		
	Thyreophoridae (Diptera)		
	Nicrophorus (Coleoptera: Silphidae)		
	Silpha (Coleoptera: Silphidae)		
	Hister (Coleoptera: Histeridae)	Remaining body fluids absorbed	
	Saprinus (Coleoptera: Histeridae)		
6th wave	Acari		6 to 12 months
7th wave	*Attagenus pellio* (Coleoptera: Dermestidae)	Completely dry	1 to 3 years
	Anthrenus museorum (Coleoptera: Dermestidae)		
	Dermestes maculates (Coleoptera: Dermestidae)		
	Tineola biselliella (Lepidoptera: Tineidae)		
	T. pellionella (Lepidoptera: Tineidae)		
	Monopis rusticella (Lepidoptera: Tineidae)		
8th wave	*Ptinus brunneus* (Coleoptera: Ptinidae)		3 years plus
	Tenebrio obscures (Coleoptera: Tenebrionidae)		
Burried corpses			
1st wave	*Calliphora* (Diptera)		1 year
	Muscina stabulans (Diptera: Muscidae)		
2nd wave	*Ophyra* (Diptera: Muscidae)		
3rd wave	Phoridae (Diptera)		
4th wave	*Rhizophagus parallelocollis* (Coleoptera: Rhizophagidae)		2 years
	Philonthus (Coleoptera: Staphylinidae)		

There are several insect succession studies carried out across the globe. In Ambleteuse (northern France), during the spring seasons, Bourel et al. [64] studied the insect succession in sand dune habitats using rabbit carrion. They presented a checklist of 66 arthropod species representing 7 orders and 25 families and a chronology of its appearance and relative abundance for 110 days of decomposition. This study recorded 38 species belonging to 10 Coleoptera families.

From May to November of 2001, Grassberger and Frank [91] conducted arthropod succession studies using clothed pig carcasses in an urban backyard in the city of Vienna, Austria. The paper, result of the study, presents a checklist of 42 arthropod species which belong to 3 orders and 20 families, plus species from the orders Isopoda and Acari. They also present a diagram with the chronology and relative abundance of insects visiting the carcasses for 60 days. Regarding the Coleoptera specimens, there were recorded 13 species belonging to 5 families.

In Murcia, Arnaldos et al. [52] carried out an exhaustive study of the carrion arthropods. As a result, they presented a checklist of data for all four seasons and stages of decomposition. It is also described the relative abundance and the differences in seasonal succession patterns. As for beetles, there were recorded 32 species belonging to 20 families.

Another work, during a one-year period study in Ancara, Turkey [50], collected 40 species belonging to Staphylinidae, Histeridae, Dermestidae, Silphidae, Nitidulidae and Cleridae families of Coleoptera which were found in 12 pig (*Sus scrofa*) carcasses. The results were as follows: the first specimens collected belonged to three families of beetles previous reported as predators [46,92], Silphidae, Staphylinidae and Histeridae. They were found feeding on fly larvae while it was abundant, i.e. in the first stages of decomposition (fresh, bloated and active decay). As for the other three families (Dermestidae, Nitidulidae and Cleridae), they were mostly found in the later stages of decomposition: the advanced decay and dry stages. While Dermestidae specimens are exclusively necrophagous, feeding on the dry tissue, the nitidulids (Coleoptera: Nitidulidae) have both necrophagous and saprophagous feeding habits. Lastly, Cleridae specimens have a completely different behaviour: they are predators [48,92] and feed on dermestid larvae and some fly larvae.

In North American studies, the first families of beetles recorded on the body are species of Silphidae (carrion beetles), Staphylinidae (rove beetles) and Histeridae (clown beetles). Amongst the later colonizers are the dermestids, found 21 days after death, when the body was in early advanced decay [48,93]. In South America, Oliva's work [94] found dermestids colonizing bodies 10 to 30 days after death. In addition, the beetles *Carpophilus hemipterus* and *Necrobia rufipes* were found in the later stages of decomposition. Oliva also was able to link silphids of the genus *Hyponecrodes* (e.g. *Hyponecrodes erythrura*) with corpses discovered in rural outdoor environments.

2.4. Medico-legal entomology in Portugal

Despite the importance of forensic entomology in criminal and civil cases, this branch of forensic sciences, unfortunately, still has a long way to go in Portugal. It is possible that the lack of trained forensic technicians is due to the little information concerning the bioecology of species with forensic interest. In addition, the short number of effective identification keys for the rapid and accurate classification of species is also a problem and the cause of the disuse of this science in Portugal.

However, there are researchers who struggle with this lack of investment in this area and, despite this, have contributed to increase the knowledge of insect species with forensic interest. Although most studies are focused in the Diptera species with forensic interest [95–107], there has been an increase in the interest of the Coleoptera study in order to understand better this order and its potential in forensic cases.

Catarina Prado e Castro *et al.* [108] conducted experiments in order to study insect species composition and seasonal succession using piglet carcasses. There were a total of 35 Staphylinidae species collected, five of which were reported for the first time in Portugal, thus enlarging the faunistic knowledge of this group: *Anotylus pumilus*, *Stenus (Metastenus) bifoveolatus*, *Bryophacis maklini*, *Mycetoporus mulsanti* and *Mycetoporus piceolus*. These experiments helped to push forward the knowledge of Portuguese entomosarcosaprophagous fauna which are especially relevant to forensic investigations.

Since an accurate identification of insect specimens is an essential step in forensic entomology, Susana Ferreira *et al.* [109] made also an important contribution to forensic entomology by studying nuclear and mitochondrial markers for Diptera and Coleoptera identification. Also concerning molecular identification is Susana Lopes masters' thesis [110]. The main goal of her study was to perform a molecular identification of Coleoptera species with forensic interest. Using two mitochondrial markers, COI and CytB, a total of 35 specimens belonging to the families Staphylinidae, Silphidae, Histeridae, Nitidulidae, Scarabaeidae and Carabidae were identified.

Another relevant investigation was done by Catarina Prado e Castro *et al.* [111]. Because insect species and their timings of appearance in cadavers diverge according to geographic location, it is relevant to know their succession patterns, as well as seasonality at a regional level. Using piglet carcasses as animal models, this study contributed to broaden this knowledge by surveying Lisbon beetle communities during the four seasons of the year. As result, there were collected 1.968 adult Coleoptera and identified 82 species, belonging to 28 families. The highest values of species abundance and richness were recorded in autumn, while winter yielded the lowest values. Staphylinidae was the family with most specimens in all seasons, although in spring and summer Dermestidae was also quite abundant. In general, most species were present in the active and advanced decay stages, particularly *Margarinotus brunneus* (Histeridae), *Creophilus maxillosus* (Staphylinidae), *Saprinus detersus* (Histeridae) and *Thanatophilus sinuatus* (Silphidae). Despite this, there were a few related to the dry stage, namely *Oligota pusillima* (Staphylinidae) and Dermestidae spp. larvae. Finally, *Anotylus complanatus* and *Atheta pertyi* (Staphylinidae) were more associated with the fresh and bloated stages, respectively. The occurrence of some species was clearly seasonal which allows a season characterisation based on the occurrence of certain *taxa* and this can be useful for forensic purposes.

3. The Coleoptera Order

3.1. General morphological characteristics

The order Coleoptera is the largest one, containing about a third of all known insects, approximately 300.000 species. Members of this order all share a few morphology features but the presence of hard wing covers (called *elytra*) is perhaps the most characteristic. These hardened wings serve as cover to protect the membranous hind wings that are used for fight [112]. Additionally, all adult beetles possess biting mouthparts (*mandibles*), their *antennae* characteristically have 11 segments (except in some species, which may be fewer than this) and the majority of them are able to fly. Their exoskeleton is formed from hardened plates: the ones on the dorsal surface are called *tergites*, on the ventral surface are *sternites* and on the lateral are called *pleurites* [113].

Beetles are holometabolous insects, i.e. they undergo a complete metamorphosis during their life cycle, passing through four stages: egg, larva, pupa and finally the adult, or imago. Each one of these stages is morphologically very different from one another and the whole length of the life cycle varies upon the family and species [114]. For example, beetles belonging to the Staphylinidae family can take 7 to 10 days from egg to adult, whereas Carabidae life cycle may take a year and the adults may live for 2 to 3 years [1].

Beetles' eggs are laid singly or in masses and, if the conditions are favourable, can hatch after several hours. Although they vary in shape (oval, spherical or spheroid), are usually considered very similar, regardless of family [115]. Regarding their larval stage, it comprises three to five growth instars, depending on species or environmental conditions. Morphologically, beetle larvae have more characteristic features than Diptera larvae: the presence of a sclerotized head capsule and mouthparts with mandibles are the most distinctive. Also, there are some families which larvae have legs (on thoracic region) or *prolegs* (on abdominal region). Despite this characteristic features, it is difficult to distinguish between growth instars [1,113,115]. However, using multivariate analysis, Watson and Carlton (2005) identified three larval stages for three species of American silphid (*Oiceoptoma inaequale*, *Necrophilia americana* and *Necrodes surinamensis*). Additionally, for *N. surinamensis* the results showed that the first instar had an average duration of 12 days, 10 days for the second instar and 11 days for the third instar. These results are relevant when dealing with insect succession patterns in forensic death investigations since they show that the larvae were present on days 9 to 22 of decomposition [116].

When ready to pupate, beetles usually burry themselves in the ground in a specially constructed chamber or form a cocoon. Beetles' pupa has distinctive characteristics: it is *exarate* (i.e. the pupal appendages are free and visible through the pupal coat) and the mouthparts are *adecticous* (i.e. there are no mandibles). However, Staphylinidae pupa is different since it is *obtect* (i.e. covered by a hardened coat and the pupal appendages are held in place by secreted material). There is also another pupa type called *coarctate*: the pupa is retained within the final larval coat, the *puparium* (similar to Diptera pupae) [117].

The body of an adult beetle (Figure 2) is composed by three parts: head, thorax and abdomen. The head can either be projected forwards horizontally (*prognathous*) or leaning downwards (*hypognathous*). On the head are located the eyes (which can be compound or not), the chewing mouthparts and the *antennae* with a wide variety of receptors. The antennae vary extremely in form: some are filiform or lamellate and other may be geniculate or clavate [1,113].

The thorax is composed by three parts all fused together (pro-, meso- and metathorax). Its dorsal surface is also divided into three segments: *pro*-, *meso*- and *metanotum*. The *pronotum* is the surface of the first thoracic segment and the biggest of the thoracic segments; part of the *mesonotum* is located between the base of the *elytra*, behind the *pronotum*; there are also a small plate, called *scutellum*, located in the *pronotum* posterior border. Correspondingly, the ventral surface of the thorax has three parts as well: *pro*-, *meso*- and *metasternum*. The well developed prothorax, together with the head, forms the anterior section of the body. As said before, beetles possess two pairs of wings: the *elytra* are supported by the mesothorax and the membranous wings are attached to the metathorax. The legs are positioned on the ventral portion of the thorax and abdomen (*sternum*) and are designed for running, walking or digging [1,113].

The Coleoptera order is divided into four suborders: Archostemata, Myxophaga, Adephaga and Polyphaga. The first two are made up of wood and aquatic *algae* feeders, respectively, and have minor forensic importance. The remaining two suborders, Adephaga and Polyphaga, contain beetles' families which are most commonly found at crime scenes and, therefore, have forensic potential. The Adephaga suborder includes 10 families and comprises predatory beetles which are present in both terrestrial and aquatic habitats. Within this suborder, it is the Carabidae family with more species recorded. There are two main morphological features used to distinguish Adephaga beetles: the positioning of their hind legs, which are fused to the metasternum causing the division of the first visible abdominal sternite; and the presence of visible notopleural sutures. The Polyphaga suborder is the largest and most diverse suborder of beetles containing 149 families including the Cleridae, Dermestidae, Histeridae, Nitidulidae, Scarabaeidae, Silphidae and Staphylinidae families. The Polyphaga beetles are characterized by the hind coxa not being fused to the metasternum and, therefore, does not divide the first visible abdominal sternite and the absence of notopleural sutures [117].

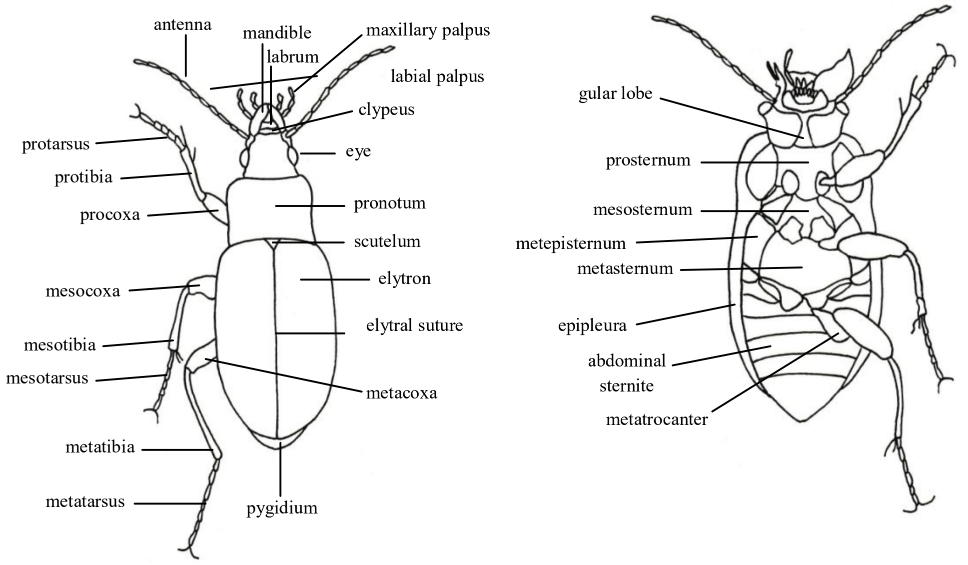

Figure 2. Coleoptera general morphological characteristics (dorsal and ventral view, not at scale). Hand drawing adapted from [1].

