CONTENTS

RIASSUNTO

I colori e i pattern cromatici presenti sui tegumenti degli insetti rappresentano da tempo un soggetto di interesse per numerosi ricercatori. Tuttavia, nonostante pattern cromatici complessi si riscontrino presso numerosi ordini di insetti, lo sforzo di ricerca è stato distribuito in modo eterogeneo, concentrandosi in particolar modo sui lepidotteri. Grazie a una lunga tradizione di ricerca, infatti, i complessi disegni che si sviluppano sulle ali delle farfalle sono oggi conosciuti con notevole approfondimento negli aspetti biochimici e ultrastrutturali, nei meccanismi di sviluppo e nelle loro basi genetiche, nella composizione ed evoluzione degli elementi discreti che li compongono, nonché negli aspetti di interazione con l'ambiente. In tempi più recenti, anche i pattern cromatici che caratterizzano i ditteri drosofilidi sono stati studiati in dettaglio negli aspetti strutturali, morfogenetici ed evolutivi.

Meno noti sono i fenomeni cromatici che si riscontrano presso altri gruppi di insetti, fra cui i coleotteri, ordine a cui si ascrivono circa 350.000 specie note. In particolare, sono stati ampiamente trascurati studi comparativi, interpretativi o evolutivi condotti a livello interspecifico e paragonabili a quelli condotti sugli altri gruppi citati. L'esplorazione della bibliografia specifica, infatti, ha permesso di individuare solo una scarna serie di lavori a riguardo, alcuni dei quali risalenti ai primi del '900. Questi si rivelano del tutto insufficienti a fornire un quadro generale sui fenomeni che governano i pattern cromatici dei coleotteri. Ad oggi non abbiamo che pochissime notizie sui meccanismi di controllo e di sviluppo dei loro pattern cromatici, sulle loro eventuali relazioni con le venulazioni delle ali (ampiamente verificate in lepidotteri e ditteri) o con altri elementi morfoanatomici, così come sulle loro capacità e tendenze evolutive.

Con questa tesi si sono voluti muovere i primi passi verso una riduzione dell'enorme lacuna di conoscenza ora delineata. Lo studio è stato condotto su due livelli diversi. Ad un primo livello, di carattere più generale, si sono voluti esplorare, dopo una accurata valutazione della letteratura, alcuni fenomeni di interesse generale, a cominciare dall'analisi di quelli più basilari: quelli, cioè, che determinano la produzione dei colori. Sono stati privilegiati, in questo caso, i diversi fenomeni che determinano la produzione di colori fisici in quanto, oltre a collegarsi direttamente con la seconda parte dello studio, sono quelli che presentano le maggiori potenzialità evolutive. Questa sezione (*Colours and chromatic effects in beetles*) si basa principalmente su un'accurata rassegna e analisi critica della bibliografia, che è stata integrata con alcune osservazioni originali. Nella sezione successiva (*Pattern evolution and Evo-Devo aspects*) si indagano alcuni aspetti dei pattern cromatici secondo una prospettiva "Evo-Devo" (così come viene comunemente detta la moderna la biologia evoluzionistica dello sviluppo), in particolare discutendo le relazioni che essi intraprendono con elementi anatomici del tegumento. In questi capitoli viene suggerito che le attività di induzione/controllo/alterazione dei pattern (sia pigmentari che di origine fisica) prodotte dagli elementi morfologici dell'elitra (punti, strie, vene) rivestano un ruolo di prim'ordine nello sviluppo e nell'evoluzione dei pattern. Lo stesso viene

suggerito a proposito delle inserzioni muscolari, per le quali viene verificata la capacità di induzione di un pattern cromatico, confermando e ampliando la documentazione di un importante dato bibliografico risalente ai primi del 1900, ma in seguito apparentemente dimenticato. Infine, viene discusso un interessante, ricorrente fenomeno di convergenza cromatica intraspecifica legata alla distribuzione geografica, per il quale sembrano improbabili spiegazioni legate alla selezione darwiniana.