3.2. Family characteristics

3.2.1. Carabidae

Carabids are commonly known as ground beetles; their habitats vary widely, from grassland to forests and are more than 40.000 species worldwide. Their *antennae*, usually filiform, can also be moniliform in some species, and are located on the *prognathous* head, between the eyes and jaws. Carabids possess sculptured *elytra* with *striations*, so that nine regular ridges and furrows can be seen across the *elytra* [1]. Nearly all species are predaceous on other insects and adult ground beetles range from 1 to 40mm long; most of the times they have a dark coloration, but a considerable number of species is metallic or otherwise brightly colored [118].

Carabid *larvae* have a linear or elongated form and a ten segment abdomen (the segment nine has a pair of *cerci*). In the head, the larva possesses a pair of pincer-like mandibles and six simple eyes (*ocelli*) on either side. There is also the presence of legs which end in two claws. Since carabid *larvae* have quick movements and tend to be nocturnal, they may not be obvious members of the carrion assemblage [117].

3.2.2. Cleridae

Commonly known as checkered or bone beetles, this family comprises approximately 3.500 species worldwide. Adult clerids range from 3 to 12mm in length, are elongated and cylindrical in shape, usually brightly colored and sometimes hairy. Another characteristic feature of this family is that they appear to have a 'neck', because the pronotum is less broad than their elytra [112]. Larvae measure between 9 and 13mm long, are soft-bodied and pale white to purple [117,118].

Members of the Cleridae family, such as *Necrobia rufipes*, feed on carrion and have been found from bloat through to the dry stage of decomposition. They begin to be attracted to a body when it has become saponified and volatile fatty acids and caseic breakdown products start to getaway [119].

A series of studies have been carried out in order to understand the role of *N Necrobia rufipes* in the decomposition process. In Hawaii, Goff and Flynn [120] found this species in the soil under a corpse, 34 to 36 days after death and concluded that it is a fly larvae predator. In India, Kulshrestha and Satpathy [60] recognized that families Cleridae and Dermestidae are the most common beetles present in the dry stage of human decomposition. Another study was carried out by Turchetto *et al.* [119], in Venice, in the fall of 1997, where the body of a strangled young woman was found in a corn field. The body was badly decomposed and had *postmortem* damages done by a farm tractor. *N. rufipes* was found on the body and they studied its association with the stage of decomposition. In Peru, between July and October 2000, Iannacone [121] made a succession study of arthropods and concluded that *N. rufipes* was a predatory species and made up 0,45% of the total insects (4.405 specimens) recovered. Carvalho *et al.* [65] concluded that, in south-eastern Brazil, *N. rufipes* served as a forensic indicator for both PMI and for the geographic area.

Mark Benecke [122] reports that clerids may influence in the cause of death interpretation. It was observed that members of this family can damage the cadaver skin and these *postmortem* damages, at first sight, may be mistaken by gunshot wounds.

3.2.3. Dermestidae

Beetles belonging to Dermestidae family are worldwide distributed with over 500 species described and have numerous common names, based on their food preferences: skin beetles, leather beetles, hide beetles, carpet beetles and larder beetles [112]. Adults range from 1,5 to 10mm in length and have an oval to elongated shape. Their *larvae* are 6 to 13mm long and have two *urogomphi* on the terminal segment. Forensically relevant dermestid larvae have a brown to black coloration and have *setae* of varying lengths over the dorsal surface [3,118].

There are several dermestid species with a role in the succession pattern of a dead body but *Dermestes ater*, *Dermestes maculatus*, *Dermestes lardarius* and *Dermestes frischii* are the most important ones [123]. They are usually related to the final stages of decomposition and, in one case, were responsible for the skeletonization of a human body in less than 5 months in an apartment with windows closed and a room temperature between 19,4 and 25,8ºC [72]. When dermestids are no longer present on a body, their frass (fecal material) can serve as an indicator that they were formerly present and has been found on 10 year-old mummified bodies. Frass consists on undigested food encased in a white peritrophic membrane with a twisted shape [1,112].

The presence of dermestid species is believed to be determined by ecological conditions. In south-eastern Spain, Arnaldos *et al.* [124] recorded few dermestid species in the initial decomposition stages, during spring and summer. As the remains began to dry out, the number of dermestid species increased. Another work, in south-eastern Brazil [65], recognized *Dermestes maculatus* as a forensic indicator of PMI but not as a forensic indicator for the geographic area.

Regarding weather conditions, beetles belonging to the Dermestidae family are able to tolerate a range of temperatures and relative humidity. In India [60], there are records of dermestids recovered from corpses at an ambient temperature of 16,5ºC and 71% average humidity; nevertheless, dermestids were also observed on a body at an ambient temperature of 20ºC and an average humidity of 46%.

3.2.4. Histeridae

Histeridae is a large family with over 3.500 species recorded worldwide. Histerids or clown beetles, as they are commonly known, are small (between 0,5 and 10mm in length), shiny black beetles with a hard exoskeleton and a more or less oval shape. They have geniculate *antennae* with the final segments forming a club and their legs have flat *tibiae*. The most distinctive morphological feature of histerids is the square-cut to the ends of the elytra, which, when viewed from above, reveal the last two abdominal segments [117,118]. Histerids have a strongly predator behavior: both *larvae* and adults feed upon the carrion insects, not only maggots and fly *puparia* but also dermestid *larvae* [112].

Since they feed upon insects present on the body, histerids are associated to the stages in which there are more carrion insects: the bloat and the decay stages. Payne [74] findings support this fact: clown beetles were recorded not only during bloat stage, but also in active and advanced decay stages. However, the results of Wolf *et al.* [46] may reveal that the arrival time of histerids may be dependent upon geography and weather conditions: the arrival of adult specimens was recorded 7 to 12 days after death and larvae were present on days 77 to 118, at the final decomposition stages. Likewise, Richards and Goff [30] confirmed the body's colonization by histerids at the end of the bloat stage.

Histeridae beetles could have a greater forensic utility if further studies were performed. These should explore the possibility of a correlation between the duration of the metamorphosis stages and histerid colonization of the body at specific temperatures. These findings could assist in the PMI calculation [1].

3.2.5. Nitidulidae

Nitidulidae is a large cosmopolitan family with more than 2.500 species worldwide distributed. These beetles, commonly called sap-feeding beetles, are small (4 to 12mm long) and the 11 segments *antennae* end in a three-segmented club. The *elytra* are usually truncated, but with rarely more than three abdominal segments visible dorsally [117,118]. Although the majority is found near fermenting plant fluids, some occur on or in close proximity to dried carcasses of dead animals and appear to prefer moister environments [112].

Little work has been done to understand the value of nitidulids as forensic evidences. Wolff *et al.* [46] findings showed that 0,2% off the total number of families visiting a dead pig was sap-feeding beetles. All of these specimens were collected during the advanced stage (PMI of 13 to 51 days).

3.2.6. Scarabaeidae

The Scarabaeidae, commonly known as dung beetles, is a large beetle family with over 30.000 species described worldwide. Scarabs vary significantly in size (0,5cm to more than 8cm), shape, and color but generally are elongate, robust and convex in profile. The most distinctive feature is the segmented *antennae* which ends in a large club of 3 to 7 segments that can be closed or expanded like a fan. Scarabs usually inhabit tunnels constructed by them beneath the corpse and, because of that, their presence can be missed [112,118].

In forest environments near Campinas City, Brazil, Carvalho *et al.* [65] concluded that Scarabaeidae beetles were the second most frequent colonizer on pig carcass. Three species were considered to have forensic significance: *Deltochilum brasiliensis*, *Eurysternus parallelus* and *Coprophanaeus ensifer*. However, the authors came to the conclusion that the crucial factor that determines the presence of scarabs on a body is the presence of suitable food instead of the decomposition stage.

3.2.7. Silphidae

Silphidae family has a practically worldwide distribution and more than 1.500 species have been described so far. Silphids or carrion beetles possess a flat body with sharp margins (that can measure from 3 to 35mm) and small heads, comparing to the thorax size. Their *antennae* segments tend to thicken as they progress to the end; they can also be distinctly clubbed. Some have orange or red markings on their *elytra*, such as *Nicrophorus vespilloides*, while others, such as *Nicrophorus humator*, are black. The main identification feature of this family is that the abdominal segments protrude from the *elytra*, and, from a ventral view, six abdominal sternites are visible [112,118].

Beetles of this family can belong to one of its two subfamilies, Silphinae or Nicrophorinae [125], and are mostly present in the decay stages of decomposition. In one hand, Barreto *et al.* [126] findings support this: near Cali, 75% of 16 bodies brought to the Cali Institute of Legal Medicine were infested with *Oxyletrum discicollis* adult beetles. On the other hand, Wolff *et al.* [46] recorded this same species on pig carcasses later in decomposition.

There seems to be two important variations that influence the Silphidae colonization. The first one is the size of the corpse. Silphinae appears to prefer larger corpses, while Nicrophorinae tend to colonize small carcasses of voles and mice. The other factor is the differences in the distribution of Silphidae species. Some investigators point the nature of the soil as the reason for the difference in distribution. Experiments around Frankfurt, Germany, linked *Nicrophorus vespilloides* to favored drier soil [1].

3.2.8. Staphylinidae

Rove beetle is the common name given to the Staphylinidae beetles, a family which comprises roughly 63.000 species. They can be found in a wide range of habitats, where they feed upon carrion, plant debris and fungi. They have an atypical body form (length varying from 1 to 25mm) but most staphylinid adults are slender, elongate and possess short *elytra*, which appear square. The membranous hind wings stay folded and completely concealed beneath the small *elytra* and so, six to seven abdominal segments are visible. Adult staphylinids are strong flyers and run in a distinctive way, with the abdomen raised in the air like scorpions, as if they were capable of stinging, which they cannot. Staphylinid *larvae* are normally long, slender, and pale and sometimes may have a darker head [112,118].

There is a minority of staphylinid species with necrophilous feeding habits and the majority has a predacious lifestyle, hunting fly eggs and *larvae* during the bloat stage of decomposition. Additionally, specimens belonging to the genus *Aleochara* have a particular behavior: their *larvae* parasite fly *pupae* and feed on it slowly, without harming its vital organs or tissues. In the end of *larvae* growth, they feed freely, killing the host and pupate underground or in the fly *puparium* itself [3].

Several studies had been made which highlight the forensic potential of staphylinids. Chapman and Sankey [127], in a study done in Surrey, UK, collected *Creophilus maxillosus* from rabbits left outside, exposed to the elements. This same specie was also recorded by Centeno *et al.* [123] almost 50 years later, in Argentine. In Hawaii, Goff and Flynn [120] recorded adult *Philonthus longicornis* specimens on the cadaver of a 23 year-old caucasian male. Centeno *et al.* [123] also carried out a series of experiments to study the staphylinid colonization on different decomposition stages throughout summer and autumn on sheltered and unsheltered corpses.

4. Interactive Keys

Regardless of the forensic entomology field of study, the identification process is the first step in the chain of procedures because, once identified, that knowledge will provide information regarding the species bioecology. However, nowadays, the most used tool for species identification are still conventional keys which can easily lead to errors in identification.

To overcome this problem, interactive keys have been increasingly developed since they have several advantages over conventional keys [128]:

- ✓ All morphological characteristics can be used and their values can be changed;
- ✓ The correct identification may be achieved in spite of errors made by the user or the inputted data;
- ✓ The numeric characters can be used directly, without being divided into intervals;
- ✓ The user can express uncertainty by inserting more than one state value or a range of numeric values.

Nevertheless, if appropriate strategies for their use are not adopted, the main advantage of interactive keys, their flexibility, can also lead to incorrect outcomes. The goal is to maximize the probability of obtaining a correct identification and this is equal to the product of the probabilities of properly apply each feature observed and inserted to the correct species. Thus, the user should aim to select features for which there is a low probability of error which will tend to minimize the number of necessary features to complete the identification. The minimization of the number of characteristics is accomplished by the use of characters that eliminate maximum taxa [128].

II. Thesis Context and Objectives

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Being a well developed forensic science throughout the world, the interest in medico-legal entomology is increasing in Portugal. However, the majority of the studies are focused primarily on the Diptera species with forensic interest, leaving the Coleoptera order at best under-emphasized.

In this study, aimed for the Coleoptera species with forensic interest, the focus lies in eight important families of Coleoptera present in Portugal, strongly related with forensic entomology cases: Carabidae, Cleridae, Dermestidae, Histeridae, Nitidulidae, Scarabaeidae, Silphidae and Staphylinidae. The main goal of this study is to characterize the most representative species belonging to these eight families which will allow an easy identification of relevant species for forensic technicians without expertise in entomology.

An accurate identification of insect specimens is an essential step in forensic entomology and, until these days, dicothomous keys are the main tool used to accomplish this task. However, these keys are, most of the times, very difficult to follow since they use morphological and technical terms that are unfamiliar to non-expert forensic technicians. In order to overcome these limitations, interactive keys have emerged, which use photographs to help in the identification of relevant morphologic characteristics. The pictorial keys and characterizations presented in this work, available online in an interactive tool, became, therefore, an easy and intuitive way to achieve a correct identification not only of Coleoptera families but also genus and even species.

III. Materials and Methods

III.Materials and Methods

For organizational purposes, three major phases may be considered in the methodology of this project: (1) sampling; (2) assembly and identification; and (3) photographic record and key elaboration.

1. Sampling

The specimens' sampling and collection were carried out in two distinct masters' theses. Gusmão [129] experimental field work occurred in three geographic sites in Gouveia, Portugal, and in two different seasons of 2007: in the winter (February 12th – March 26th) and in the spring (May 22nd – July 6th). EFD, specifically design for this study, held a Dexion structure which supported the insect traps and protected them against vertebrate predators. There were placed two EFD in each of the three zones.

Several wild animals were selected to be placed in the EFDs: two common genets, *Genetta genetta*; one red fox, *Vulpes vulpes*; one common buzzard, *Buteo buteo*; and one wild boar, *Sus scrofa*. Among the domestic animals chosen were: four rock pigeons, *Columba livia*; and four common rabbits, *Oryctolagus cuniculus*. Whenever possible, in each zone and for the two seasons, it was placed one bird's corpse in one EFD and a mammal corpse in the other.