Nella seconda parte della tesi (*Structural and evolutionary aspects in* Chrysolina) si è affrontata una ricostruzione dell'evoluzione dei pattern cromatici all'interno di un gruppo di coleotteri. A questo scopo, è stato individuato come utile candidato il genere *Chrysolina* (Chrysomelidae Chrysomelinae) inteso in senso lato, cioè comprendente alcuni generi affini di incerta collocazione tassonomica. La possibilità di accedere ad ampie collezioni entomologiche mi ha permesso di condurre una vasta ricognizione sulla quasi totalità dei sottogeneri e delle specie esistenti a livello mondiale. Le condizioni cromatiche osservate su decine di migliaia di esemplari sono state ricondotte a un numero limitato di pattern cromatici e mappate all'interno di una tabella sinottica con risoluzione a livello di sottogenere (attualmente si considerano validi circa 65 sottogeneri per il solo genere *Chrysolina* s. str.). Poiché non esiste, in letteratura, alcuna filogenesi del gruppo indagato, è stato intrapreso un tentativo di filogenesi su base morfologica condotto su 59 specie rappresentative di 4 generi e 52 sottogeneri. Nonostante il prolungato sforzo di ricognizione e codifica, il risultato ottenuto è stato complessivamente deludente, a causa, presumibilmente, dell'estrema uniformità morfologica riscontrata nel gruppo in esame. Ciononostante, alcuni cladi sono risultati supportati abbastanza da permettere alcune interessanti considerazioni. Ciascuna condizione cromatica è stata discussa dal punto di vista morfo-strutturale e, quando possibile o pertinente, evolutivo. Il risultato più interessante, in questo senso, è rappresentato dal riconoscimento di alcune forme cromatiche, distribuite fra 2 generi (*Oreina* e *Chrysolina*) e 8 sottogeneri diversi, quali espressioni di un unico pattern fondamentale (chiamato *fastuosa-like pattern*) che risulta riconducibile a un'unica innovazione evolutiva: le specie che ne sono interessate, infatti, a dispetto della tassonomia corrente, appartengono tutte a un medesimo clade.

La conduzione di uno studio sull'ultrastruttura della cuticola ha permesso, infine, di verificare l'origine del polimorfismo che caratterizza molte delle specie interessate dallo studio. In particolare, è stato individuato un meccanismo inedito che permette la frequente comparsa di forme nere (note anche come forme *nigrine*) presso gran parte delle specie a colorazione metallica, funzionando come un interruttore il cui azionamento (su scala filogenetica) permette di rendere visibile la colorazione pigmentaria oppure quella di origine fisica.

In conclusione, vengono proposte alcune osservazioni sulla grande versatilità dei meccanismi che producono colori o pattern di origine fisica, dalla quale può forse conseguire una difficoltà di controllo degli stessi, così come sulla versatilità degli elementi morfologici del tegumento, che si possono interfacciare

con meccanismi cromatici molto diversi, producendo interessanti fenomeni di convergenza di pattern anche fra gruppi filogeneticamente molto lontani.

ABSTRACT

Colours and chromatic patterns of insects are, since long time, a subject of particular interest for researchers. However, despite several orders exhibit complex colour patterns, few groups received most of the attention, i.e. butterflies (Lepidoptera) and *Drosophila* fruit flies (Diptera). Today, colour patterns found in each one of these two groups are known in great detail under various aspects, from those of the comparative morphology of pattern elements to those of developmental processes and genetics.

Surprisingly, studies on the chromatic patterns of beetles, despite the large variety of chromatic patterns found among their members, have been widely neglected. An accurate perusal of literature revealed that works dealing with interspecific evolution of patterns are very scanty. In addition, information is lacking also about the putative developmental or topological relationships between colour patterns and integument morphology, with particular reference to the role of wing veins, which were demonstrated to be a fundamental patterning device in other groups.

This research meant to move the first steps towards filling some of the major gaps in the present knowledge of evolutionary phenomena occurring in beetles colours and colours patterns.

The first section of the thesis (*Colours and chromatic effects in beetles*) deals with some aspects of colours and colour patterns which are of general interest, at level of the whole order. A review of colour-producing phenomena is presented, with particular attention to the colours of physical origin (i.e., those which stronger evolvabilty), and some original observations are presented. In the following section (*Pattern evolution and Evo-Devo aspects*) the focus is brought on aspects of the interactions between anatomy and colour patterns, for which several original observations are presented and discussed. The limited information available from the literature is reviewed, and original data and case studies are presented and discussed. Induction- or control-like effects from morphological elements towards colour pattern elements are exemplified, discussed and proposed as a major device active in the colour patterning of the integument.

In the third section of the thesis (*Structural and evolutionary aspects in* Chrysolina), the focus is brought on a circumscribed group of leaf-beetles, the genus *Chrysolina* and the allied ones. A wide survey of their colour pattern is performed, aiming at a reconstruction of the main evolutionary changes occurred in this genus. In order to achieve a necessary phylogenetic framework, which was unavailable from the literature, a morphology-based phylogeny attempt is presented, although the results did not provide a good resolution. However a few interesting considerations were allowed by the more resolved branches of the tree. In particular, similar-looking species scattered among different genera and

subgenera are recognised as belonging to a monophyletic clade, and thus their peculiar pattern (*fastuosa-like pattern*) is demonstrated to derive from a single evolutionary event.

Finally, investigations on the ultrastructure of the epicuticle allowed to recognize the morphological basis for the chromatic polymorphism found in many of the species showing physical colours, and to observe an up-to-date unknown morphological arrangement of the epicuticle, which account for the widespread comparison of black phenotypes.

In conclusion, some considerations are proposed on the large evolutionary potential of mechanism producing physical colours, and of the morphological elements capable to interact with the colour pattern.

I cannot pretend to feel impartial about colors.
I rejoice with the brilliant ones and am
genuinely sorry for the poor browns.

Winston Churchill

INTRODUCTION

Colours and chromatic patterns of insect integuments have attracted attention from researchers since long time, for reasons going from a naive fascination about their beauty to serious, challenging questions about their adaptive value, development and evolution. Nevertheless, after centuries of investigations, many old questions still await answer, and new ones keep on arising.

Chromatic patterns of often conspicuous complexity are observed across several orders of insects, especially Lepidoptera, Coleoptera, Homoptera and Heteroptera. However, research effort has been distributed in uneven manner. Butterflies (Lepidoptera), whose members display the patterns of most extreme complexity, have been the favourite subject for scientist studying insect colours and patterning since decades (Parchem et al., 2007). In more recent times, a great deal of knowledge has been also gathered around Diptera. But the colour patterns of fly body and wings cannot be regarded as particularly exciting in terms of variation and complexity, their study arising mainly as a spin-off of the choice of *Drosophila melanogaster* as a model organism.

Therefore, we have come to a quite uneven level of knowledge for the different groups of insects. Nowadays, the complex patterns of butterfly wings are known in detail under several aspects, including evolutionary and developmental ones (see Nijhout, 1991 for a broad review). Many of the earlier workers focussed their efforts on the elaboration of interpretative models, aimed at explaining the diverse patterns as different expressions of a single, fundamental architecture shared by most of the Lepidoptera and organized as a complex of independent modules. This interpretative approach is today well extablished, and is accompanied and supported by results deriving from modern genetic and developmental studies. These are shedding light on the biological processes leading to the shape of the final products, and were able to uncover relationships linking genetic information, developmental pathways and environmental influences to some aspects of the colour pattern. In addition to studies focused on the determination of the pattern at the specific level are some studies devoted to the transspecific evolution of patterns (e.g.: Brower, 1996; Descimon, 1986; Jiggins, 2001).