Two types of traps were used: pitfall and malaise. The first one consists in containers buried in the ground, leaving the edge at the surface level. Pitfalls are specially directed for sampling of ground-dwelling arthropods [130,131]. The collection of specimens and replacement of pitfall containers was performed weekly. Regarding the malaise traps, they simply intersect insects in flight and change their routes to a collection vessel placed on top of the trap [132]. The collection of specimens captured in these traps was performed in shorter intervals, from 1 to 3 days.

In Centeio [133] master thesis, the sampling process took place in Campo Grande, Aroeira and Sertã, using baited traps with pork liver and fish. After the collection, all specimens were duly preserved in vials with 70% alcohol and properly labeled.

2. Assembly and identification

As previously stated, all received specimens were preserved in vials with 70% alcohol and had a label with all the sampling details: local, experiment day, day of the year, and season.

After carefully removing every specimen from the vial with the help of entomological tweezers, each one was pinned using black enamelled insect pins ENTO PHINX No.1, placed inside a box and left one day to dry.

When completely dried, the specimens were identified using the stereomicroscope Olympus SZX7 coupled with an Olympus SC30 digital camera and with the Lab Sens (version 1.1) software. The identification was carried out using several identification keys, photo galleries from entomology collections and listings [25,71,134–144].

3. Photographic record and key elaboration

The main method used for the photographic record was the stereomicroscope Zeiss LUMAR.V12 SteREO coupled with The Imaging Source digital camera. To analyze and save the images obtained was used the IC Capture 2.4 Ink software.

To process the images of the morphological characteristics was used the Adobe Photoshop CS6 13.1 Portable software.

The pictorial keys and characterizations presented in the next chapter were made available online through the Weebly platform.

IV. Results and Discussion

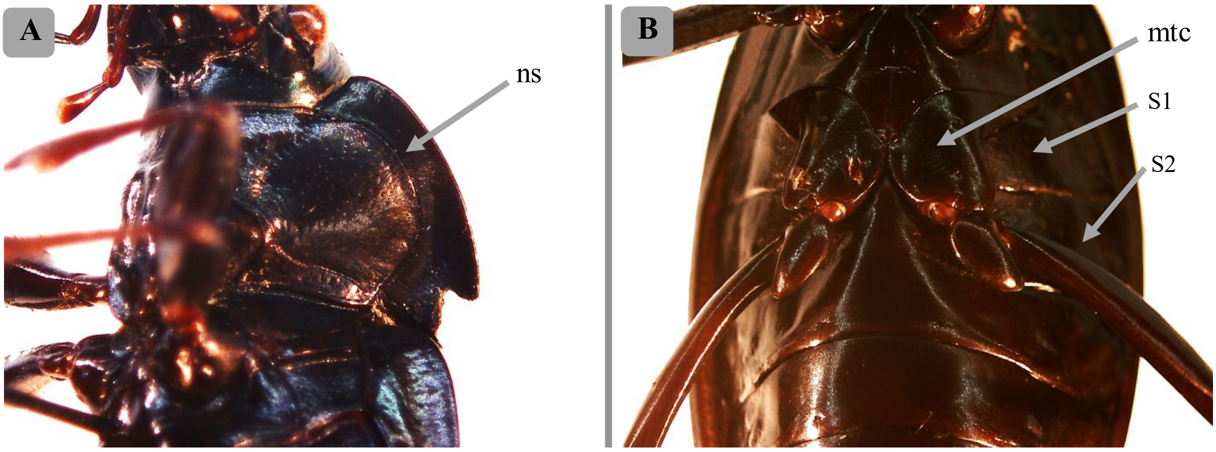
IV. Results and Discussion

The photographic record presented below characterizes several groups belonging to eight Coleoptera families with forensic interest. The specimens selected to this work came from two master theses and were captured using baited traps. Through the use of optical microscopy, it was possible to create two pictorial keys and five pictorial characterizations, presented below.

1. Key to eight Coleoptera Families with Forensic Interest

This family key begins to separate the two main Coleoptera suborders, Adephaga and Polyphaga. Since this work contains only one family belonging to the Adephaga suborder, Carabidae, the first step of this key allows its identification through the observation of two morphological characteristics: Adephaga beetles have the notopleural suture visible and the posterior border of the *metacoxae* passes the first abdominal *sternite* (Figure 3 A and B); on the other hand, Polyphaga beetles do not have the notopleural suture visible and the posterior border of the *metacoxae* does not pass the first abdominal *sternite* (Figure 3 C and D).

1	Presence of notopleural suture (Figure 3A); posterior border of the *metacoxae* passes the first abdominal *sternite* (Figure 3B).	Carabidae Suborder Adephaga
	Absence of notopleural suture (Figure 3C); posterior border of the *metacoxae* does not pass the first abdominal *sternite* (Figure 3D).	2 Suborder Polyphaga



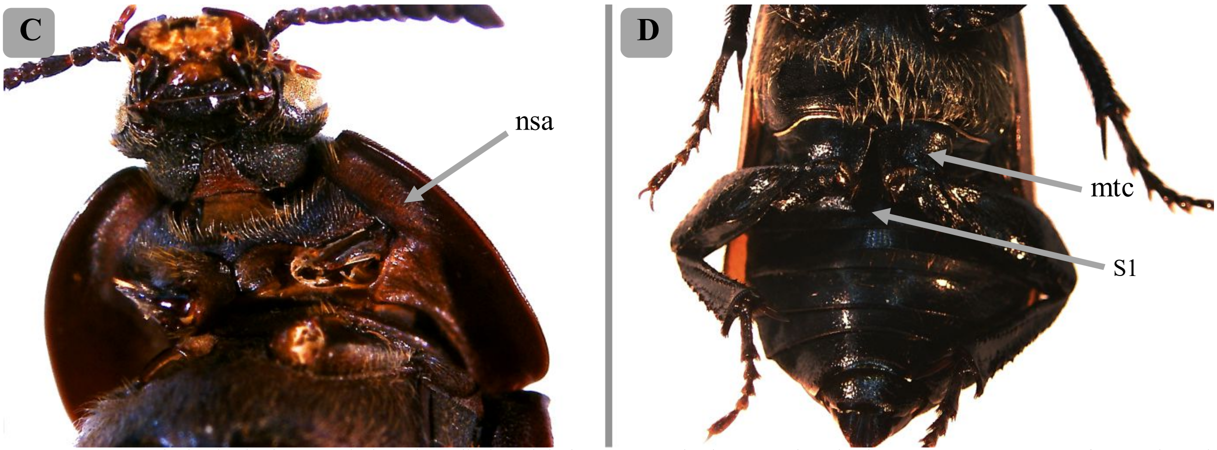


Figure 3. Morphological characteristics that distinguish between Adephaga and Polyphaga: **A** – presence of notopleural suture (lateral view, mag. 9,6x); **B** – posterior border of the *metacoxae* passes the first abdominal *sternite* (ventral view, mag. 9,6x); **C** – absence of notopleural suture (ventral view, mag. 21,5x); **D** – posterior border of the *metacoxae* does not pass the first abdominal *sternite* (ventral view, mag. 9,6x).

The Scarabaeidae family is then easily identified through the observation of its lamellated *antennae* and, in dorsal view, its large clypeus covers the *labrum* and mandibles (Figure 4). None of the other families have lamellate *antennae* (Figure 5 A to D).

2 **(1)**	Lamellate *antennae* (Figure 4A) and large *clypeus* covering *labrum* and mandible in dorsal view (Figure 4B)	Scarabaeidae
	Antennae not lamellate (Figure 5 A to D)	3

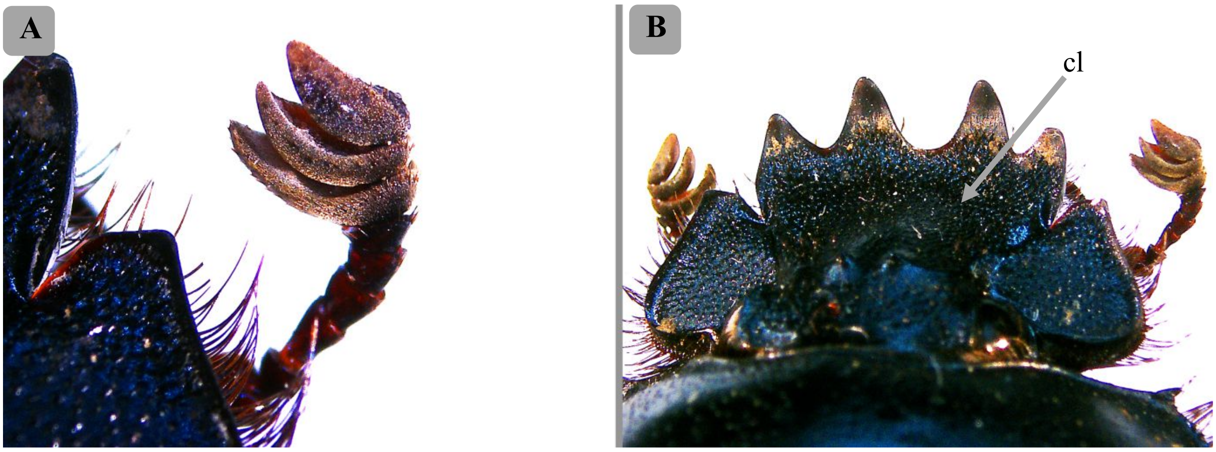


Figure 4. Morphological characteristics of Staphylinidae: **A** – lamellate antennae (dorsal view, mag. 23,0x); **B** – large *clypeus* covering *labrum* in dorsal view (dorsal view, mag. 9,6x).

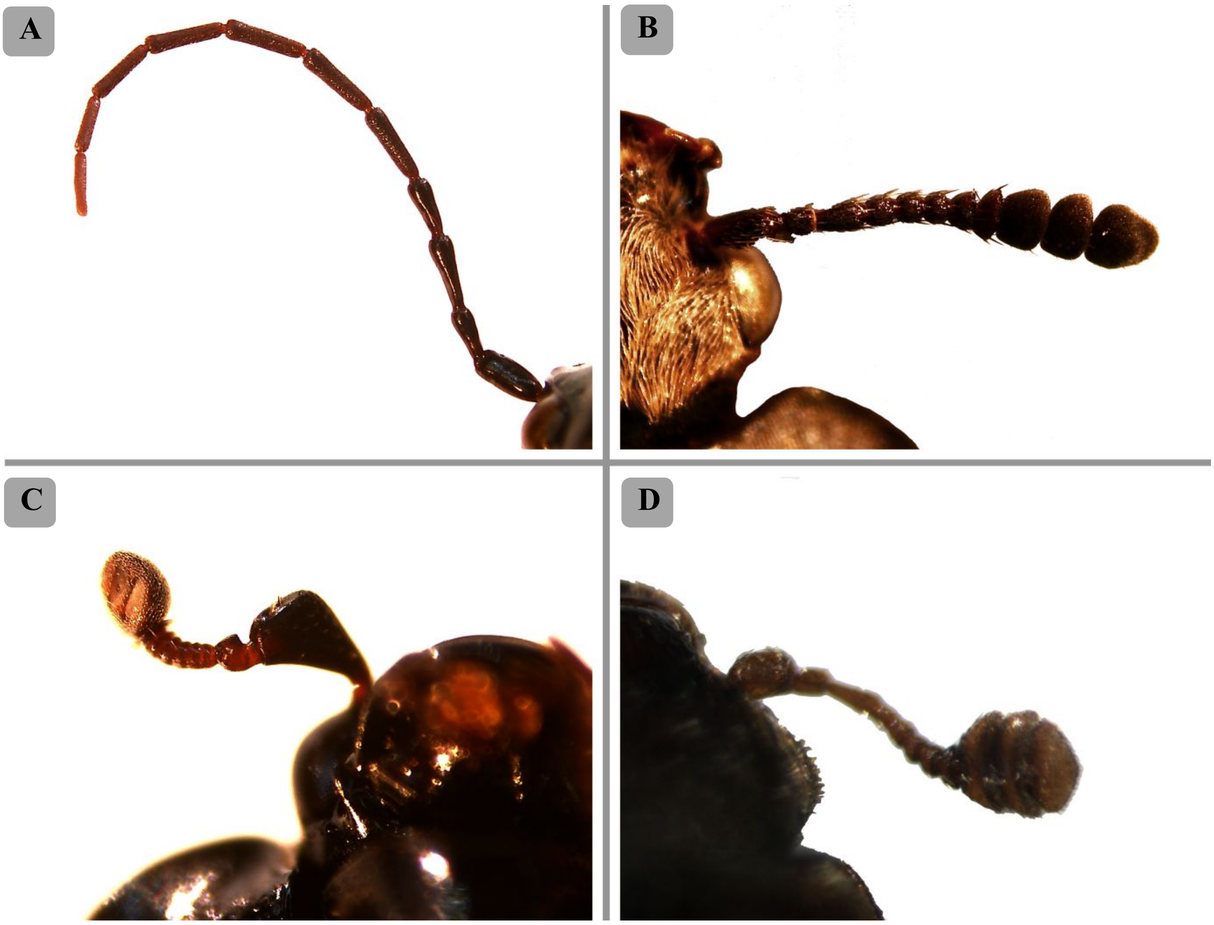


Figure 5. Different types of *antennae*: **A** – Carabidae filiform *antennae* (dorsal view, mag. 12,0x); **B** – Silphidae slightly club *antennae* (dorsal view, mag. 18,6x); **C** – Histeridae elbowed *antennae* (ventral view, 52,0x); **D** – Nitidulidae distinctly capitate *antennae* (dorsal view, mag. 96,0x).

The Staphylinidae family is identified by its e*lytra*, which are very short and truncate exposing at least three abdominal *tergites*, and its *antennae* are filiform or moniliform (Figure 6).