Some evolutionary reconstructions of traits of the colour patterns in *Drosophila* fruitflies are also available (Hollocher et al., 2000). For this group, indeed, our understandig of the genetics and development of chromatic phenomena is even better than in butterflies, and resolves into a step by step reconstruction of the developmental process details (e.g., Gompel et al., 2005).

Although it is still not known whether the genes controlling wing patterns in Lepidoptera and *Drosophila* are the same (Parchem et al., 2007), these studies were able to uncover some traits which are common to both groups, among which are the fundamental importance of wing veins as organizers of pattern (Nijhout, 1991; True et al., 1999; O'Grady and DeSalle, 2000) and the existence of a sort of prepattern marking extablished in a manner not dependent from wing veins (True et al., 1999; Reed and Gilbert, 2004).

Butterflies and fruitflies apart, studies devoted to interspecific comparisons or evolution of colour patterns are rare among other groups of insects, although a few examples can be cited, e.g. among Phasmatodea (Crespi and Sandoval, 2000), Hemiptera (e.g.: Zrzavý and Nedvěd, 1999 and references therein) and Hymenoptera, which were discussed, among others, in a notable paper by Williams (2007) on the colour patterns of *Bombus*.

Rather surprisingly, among the groups poorly covered by evolutionary studies, are the beetles. Beetles, in fact, represent the largest among the orders of insects, comprising no less than 350.000 valid species and accounting for 40% of all insects (Grimaldi & Engel, 2005). Their body and their first pair of wings are often characterized by bright colours, and the occurrence of polychromous patterns, strongly diversified from each other, is common too (Evans, et al., 2000). Patterns observed on the wings of Coleoptera do not reach the peak of complexity displayed by of butterfly wings, and commonly arise by the combination of only two colours as a rather simple series of simply shaped patches. Such a condition is typical, for example, of many Coccinellidae. Nevertheless, patterns of higher complexity can be retrieved even among beetles, most commonly within groups whose members are covered by hairs or scales (e.g.: Cerambycidae, fig. 1), but also among species owing their colours to cuticular pigments or to physical phenomena (e.g.: Chrysomelidae, figs. 2-3). In any case, whatever the degree of complexity, mechanisms controlling the production and the shaping of beetle colour patterns, as well as their evolution, are almost completely unknown.

Despite the undeniable appeal of the topic, studies dealing with the evolution of colour patterns of beetles or, at least, proposing reasoned comparisons between the intraspecific patterns of related species are very scanty. Of the older works, remarkable are Tower's (1906) detailed research on the evolution of the genus *Leptinotarsa*, containing many pages on the variation of the colour pattern in that genus, and Shelford's (1917) study on the colours of tiger beetles, focussed on the ecological and environmental factors influencing development and evolution of the patterns. Among the recent contributions providing a sound phylogenetic background to the study of evolution of colour patterns, three works can be listed, dealing with a genus of Carabinae (Okamoto et al., 2001), the whole family Erotylidae (Robertson et al., 2004) and a genus of Staphylindae (Chatzimanolis, 2005), respectively.

Consequently, our understanding of the structure of colour patterns in beetles is still very poor, as is our knowledge of the aspects of chromatic

evolution. Comprehensive studies on the architecture of colour patterns, intended as a complex of modular elements largerly independent from each other, have been largerly neglected, and the same is true of studies on the mechanisms controlling their shape or driving their evolution. At a difference with other mentioned groups, we have practically no knowledge of how patterns interact (if they do) with the integumentary morphological structures, or of how they interact with wings veins, which seem to strongly take part in pattern shaping in the better known groups.