3 **(2)**	*Elytra* very short and truncate, exposing more than three abdominal *tergites* (Figure 6A); *antennae* filiform or moniliform (Figure 6B)	Staphylinidae
	Without the above combination of characters	4

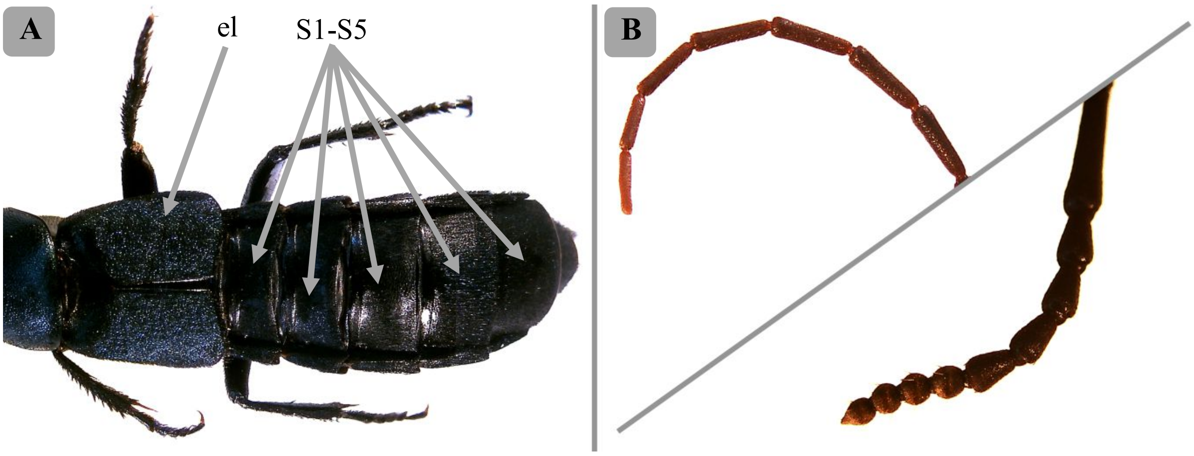


Figure 6. Morphological characteristics of Staphylinidae: **A** – *elytra* very short and truncate exposing more than three abdominal *tergites* (dorsal view, mag. 10,8x); **B** – *antennae* filiform (up, mag. 23,0x) or moniliform (down mag. 15,3x).

The next step separates the nitidulids and histerids from the remaining families: both have *antennae* with a compact club (Figure 7A) and *elytra* short and truncate exposing abdominal *tergites* (Figure 7B).

4 **(3)**	*Antennae* with a compact club (Figure 7A); *elytra* short and truncate exposing abdominal *tergites* (Figure 7B)	5
	Without the above combination of characters	6

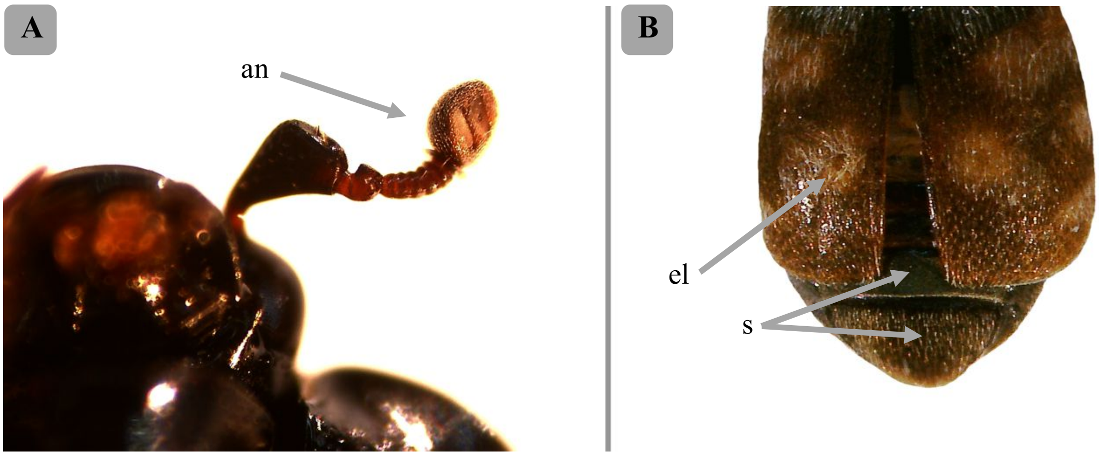


Figure 7. Morphological characteristics of Nitidulidae and Histeridae: **A** – *antennae* with a compact club (ventral view, mag. 52,0x); **B** – short and truncate *elytra* exposing abdominal *tergites* (dorsal view, mag. 32,5x).

The main morphological structure that allows the Nitidulidae/Histeridae separation is the trochantin: while the first ones have a *procoxae* transverse with exposed *trochantin* (Figure 8), the *procoxae* of histerids do not expose the *trochantin* (Figure 9A). In addition, Histeridae beetles have short and truncate *elytra* exposing the last two abdominal tergites, *pygidium* and *propygidium* (Figure 9B) and their *tibiae* are flattened with spines or teeth (Figure 10).

5 **(4)**	*Procoxae* transverse with exposed *trochantin* (Figure 8)	Nitidulidae
	Procoxae transverse without exposed *trochantin* (Figure 9A); short and truncate *elytra* exposing *pygidium* and *propygidium* (Figure 9B); *tibiae* flattened with spines or teeth (Figure 10)	Histeridae

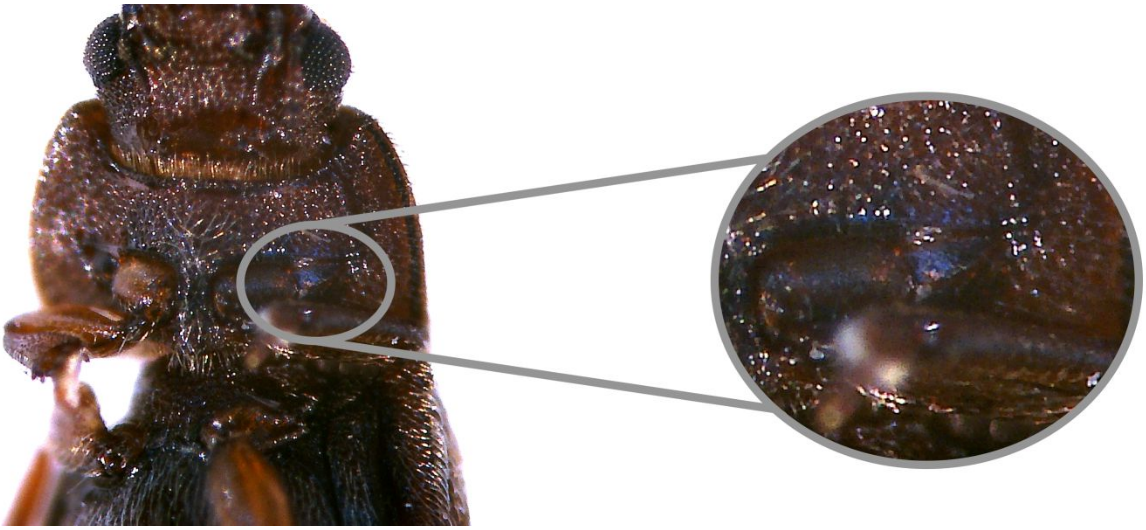


Figure 8. *Procoxae* transverse with exposed *trochantin* on Nitidulidae (ventral view, mag. 64,0x and 103,0x).

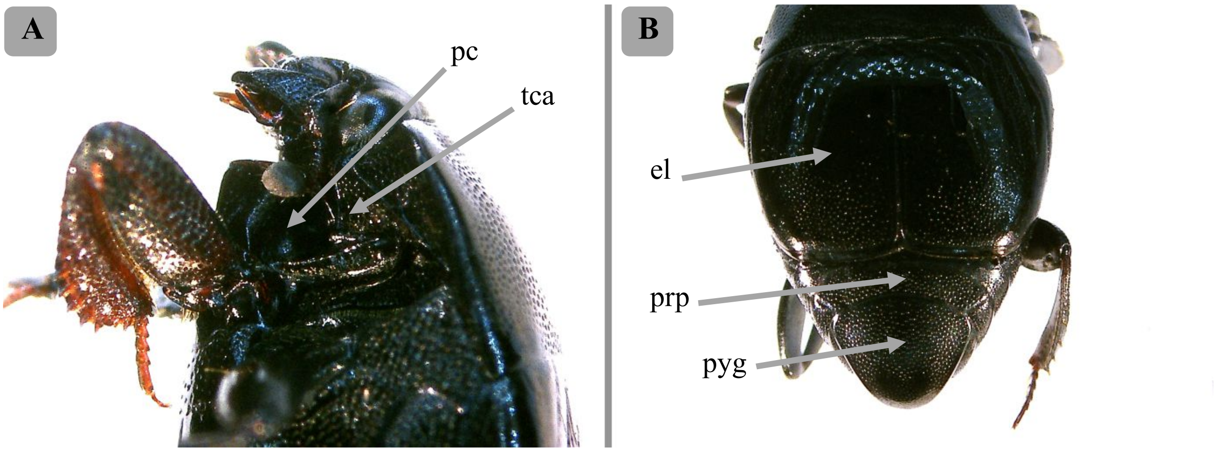


Figure 9. Morphological characteristics of Histeridae: **A** – *procoxae* transverse without exposed *trochantin* (lateral view, mag. 27,5x); **B** – short and truncate *elytra* exposing *pygidium* and *propygidium* (dorsal view, mag. 16,0x).

Figure 10. *Tibiae* flattened with spines on Histeridae (ventral view, mag. 27,3x).

Silphidae family is then identified through the observation of (1) the *antennae* which is clubbed with segments 9-11 presenting dense pubescence (Figure 11A); (2) very characteristic black *elytra* with orange markings and longitudinal *striae* (Figure 11B); (3) *pronotum* tomentose (Figure 11C); and (4) exposed *trochantin* (Figure 12). The silphids have a prognathous head (Figure 11C), while the other families have *hypognathous* head (Figure 13).

6 **(4)**	*Antennae* clubbed with 10 or 11 segments, segments 9-11 with dense pubescence (Figure 11A); *elytra* black with orange or yellow markings and longitudinal *striae* (Figure 11B); head prognathous and *pronotum* tomentose (Figure 11C); exposed *trochantin* (Figure 12)	Silphidae
	Head *hypognathous* (Figure 13)	7

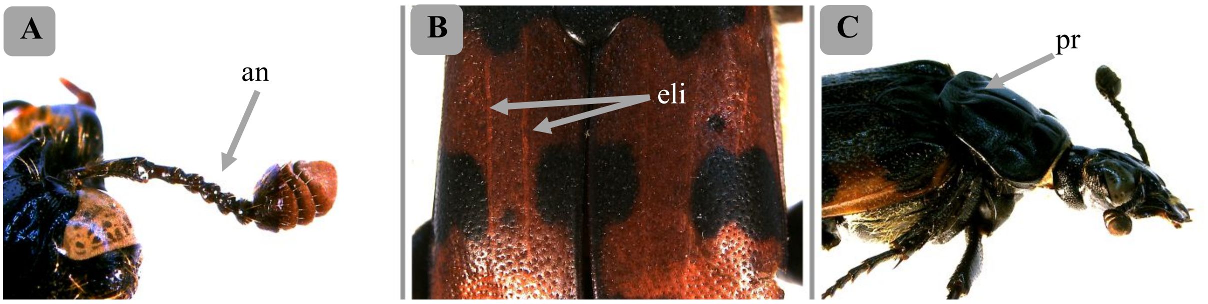


Figure 11. Morphological characteristics of Silphidae: **A** – capitate *antennae* with 10 or 11 segments (dorsal view, mag. 26,0x); **B** – *elytra* with orange markings and longitudinal *striae* (dorsal view, mag. 12,6x); **C** – head *prognathous* and *pronotum* tomentose (lateral view, mag. 10,1x).

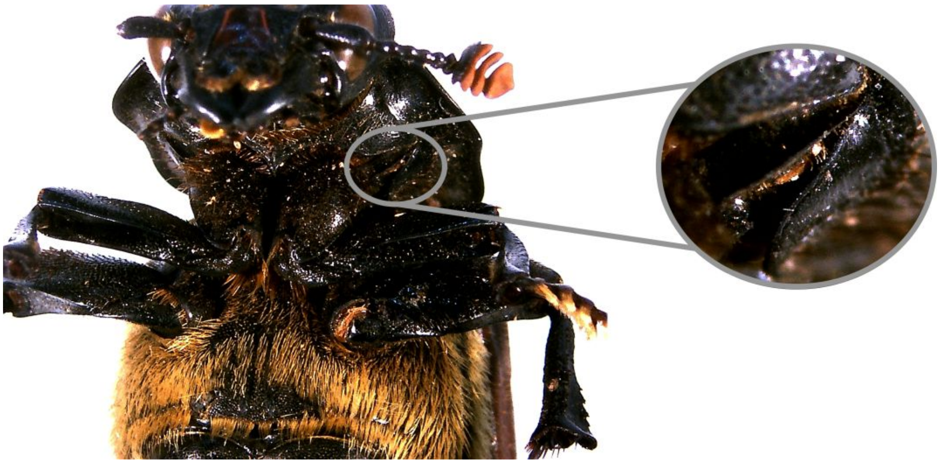


Figure 12. Exposed *trochantin* on Silphidae (ventral view, mag. 9,6x and 17,0x).

Figure 13. Head *hypognathous* (lateral view, mag. 25,0x).

Finally, there are only two families remaining: Dermestidae beetles have a clubbed and short *antennae* received into grooves on underside of *prothorax* (Figure 14A) and five visible *sternites* (Figure 14B); while Cleridae beetles have a distinct elongated body, covered by bristly hairs (Figure 15), the *antennae* are not received into grooves on underside of *prothorax* (Figure 16A) and have conical *procoxae* (Figure 16B).