The Coleoptera are holometabolous insects as are the Diptera and the Lepidoptera, but show some major difference in the pattern-related problems with respect to the other two orders. Different from the Lepidoptera, their pattern does not involve the hindwings (which are not in sight), but, instead, is strongly dependent on the colour and colour patterns of the fore-body (head and pronotum). Moreover, the shape of their fore wings (elytra) is not flat, but mostly convex, and the wing veins are commonly invisible on the surface.

My research was meant to identify the major gaps in the current knowledge about the evolution of colour patterns in Coleoptera, and to move the first steps towards filling some of them.

I started with a broad, critical perusal of the literature. This is mirrored by the first section of this thesis (*Colours and chromatic effects in beetles,* p. 13), where some aspects of pigmentary colouration are briefly reviewed, followed by a wider review, including original observations, about the production of physical colours, which appear of notable interest for their capability to produce quick major evolutionary changes of the overall body aspect. In the next section (*Pattern evolution and Evo-Devo aspects*, p. 25) the focus is brought on aspects of the interaction between anatomy and colour pattern. The limited information available from the literature is reviewed, and original data and case studies are proposed and discussed.

Against this background I moved to the study of the evolution of the colour patterns in a model group (*Structural and evolutionary aspects in* Chrysolina, p. 39), which was investigated also taking into account the notions gathered in the previous, more extensive, survey. The study group was identified in the Chrysomelidae of the genus *Chrysolina*, together with some close genera whose separate identity is a matter of debate. This group seemed to fulfill needs both intrinsic and logistic (i.e. access to some important collections of Chrysomelidae). The genus *Chrysolina*, in fact, is notable for its huge chromatic variations, thus providing a large array of phenotypic plasticity examples, whose comprehension is a key challenge in evolutionary biology. These, encompass both pigmentary and physical colours, as well as both monochromous, polychromous and differently patterned species. In addition, this group is noteworthy for expressing its chromatic variation at a low taxonomic level, given the great diffusion of chromatic variations even within populations of a single a species.

A case of actually impressive polymorphism is represented by species of the genus *Oreina* and close *Chrysolina* species. Members of a single species, and

even of a single population can show extreme variations, commonly ranging from brilliant and uniform colour, to polychromous striped specimens; less commonly, black phenotypes are known too.

The expression of such a polymorphism is noteworthy even among beetles, and call for explanations accounting both for its biological significance and the related developmental processes. According to the modern perspective of the evolutionary developmental biology (evo-devo), in fact, one must keep in mind that the phenotype is the contact point between the drive of the selective pressure, which is imposed by the interactions with the environment, and the results of a developmental process, which can impose constraints and, eventually, bias further evolution.

MATERIALS AND METHODS

Sources of study material

The present research was based primarily on the Chrysomelidae collection of the Museo Civico di Storia Naturale di Verona (in particular for the examination of long series of European Chrysomelidae) and on the private collection M. Daccordi (Verona) (in particular for the examination of exotic Chrysomelinae). In addition, a minor number of specimens of Chrysomelidae and other families of Coleoptera have been sampled from the collection of the Museo di Storia Naturale, Venezia and from the private collections of A. Minelli (Padova) and M. Uliana (Codevigo, PD). On the whole, about 40.000 specimens of Chrysomelinae have been evaluated, mostly belonging to *Chrysolina* and allied genera, but also including representatives of all Chrysomelinae genera regarded as valid and over 95% of the Palaearctic species.

Occasionally, samples of fresh Chrysomelinae specimens were collected, their use however being eventually relevant only for the observation of the muscular system on fresh specimens of *Leptinotarsa decemlineata*.

Microscopy and imaging

Light microscopy observations were performed with a Leica MZ12.5 stereoscopic miscroscope with magnification ranging from 8x to 100x, either with reflected light (for whole specimens or elytra) or with transmitted light (for elytra or pronotum integument only).