7 **(6)**	Clubbed and short *antennae* received into grooves on underside of *prothorax* (Figure 14A); five visible *sternites* (Figure 14B)	Dermestidae
	Body elongated, covered by bristly hairs and with the *pronotum* narrower than *elytra* (Figure 15); *antennae* not received into grooves on underside of *prothorax* (Figure 16A); conical *procoxae* (Figure 16B)	Cleridae

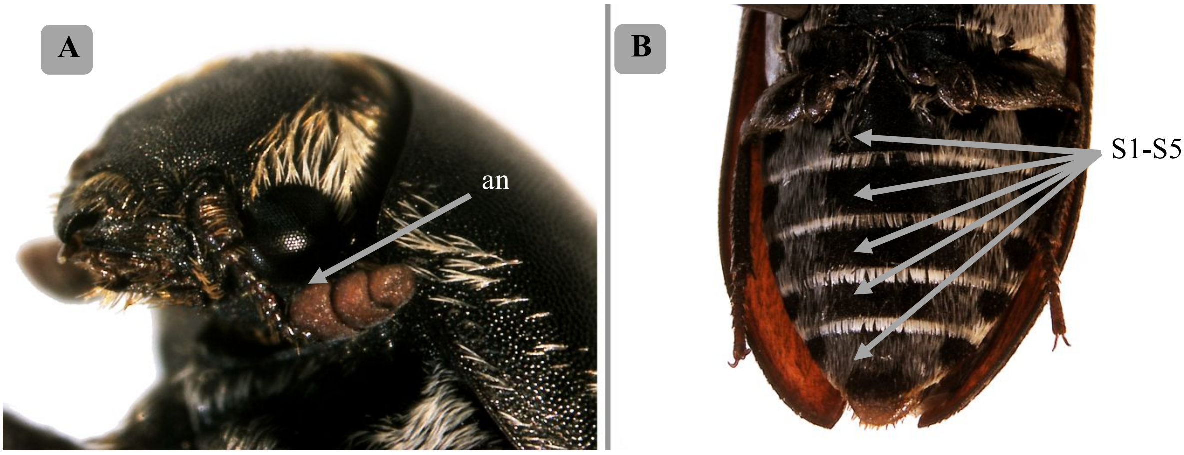


Figure 14. Dermestidae morphological characteristics: **A** – clubbed and short *antennae* received into grooves on underside of *prothorax* (lateral view, mag. 40,0x); **B** – five visible *sternites* (ventral view, mag. 15,6x).

Figure 15. Body elongated with bristly hairs and *pronotum* narrower than *elytra* (dorsal view, mag. 26,0x).

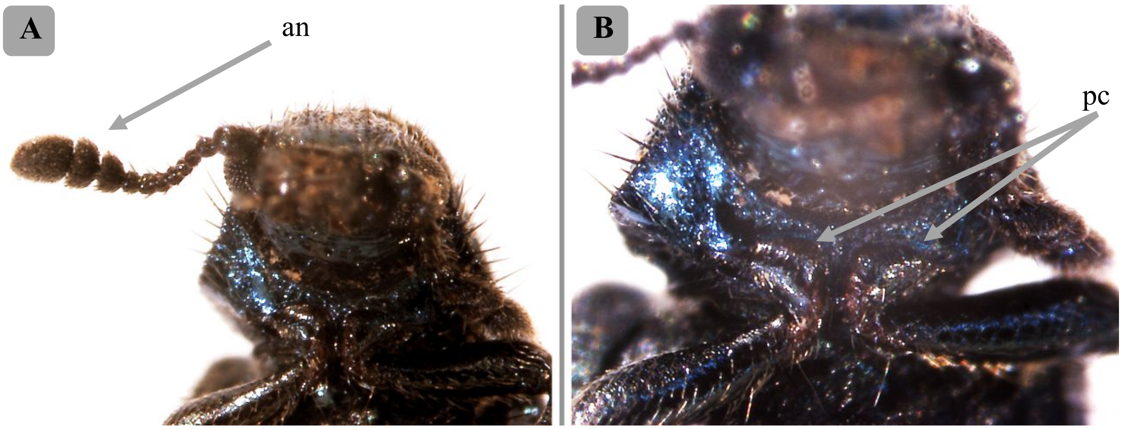


Figure 16. Cleridae morphological characteristics: **A** – *antennae* not received into grooves on underside of *prothorax* (ventral view, mag. 57,0x); **B** – conical *procoxae* (ventral view, mag. 82,0x).

2. Silphidae Key to Three Species of Nicrophorus Genus

The Silphidae key begins to identify the *Nicrophorus* genus. The main morphological characteristic of this genus is the *elytra* apex, which looks as if they have been cut off (i.e. are not rounded) leaving, in most species, the last three or four segments of the abdomen exposed (Figure 17); also, the *antennae* are distinctly clubbed (Figure 18A) and the front *tibiae* have a strong tooth towards the *apex* (Figure 18B).

1	*Elytra* appearing cut off at the *apex* (i.e. not rounded) and four abdominal segments exposed (Figure 17); clubbed *antennae* (Figure 18A); *protibiae* with a strong tooth towards the *apex* (Figure 18B)	2 Genus *Nicrophorus*
	Without the above combination of characters	Not found in the sampling

Figure 17. *Elytra* appearing cut off at the *apex* and four abdominal segments exposed (dorsal view, mag. 9,6x).

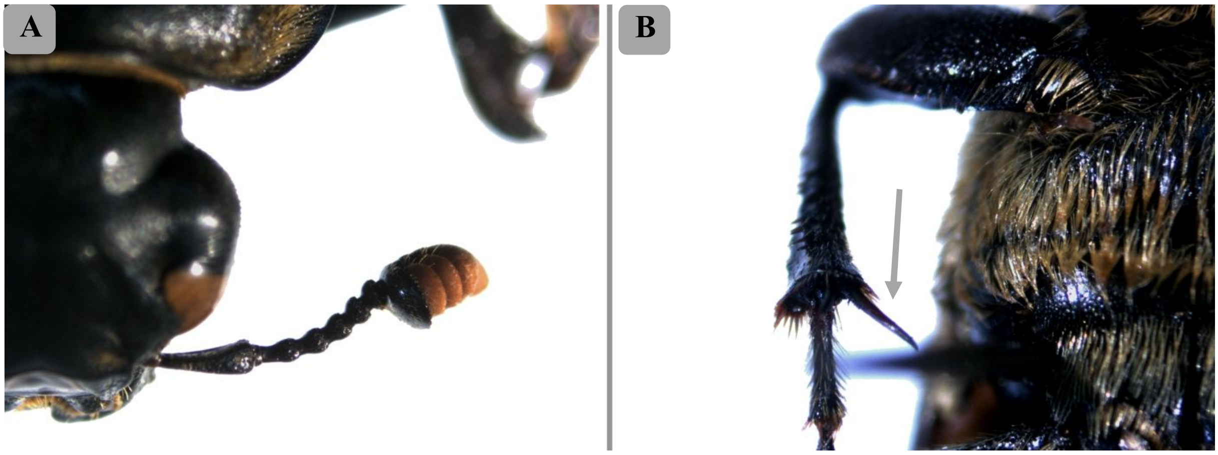


Figure 18. Morphological characteristics of *Nicrophorus* genus: **A** –clubbed *antenna* (dorsal view, mag. 15,6x); **B** – *protibia* with a strong tooth towards the *apex* (ventral view, mag. 20,5x).

There are one morphological characteristic that separates *Nicrophorus vestigator* (Figure 22) from the other two species: *N. vestigator* has long golden brown hair near the *pronotum* front and rear margin (Figure 21A); the other two species have a hairless *pronotum* (Figure 21B). *N. vestigator* has also two transverse orange marks on the *elytra* (Figure 19) and the last three segments of *antennae* club are reddish-yellow (Figure 20).

2 **(1)**	Two transverse reddish to orange-brown marks present on *elytra* (Figure 19); *antennae* with the last three segments of club reddish-yellow (Figure 20); front and rear margin of *pronotum* with long golden hair (Figure 21A)	*Nicrophorus vestigator* (Figure 22)
	Hairless *pronotum* (Figure 21B)	3

Figure 19. Two transverse reddish to orange-brown marks present on *elytra* (dorsal view, mag. 9,6x).

Figure 20. *Antennae* with the last three segments of club reddish-yellow (ventral view, mag. 33,5x).

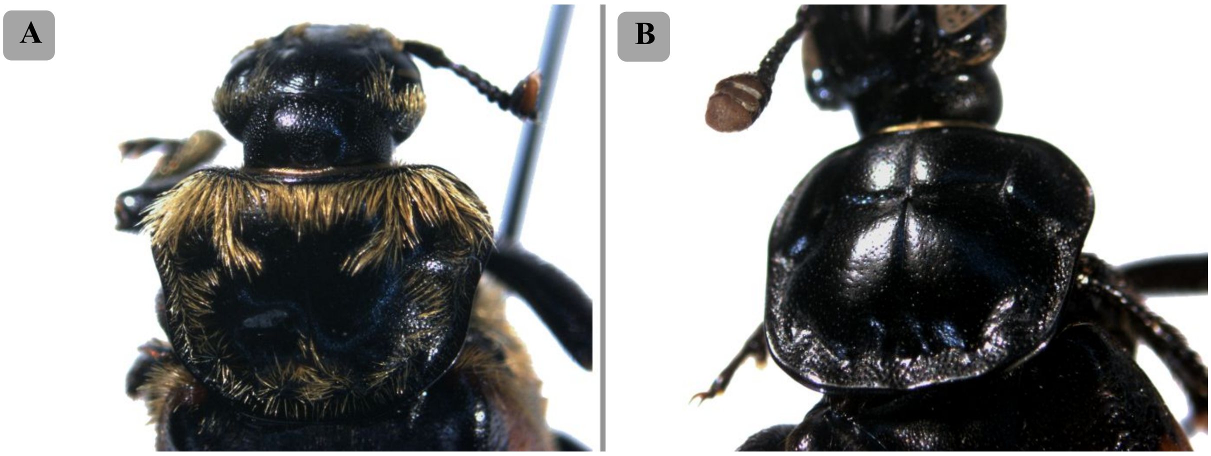


Figure 21. Morphological characteristic distinguishing *Nicrophorus vestigator* from two other species: **A** – front and rear margin of *pronotum* with long golden hair (dorsal view, mag. 12,3x); **B** – hairless pronotum (dorsal view, mag. 14,0x).

Figure 22. *Nicrophorus vestigator* whole body photograph (dorsal view, mag. 9,6x; composite photograph).

The separation between *Nicrophorus investigator* and *Nicrophorus interruptus* is simple: in the *elytra*, if the front coloured mark continues without interruption across both *elytra* (Figure 23) is *N. investigator* (Figure 24); on the other hand, if both coloured marks (anterior and posterior) are separated from one another at the suture (Figure 25) is *N. interruptus* (Figure 26).

3 **(2)**	Coloured mark from the front continues without interruption across *elytra* (Figure 23)	*Nicrophorus investigator* (Figure 24)
	Both coloured marks (anterior and posterior) separated from one another at the suture (Figure 25)	*Nicrophorus interruptus* (Figure 26)

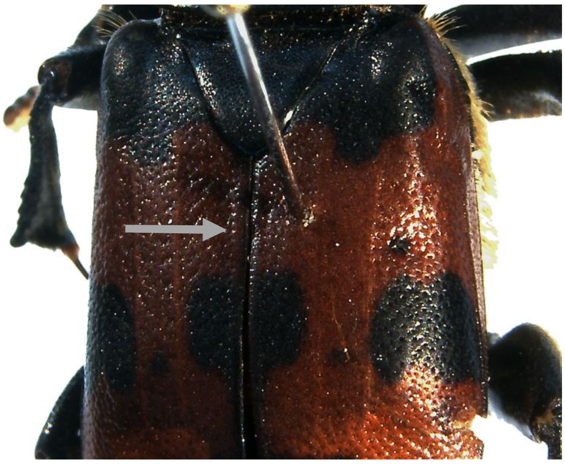


Figure 23. Coloured mark from the front continues without interruption across *elytra* (dorsal view, mag. 9,6x).

Figure 24. *Nicrophorus investigator* whole body photograph (dorsal view, mag. 9,6x; composite photograph).

Figure 25. Both coloured marks separated from one another at the suture (dorsal view, mag. 11,6x).

Figure 26. *Nicrophorus interruptus* whole body photograph (dorsal view, mag. 9,6x; composite photograph).

3. Pictorial Characterization of Carabus Genus

Carabus genus (Figure 30) is identified by several morphological characteristics. *Clypeus* is narrower than the distance between the *antennae* (Figure 27A), last segment of the *maxillary* palps well-developed (Figure 27B), *frons* without ridges and with a single puncture bearing a bristle on top of the head each side just by the eye (Figure 27C) and the head is broad and *pronotum* not oval in shape (Figure 27D). Additionally, there is an absence of waist between the *pronotum* and *elytra* (Figure 29) and the last are hairless and can have either regular *striae* but less than 11 (Figure 28A) or rows of granules with a sculpture more or less irregular (Figure 28B).

Head

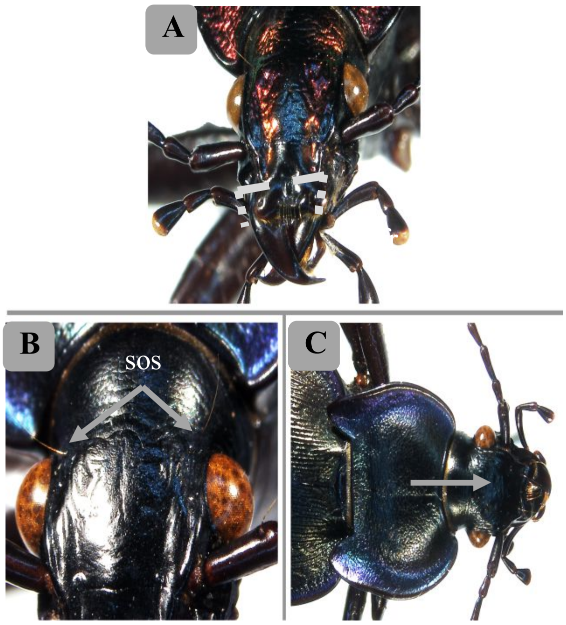


Figure 27. Morphological characteristic of *Carabus spp.*: **A** – distance between the *antennae* (long dashed) broader than *clypeus* length (short dashed) (front view, mag. 15,6x); **B** – *frons* without ridges and with two punctures, each one carrying a bristle (frontal view, mag. 9,6x); **C** – broad head and *pronotum* not oval in shape (dorsal view, mag. 9,6x).

Elytra

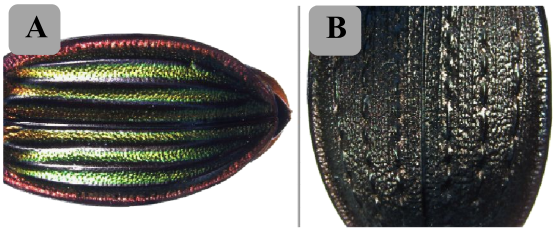


Figure 28. Hairless *elytra* with **(A)** regular *striae* (less than 11) (dorsal view, mag. 9,6x; composite photograph) or **(B)** irregular sculpture with rows of granules (dorsal view, mag. 9,6x).

Figure 29. Absence of waist between the *pronotum* and *elytra* (dorsal view, mag. 9,6x; composite photograph).

Figure 30. *Carabus sp.* whole body photograph (dorsal view, mag. 9,6x; composite photograph).

4. *Dermestes frischii* Pictorial Characterization

Dermestinae Subfamily

The subfamily characteristic which has a higher discriminatory power is the absence of *ocellus* on the frons (Figure 31).

Figure 31. *Frons* without an *ocellus* (frontal view, mag. 36,0x).

Dermestes Genus

The three main characteristics of *Dermestes* genus are the p*ronotum* broadest at the base (Figure 32A) and clubbed *antennae* (Figure 32B) with the club oval in shape (Figure 32C).

Figure 32. Morphological characteristics of *Dermestes* genus: **A** – *pronotum* broadest at the base, tapering towards the front (dorsal view, mag. 24,0x); **B** – clubbed *antennae* (ventral view, mag. 47,5x); **C** – *antenna* club oval (ventral view, mag. 99,0x).

Dermestinus Subgenus

Dermestinus subgenus is characterized by the front border of the *pronotum* with a semi-circular shape and right-angles (Figure 33A), the strongly domed *pronotum* (Figure 33B) and the presence of dense chalky white hairs on the underside (Figure 33C).

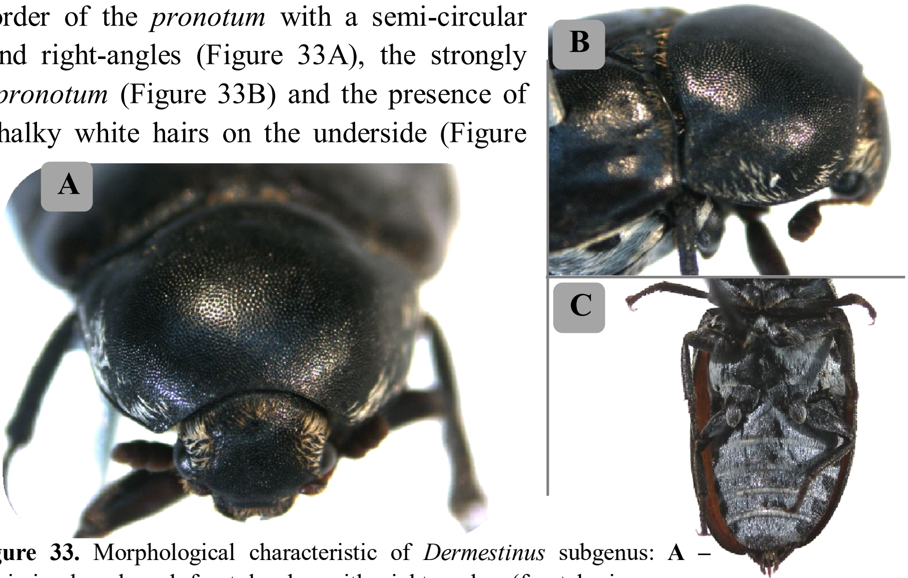


Figure 33. Morphological characteristic of *Dermestinus* subgenus: **A** – semi-circular shaped front border with right-angles (frontal view, mag. 21,5x); **B** – domed *pronotum* (lateral view, mag. 22,5x); **C** – underside with numerous white hairs (ventral view, mag. 11,0x).

Dermestes frischii

Finally, *Dermestes frischii* species (Figure 37) has white hairs angled obliquely towards the middle on the sides of the *pronotum* (Figure 34), the head and *scutellum* have patches of golden hairs (Figures 35A and 36), the apex of *elytra* by the suture do not have a sharp point and the apical margin is more or less smooth (Figure 35B) and the last *sternite* has black hair at the tip (Figure 35C).

Figure 34. Sides of the *pronotum* with white hairs pointing towards the middle (lateral view, mag. 22,5x).

Figure 36. *Scutellum* covered with yellowish hairs (dorsal view, mag. 21,5x).

Figure 35. *Dermestes frischii* morphological characteristics: **A** – head with patches of golden hair (frontal view, mag. 34,5x); **B** – apex without a sharp point and with a smooth margin (dorsal view, mag. 24,5x); **C** – black hair at the tip of the abdomen's last segment (ventral view, mag. 33,6x).

Figure 37. *Dermestes frischii* whole body photograph (dorsal view, mag. 11,3x).

5. *Saprinus subnitescens* Pictorial Characterization

Saprininae Subfamily

The Saprininae subfamily is characterized for having the p*ronotum* without a transverse furrow in the middle and broadest at the base (Figure 38), the *pronotum* and *elytra* do not have uniform ridges and are hairless (Figure 39), the club of *antenna* is oval and usually appears to be segmented (Figure 40) and finally the gular lobe is absent (Figure 41).

Figure 38. Unruffled *pronotum*, broadest at the base (dorsal view, mag. 18,6x).

Figure 39. *Pronotum* and *elytra* without uniform ridges and hairless (dorsal view, mag. 16,0x).

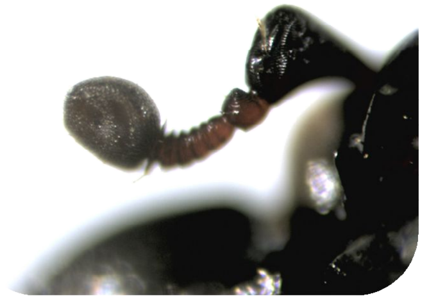


Figure 40. Club of *antenna* oval and usually appearing segmented (ventral view, mag. 80,0x).

Figure 41. Gular lobe absent (ventral view, mag. 18,6x).

Saprinus Genus

Histerids belonging to *Saprinus* genus have a distinct border separating eyes from frons (Figure 42A) which creates a discontinued rim separating the eyes from *frons* (Figure 42B). In addition, the mandibles are large and prominent (Figure 43A), the *elytra* are more or less punctured and with distinct *striae* (Figure 43B) and the front *tibiae* have distinct teeth (Figure 43C).

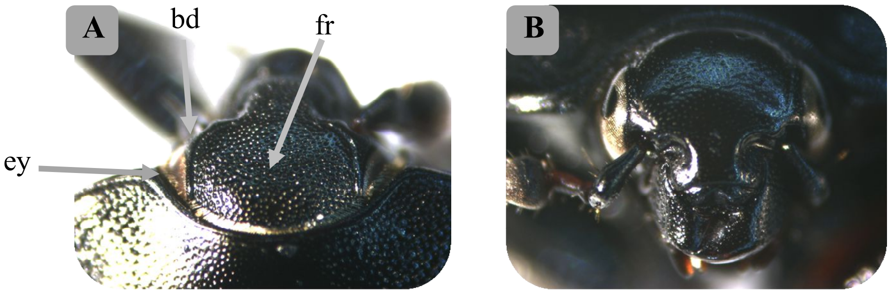


Figure 42. Morphological characteristics of *Saprinus* genus: **A** – distinct border separating eyes from frons (dorsal view, mag. 49,5x); **B** – rim separating eyes from *frons* is discontinued (frontal view, mag. 51,0x).

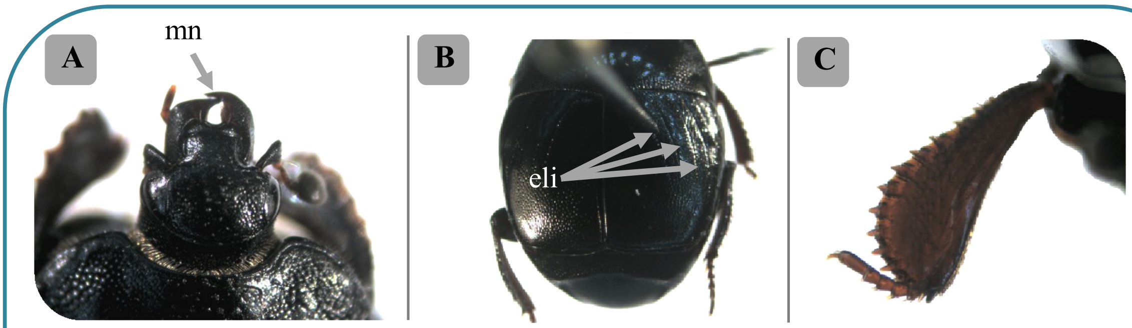


Figure 43. Morphological characteristics of *Saprinus* genus: **A** – prominent mandibles (dorsal view, mag. 27,0x); **B** – punctured *elytra* and with *striae* (dorsal view, mag. 16,0x); **C** – front *tibiae* with teeth (dorsal view, mag. 52,0x).

Saprinus subnitescens

Saprinus subnitescens species (Figure 46) have the front half of the *elytra* smooth from the suture to the margin and crossed by four or more *striae*, the innermost of which curves towards the *elytra* suture (Figure 44). Furthermore, the central part of the *mesosternum* do not have punctures (Figure 45A), the top of the *pronotum* do not have punctures and its margin is hairless (Figure 45B) and the sutural *stria* connects to the *stria* which runs parallel to the rear edge (Figure 45C).

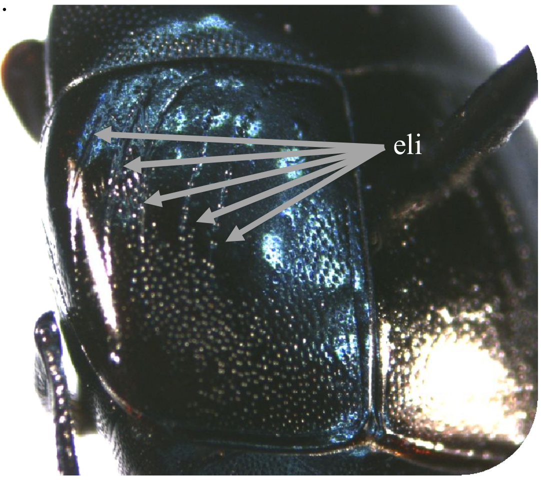


Figure 44. Front half of the *elytra* smooth yet crossed by more than four *striae*, with the innermost curving towards the *elytra* suture (dorsal view, mag. 23,5x).

Figure 46. *Saprinus subnitescens* whole body photograph (dorsal view, mag. 12,0x).

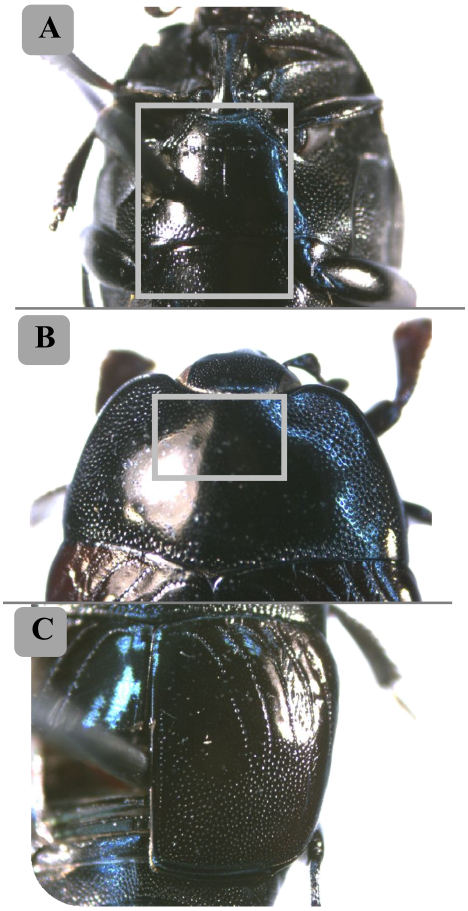


Figure 45. *Saprinus subnitescens* morphological characteristics: **A** – *mesosternum* with the central part without punctures (ventral view, mag. 21,0x); **B** – top of *pronotum* without punctures and its margin hairless (dorsal view, mag. 27,5x); **C** – *stria* running parallel to the rear edge connects to the sutural *stria* (dorsal view, mag. 25,5x).

6. *Onthophagus joannae* Pictorial Characterization

Scarabaeinae Subfamily

Scarabaeinae subfamily has the last segment of abdomen completely covered by the *elytra*, only visible from behind (Figure 47 A and B) and the *antennae* have a short club covered by minute hairs (Figure 47C). Other subfamily characteristics include the *elytra* as broad as long (Figure 48A), each hind *tibiae* with a single spur (Figure 48B) and the absence of *scutellum* (Figure 48C).

Figure 47. *Elytra* covering last segment of abdomen, only visible if viewed from behind; **A** – dorsal view (mag. 17,0x); **B** – view from behind (mag. 21,5x); **C** – *antennae* with short club covered by minute hairs (ventral view, mag. 71,0x).

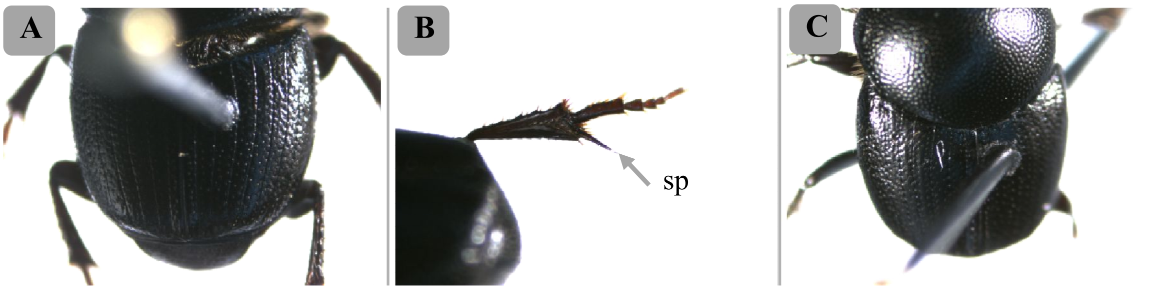


Figure 48. Morphological characteristics of Scarabaeinae subfamily: **A** – *elytra* as broad as long (dorsal view, mag. 24,0x); **B** – each pro *tibia* with a spur (ventral view, mag. 27,0x); **C** – absent *scutellum* (dorsal view, mag. 19,8x).