Microscopic photos were taken with a Leica DFC420 camera mounted on the microscope; whole specimen photos were taken with a Pentax K10D digital camera equipped with a Sigma 105 mm macro objective. Photos were edited with the Photoshop CS2 software. In addition, in order to get well focused images of considerably thick subjects, the image stacking technology was used: stack of photos of the same subject were taken with focus on different focal planes and then processed into a single image trough the Combine MZ software (Hadley, 2008).

For observations and photos under reflected light, specimens were uniformly illuminated with a fluorescent ring lamp either attached to the microscope objective or placed around the beetle. Light diffusion was strongly enhanced using a cylindric screen of white semitransparent plastic material, placed as close as possible to the beetle.

Observations and photography of elytra venation were carried out with transmitted light on samples either dry or rehydrated with 70% ethanol. As for the source of light, the lighting system of the microscope stage revealed too dim to pass through the thicker elytra, and a Leica L2 cold light source was often needed. In this case, the light beam was directed upwards across the subject with the help of a mirror. The slide carrying the elytra was covered with opaque adhesive tape in order to moderately diffuse the beam, and tightly darkened around the subject in order to protect the observer's eyes from the violent source of light and to allow the visual perception to fit on the dark tones of the subject without being dazzled by the surrounding light field.

TEM observations were meant to study physical colours not changing after death and dehydration, therefore they were carried out without problems on elytra samples taken from dry museum specimens. Ordinary treatments of fixation and postfixation were therefore unnecessary. Samples were directly dehydrated in ethanol alcoholic series, embedded in epossidic medium and cut into sections 80-110 nm thick and contrasted for 20' with uranyl acetate in alcoholic solution and then for 7' with lead citrate. Observations were performed with a Hitachi H600 microscope.

Phylogenetics

Phylogenetic analysis was performed under maximum parsimony method with TNT software (Goloboff et al., 2003) on a 2.67 Ghz processor with 2 MB RAM (1 MB was dedicated to TNT during phylogenetic reconstruction). The software choice was done taking into account its high computational speed and its unique capability to handle continuous characters, such as measurements and morphometric indices, without the need to code them as arbitrarily discrete characters.

The ingroup was selected from over 400 species of *Chrysolina* and allied genera. Coding was subsequently limited to 59 taxa, representing 52 species and 47 subgenera of *Chrysolina* (plus one *incertae sedis* species), 5 species and 3 subgenera of *Oreina*, 1 species of *Semenovia*, 1 species of *Crosita* (see *Appendix 1*, p. 100). The choice of this taxon set was made taking the following into account: availability of collection specimens of both sexes to dissect and/or to handle in a non-conservative way, inclusion of the highest possible number of subgenera, especially of subgenera particularly relevant from the chromatic point of view. Other conditions being favourable, for each subgenus the type species was chosen. Five pairs of species belonging to as many subgenera were included in the ingroup, as a basic test to evaluate the phylogenetic hypothesis produced.

As an outgroup was chosen *Leptinotarsa decemlineata*, a species belonging to a Neotropical lineage allopatric respect to the considered ingroup and since long regarded as distinct from the *Chrysolina* lineage (cfr. Daccordi, 1994), a condition which was confirmed by its position in recent phylogenies (Gómez-Zurita et. al, 2007).

The phylogenetic reconstruction was performed chiefly on morphological characters directly verified on the studied specimens; however the building of the character matrix revealed highly problematic and much more time consuming than expected. As a preliminary work, a wide screening including over 210 characters or characters coding systems was performed. A high number of characters was subsequently discarded *in itinere*, due to the absence of phylogenetic information (autapomorphies, e.g. toothed last tarsal article of *Chrysolina fastuosa*, denticulate mandibles of *Chrysolina varians*) or, most commonly, due to the impossibility to recognize sufficiently discrete states (e.g.: shape of the periocular sulcus, shape of the mandibles) or to the high interspecific variability of the character taken into account (e.g.: presence of the apical medial tubercle of the prosternum).