Onthophagus Genus

Onthophagus genus has the front margin of *pronotum* very strongly notched (Figure 49A) and each *elytron* has eight striae (Figure 49B).

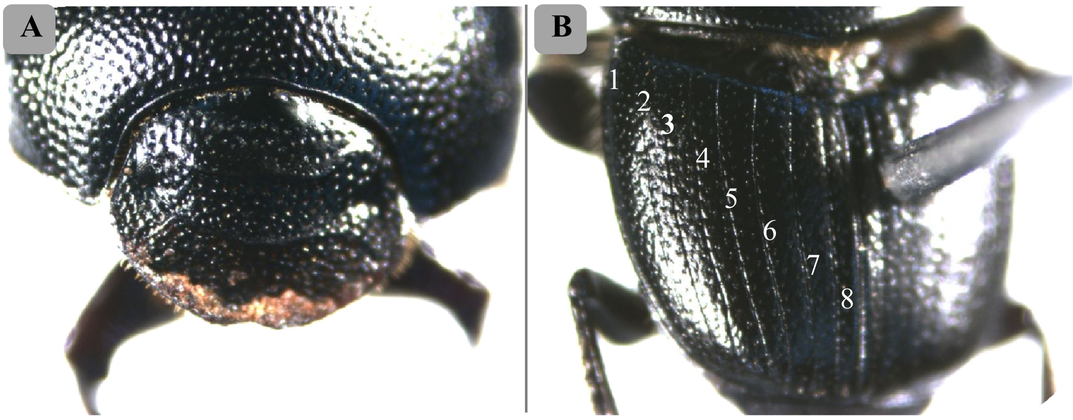


Figure 49. Morphological characteristics of *Onthophagus* genus: **A** – *pronotum* front margin strongly notched (frontal view, mag. 36,0x); **B** – eight striae on each *elytron* (dorsal view, mag. 28,0x).

Onthophagus joannae

Finally, *Onthophagus joannae* (Figure 51) has the front of the head slightly sinuate in the middle and the males have a raised transverse margin in front of the eyes (Figure 50).

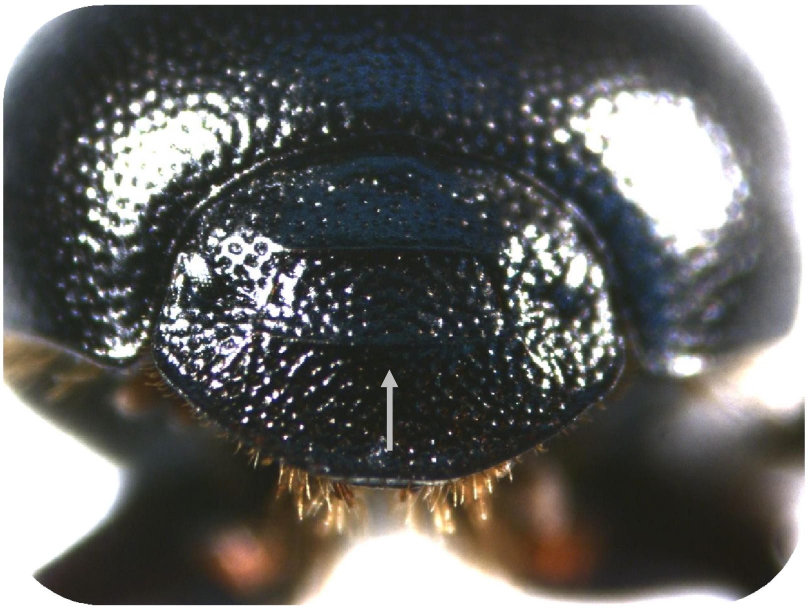


Figure 50. Front of head sinuate in the middle and males with a transverse margin located in front of the eyes (frontal view, mag. 39,5x).

Figure 51. *Onthophagus joannae* whole body photograph (dorsal view, mag. 9,6x; composite photograph).

7. *Creophilus maxillosus* Pictorial Characterization

Staphylininae Subfamily

There are several morphological characteristics of the Staphylininae subfamily: e*lytra* shortened with more than three segments of the abdomen exposed and last segment of abdomen with long styles (Figure 52A); *tarsi* with five segments (Figure 52B); *antennae* not clubbed with eleven segments, inserted under a ridge on the front (Figure 52C); *prosternum* does not project forwards under the neck (Figure 53A); labial palps have the last segment about equal in thickness with the previous one (Figure 53B); the *pronotum* and *elytra* do not have ridges or longitudinal depressions and *elytra* not overlaps with one another (Figure 53C); the mandibles are smaller and without curved extensions and the *antennae* are inserted at the front of the head (inside the base of the mandibles) a long way apart, further from each other than they are from the eyes (Figure 54).

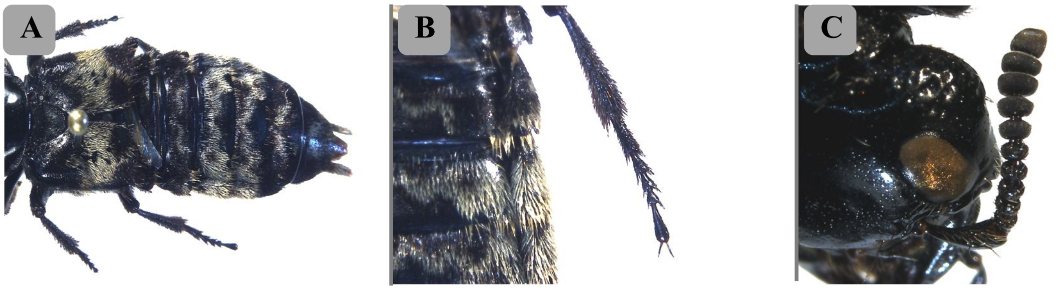


Figure 52. Morphological characteristics of Staphylininae subfamily: **A** – e*lytra* shortened with more than three segments of the abdomen exposed and the last one with long styles (dorsal view, mag. 9,6x); **B** – five segmented *tarsi* (dorsal view, mag. 13,6x); **C** – *antennae* not clubbed and eleven segmented, inserted under a ridge on the front (frontal view, mag. 25,5x).

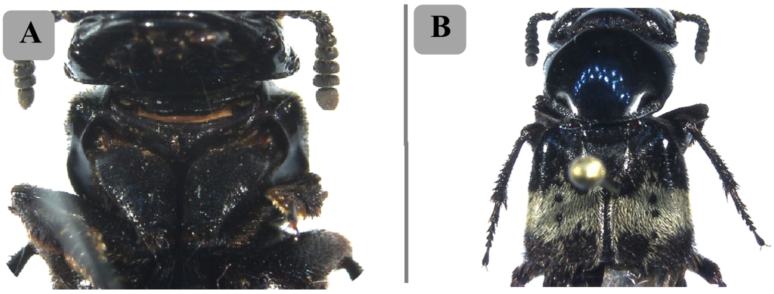


Figure 53. Morphological characteristics of Staphylininae subfamily: **A** – *prosternum* does not project forwards under the neck (ventral view, mag. 17,4x); **B** – *pronotum* and *elytra* without ridges or longitudinal depressions and *elytra* not overlapping with one another (dorsal view, mag. 9,6x).

Figure 54. Smaller mandibles and without curved extensions and *antennae* inserted at the front of the head (inside the base of the mandibles) a long way apart, further from each other than from the eyes (frontal view, mag. 17,4x).

Staphylinini Tribe

The Staphylinini tribe has the hind *tarsi* with an elongated first segment (Figure 55).

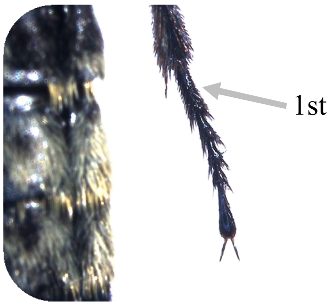


Figure 55. Hind *tarsi* usually with an elongated first segment (dorsal view, mag. 17,0x).

Staphylinina Subtribe

The Staphylinina subtribe has the *epipleura* visible (Figure 56A), punctured *pronotum* (Figure 56B) and short *antennae*, scarcely longer than the head and with a club of 5-6 segments which are broader than long (Figure 57).

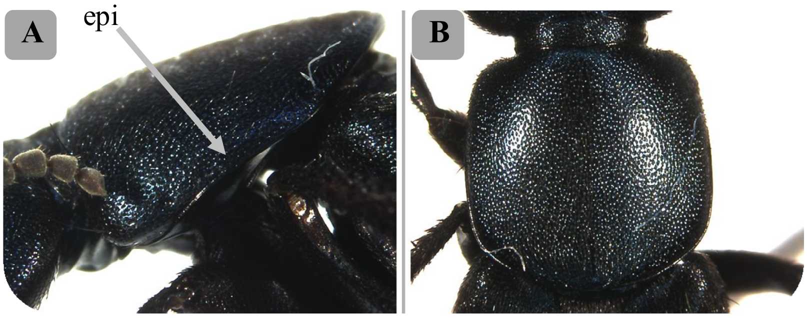


Figure 56. Morphological characteristics of Staphylinina subtribe: **A** – *epipleura* visible (side view, mag. 28,0x); **B** – punctured *pronotum* (dorsal view, mag. 20,5x).

Figure 57. Short *antennae*, longer than the head and with a club of 5 to 6 segments, broader than long (frontal view, mag. 18,0x).

Creophilus Genus

The *Creophilus* genus is characterized for having the head bare except for the area behind the eyes (Figure 58A) and the *pronotum* also bare except narrowly on the side margins and without punctures on top (Figure 58B).

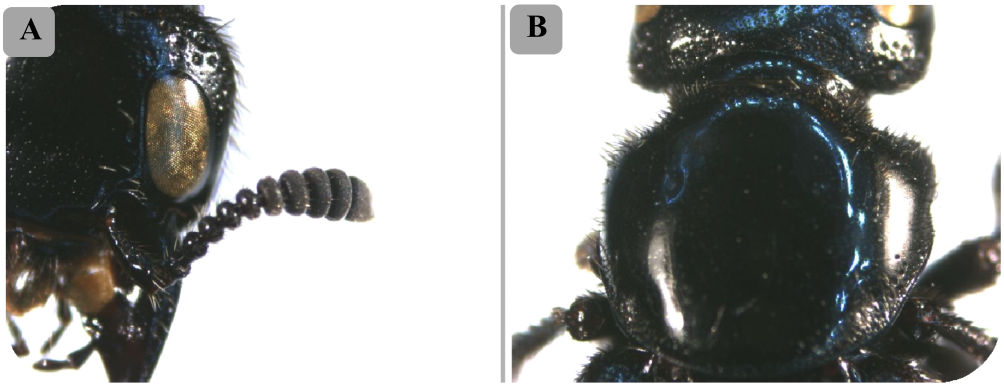


Figure 58. Morphological characteristics of *Creophilus* genus: **A** – bare head except behind the eyes (frontal view, mag. 24,5x); **B** – bare *pronotum* except on the side margins and without punctures on top (dorsal view, mag. 21,0x).

Creophilus maxillosus

At last, *Creophilus maxillosus* species (Figure 60) has distinctive white to yellow pubescence especially across middle of *elytra* and on abdominal tergites (Figure 59).

Figure 59. Distinctive yellow pubescence across middle of *elytra* and on abdominal tergites (dorsal view, mag. 9,6x; composite photograph).

Figure 60. *Creophilus maxillosus* whole body photograph (dorsal view, mag. 9,6x; composite photograph).

This work shares the same goals as other important characterization researches. For instance, regarding Diptera order, the pioneer scientist Patricia Thyssen has been characterizing immature stages of Diptera species, mainly belonging to the Calliphora family [145,146]. In another groundbreaking work, Almeida and Mise [71] provide a detailed description with both drawings and photographs, of the main families and species of South American Coleoptera of forensic importance. Also in South America, Fernando Aballay and his team [147] provide a key to 16 histerid species associated with decaying carcasses, which includes diagnoses and habitus photographs. In a broader scope, Mike's insect keys [139] is an undeniable revolutionary work regarding the photographic characterization of both Diptera and Coleoptera groups, which provides an interactive photographic key with detailed, well-marked and identified morphological characteristics. Also books have been giving increasing importance to images and photographic records, leaving aside the traditional dichotomous keys: two whole chapters of Current Concepts in Forensic Entomology provide photographs and drawings in keys for the identification of immature insects [148] and third instars of European blowfies (Diptera: Calliphoridae) of forensic importance [149]; Terry Whitworth [150] provides keys with detailed drawings to the identification of blow fly species of America and North of Mexico; finally, Gennard's book [1] covers the identification of flies and beetles that are important in forensic entomology.

The results presented in this work are of extreme importance as they give accurate and detailed morphological information regarding several groups of beetles. When became available, these results will provide a tool, simple and easy to use, which will guarantee that the process of identification will become simpler and subject to fewer errors. The rapid and accurate identification of Coleoptera species is a crucial step in every field of forensic entomology as it provides base evidence which will then allow to proceed with the investigation. For example, in cases where stored food is being consumed by a certain beetle species, knowing the taxonomic identification will provide information about this species and, therefore, a way to combat and eradicate its presence.

V. Conclusion and Perspectives

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As seen throughout this work, forensic entomology is a well developed forensic science with a wide spectrum of applications. In contrast to what is most publicized by the media, this science has an important role in civil cases like plagues present in buildings and also stored products. Despite this, is still an under evaluated forensic tool in Portugal. So, the results presented in this work are important not only to continue the development of forensic entomology as a field of work in Portugal but also to shed some light in the Coleoptera biodiversity.

The continuation of this work, unprecedented in Portugal, will bring undeniable contributions to the extension of knowledge concerning beetles' morphology and bioecology. Additionally, since dichotomous keys are a very hard identification tool based on morphological characteristics, these pictorial keys and characterizations will certainly help forensic technicians not specialized in entomology to start the identification of specimens collected in the field. Finally, the results presented in this work are also presented in a website, thus, becoming available to anyone who needs to consult them.

VI. References

VI. References

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VII. Appendices

VII. Appendices

1. Article submitted to online scientific journal Ecologi@

Pictorial characterization of Coleoptera *Carabidae*, *Histeridae, Nitidulidae, Scarabaeidae* and *Silphidae* with forensic interest

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Resumo

Os insetos podem fornecer importantes informações em investigações de homicídios uma vez que contribuem para a decomposição do cadáver. Assim sendo, quando é descoberto um corpo, os insetos permitem estimar o intervalo pós-morte (IPM) ou indicar a presença de substâncias tóxicas. A fim de estudar estes eventos, é essencial realizar uma identificação precisa das espécies encontradas no cadáver.

Este estudo centra-se em cinco famílias de coleópteros presentes em Portugal, fortemente relacionadas com casos de entomologia forense: *Carabidae*, *Histeridae*, *Nitidulidae*, *Scarabaeidae* e *Silphidae*. O seu principal objetivo é utilizar imagens para caracterizar morfologicamente as espécies mais representativas dessas famílias. Foram analisados espécimes previamente capturados em armadilhas com isco ou em carcaças de animais. As estruturas diagnosticantes (somáticas e genitais) dos espécimes são descritas e fotografadas utilizando microscopia ótica. Os dados obtidos serão utilizados para construir uma chave interativa que irá permitir uma fácil identificação de espécies relevantes por técnicos forenses sem experiência em entomologia. O desenvolvimento da chave interativa pode ainda ser otimizado através da criação de um *software* de computador a fim de tornar a identificação de espécimes mais portátil e com a possibilidade de melhoramentos quando necessário.

Palavras-chave: Entomologia forense, chaves de identificação, microscopia, Coleoptera

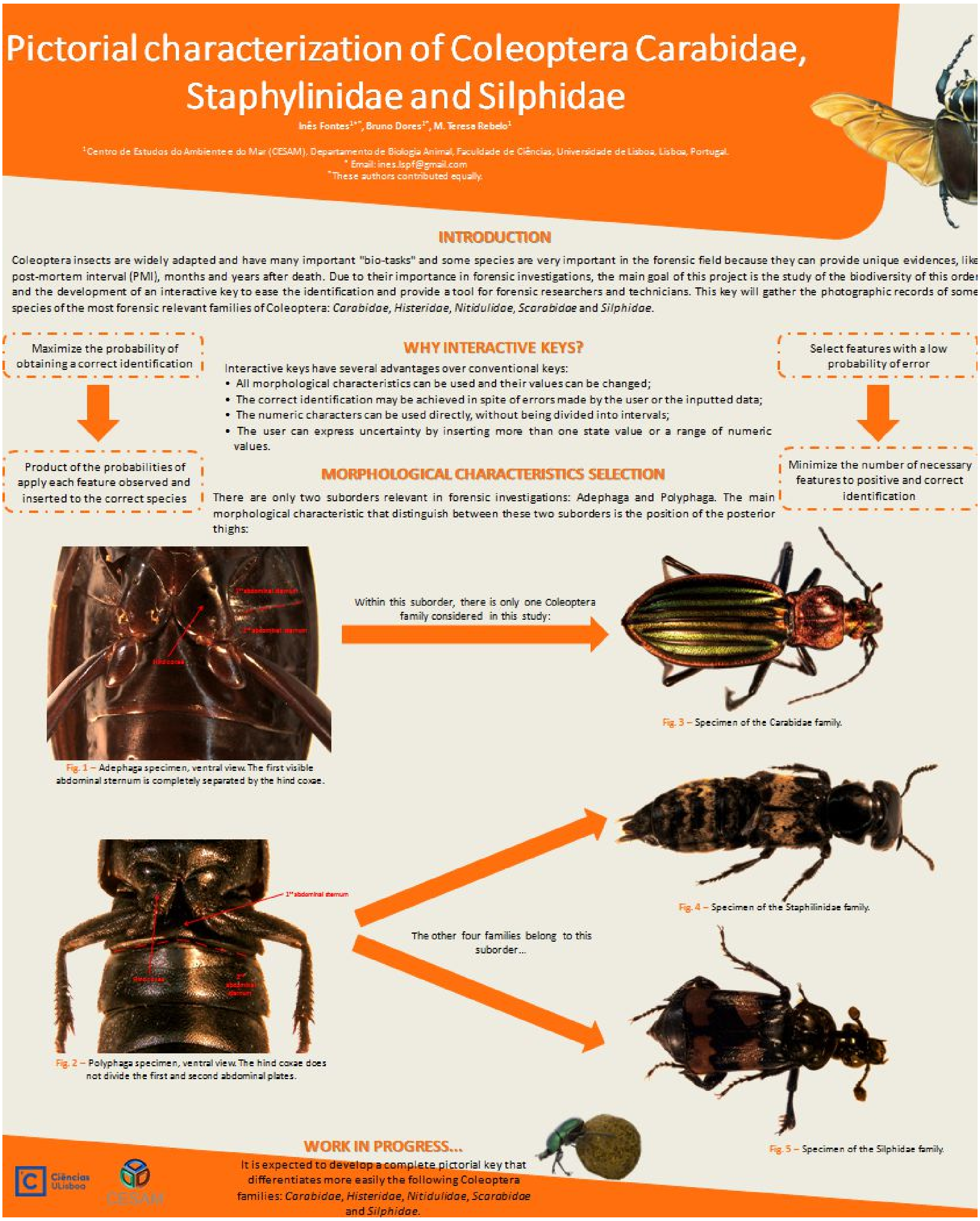
Abstract

Insects can provide significant information in death investigations because of their contribution to corpse decomposition. As so, if a dead body is discovered, insects can help in the estimation of the post-mortem interval (PMI) or point out the presence of toxic substances present in the body. In order to study these events, it is essential to accomplish an accurate identification of the species found on the corpse.

The focus of this study lies in five key families of Coleoptera present in Portugal, strongly related to forensic entomology cases: *Carabidae*, *Histeridae*, *Nitidulidae*, *Scarabaeidae* e *Silphidae*. Its main goal is to use pictures to characterize morphologically the most representative species belonging to these families using specimens previously caught in baited traps or on animal carcasses. To proceed with this analysis, the diagnostic structures (somatic and genital) of the specimens are described and photographed using optical microscopy. When finished, the data obtained will be used to build an interactive key that will allow easy identification of relevant species for forensic technicians without expertise in entomology. The development of the interactive key can still be optimized with the creation of a computer software in order to make the specimens identification more portable and with the possibility of upgrades when necessary.

Keywords: Forensic entomology, identification keys, microscopy, Coleoptera

2. Poster presented at EBCI 2015



Pictorial characterization of Coleoptera Carabidae, Staphylinidae and Silphidae

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INTRODUCTION

Coleoptera insects are widely adapted and have many important "bio-tasks" and some species are very important in the forensic field because they can provide unique evidences, like post-mortem interval (PMI), months and years after death. Due to their importance in forensic investigations, the main goal of this project is the study of the biodiversity of this order and the development of an interactive key to ease the identification and provide a tool for forensic researchers and technicians. This key will gather the photographic records of some species of the most forensic relevant families of Coleoptera: *Carabidae, Histeridae, Nitidulidae, Scarabidae* and *Silphidae.*

Maximize the probability of obtaining a correct identification

Product of the probabilities of apply each feature observed and inserted to the correct species

WHY INTERACTIVE KEYS?

Interactive keys have several advantages over conventional keys:

- All morphological characteristics can be used and their values can be changed;
- The correct identification may be achieved in spite of errors made by the user or the inputted data;
- The numeric characters can be used directly, without being divided into intervals;
- The user can express uncertainty by inserting more than one state value or a range of numeric values.

Select features with a low probability of error

Minimize the number of necessary features to positive and correct identification

MORPHOLOGICAL CHARACTERISTICS SELECTION

There are only two suborders relevant in forensic investigations: Adephaga and Polyphaga. The main morphological characteristic that distinguish between these two suborders is the position of the posterior thighs:

Fig. 1 – Adephaga specimen, ventral view. The first visible abdominal sternum is completely separated by the hind coxae.

Within this suborder, there is only one Coleoptera family considered in this study:

Fig. 3 – Specimen of the Carabidae family.

Fig. 4 – Specimen of the Staphilinidae family.

The other four families belong to this suborder...

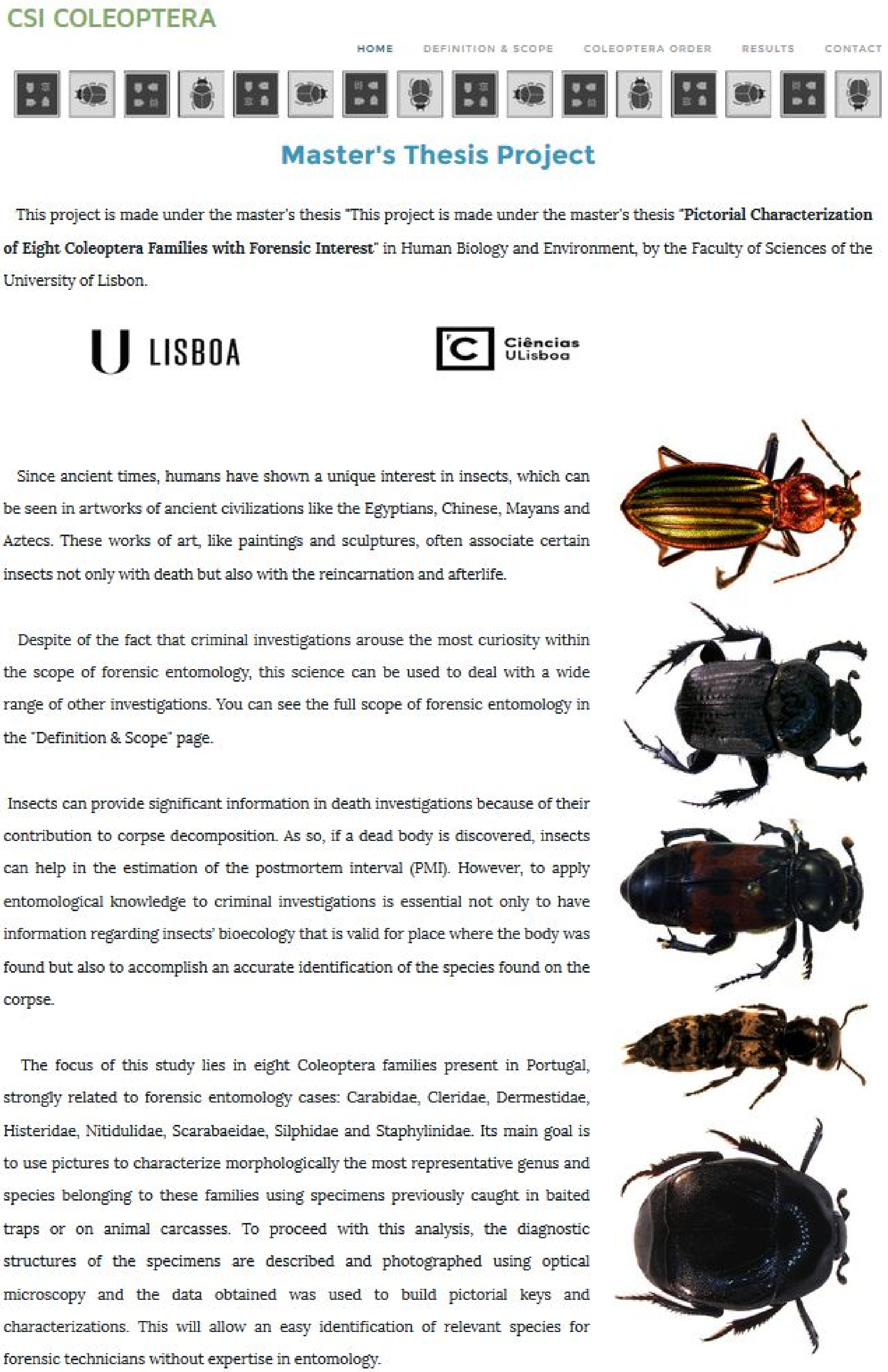
Fig. 2 – Polyphaga specimen, ventral view. The hind coxae does not divide the first and second abdominal plates.

Fig. 5 – Specimen of the Silphidae family.

WORK IN PROGRESS...

It is expected to develop a complete pictorial key that differentiates more easily the following Coleoptera families: *Carabidae, Histeridae, Nitidulidae, Scarabidae* and *Silphidae.*

3. Website CSI Coleoptera



Master's Thesis Project

This project is made under the master's thesis "This project is made under the master's thesis "**Pictorial Characterization of Eight Coleoptera Families with Forensic Interest**" in Human Biology and Environment, by the Faculty of Sciences of the University of Lisbon.

Since ancient times, humans have shown a unique interest in insects, which can be seen in artworks of ancient civilizations like the Egyptians, Chinese, Mayans and Aztecs. These works of art, like paintings and sculptures, often associate certain insects not only with death but also with the reincarnation and afterlife.

Despite of the fact that criminal investigations arouse the most curiosity within the scope of forensic entomology, this science can be used to deal with a wide range of other investigations. You can see the full scope of forensic entomology in the "Definition & Scope" page.

Insects can provide significant information in death investigations because of their contribution to corpse decomposition. As so, if a dead body is discovered, insects can help in the estimation of the postmortem interval (PMI). However, to apply entomological knowledge to criminal investigations is essential not only to have information regarding insects' bioecology that is valid for place where the body was found but also to accomplish an accurate identification of the species found on the corpse.

The focus of this study lies in eight Coleoptera families present in Portugal, strongly related to forensic entomology cases: Carabidae, Cleridae, Dermestidae, Histeridae, Nitidulidae, Scarabaeidae, Silphidae and Staphylinidae. Its main goal is to use pictures to characterize morphologically the most representative genus and species belonging to these families using specimens previously caught in baited traps or on animal carcasses. To proceed with this analysis, the diagnostic structures of the specimens are described and photographed using optical microscopy and the data obtained was used to build pictorial keys and characterizations. This will allow an easy identification of relevant species for forensic technicians without expertise in entomology.