The final matrix (*Appendix 3*, p. 105) was composed of 90 morphological characters (*Appendix 2*, p. 101). Of these, 15 are quantitative morphometry-based characters and 75 are qualitative characters. Among the qualitative characters, 39 multistatum characters have been defined, of which 18 are additive, due their belonging to an apparent morphocline. In addition, diploid chromosomic number, plant host family, and class of defensive chemical compounds, whenever available, were coded as additional characters from literature data. On the whole, a total of 95 characters were definitely processed with the software.

Evaluation of chromatic conditions and level of approximation

A survey of over 35.000 specimens of *Chrysolina* and allied genera allowed to recognize a few distinct chromatic conditions, not exclusive of each other. The observation of chromatic characters was limited to the dorsal surface.

The distribution of each of the chromatic conditions within the studied group was then outlined at a subgeneric level using four degree of presence:

(a) observed as most common condition within the whole subgenus;
(b) regularly observed within one or more species;
(c) observed as single aberrant specimens within one or more species;
(d) never observed;

The choice to approximate the study to a subgeneric level was done as a temptative to efficiently cope with such a species-rich taxon, therefore having no possibility to undertake a detailed phylogenetic reconstruction of over 400 species, but trying anyway to mantain a comprehensive evolutionary view of the same.

From an phylogenetic point of view, this correspond to consider each subgenus as monophyletic. This assumption, although a work hypothesis, is not unwarranted. Monophyly of several subgenera, infact, is suggested by the strong morphological similarity observed among their members, which often can be distinguished on the basis of fine morphological or edeagic characters only; in addition, the limited phylogenetic studies performed until now on this group, always suggested monophyly or, in a single case, paraphyly of the subgenera taken into account (see also *The Chrysomelidae: a taxonomic and phylogenetic outline*, p. 39). However, the subgenus *Pezocrosita*, was discarded from the analysis since it appeared to be strongly heterogeneous and most likely polyphyletic.

COLOURS AND CHROMATIC EFFECTS IN BEETLES

Colours producing devices: pigments

Pigmentary colours of beetles occur either embedded in the cuticle or in the underlying hypodermal cells (Crowson, 1981) The most common pigments are melanins, a large and heterogeneous family of polymerized quinone derivatives of phenolic compounds (True et al. 1999), whose production depends on the availability of tyrosine. There are two classes of melanins, eumelanins (brown to black) and phaeomelanins, which are yellow to reddish (Berthier, 2007; True et al., 1999). Beside melanins, carotenoids and ommochromes are also common in beetles, where they mostly locate in the cuticle (Crowson, 1981). They are characterised by a yellow to red appearance and are spread across different families. It seems that, in some instances at least, these pigments are not syntethized by the beetle, but acquired from the food. This is the case, for example, of Cassidinae leaf-beetles, whose pigmentary colour was indirectly observed to be dependent on the foodplant: adult beetles may vary in colour across the seasons, depending on the chemical condition of their foodplant (Jolivet, 1994). A direct evidence of acquirement of β-carotene from leaves of the foodplant was produced for at least one member of this subfamily, *Cassida murraea* (Jolivet, 1994). The presence of other classes of pigments, in particular of biliary pigments (producing green to blue hues) is supposed, but not yet ascertained (Crowson, 1981). Actually, green and blue colours of non-physical origin are rather rare in beetles, but they occur among some of the most colourful groups, such as Erotylidae and Chrysomelidae Chrysomelinae (e.g., *Platyphora gratiosa, Platyphora nigroguttata*) and Clytrinae (e.g., *Diapromorpha trifasciata*).

Darkening and sclerotisation of the cuticle

The relationship between the sclerotisation (hardening) of the insect cuticle and its darkening is known since long time. To date, despite several investigations on the sclerotization of the insects cuticle, this process is still not completely understood (Andersen, 2005; True et al., 1999).

However, sclerotisation and darkening were supposed to be independent long ago (cfr. Goodwin, 1952) and recognised as distinct processes since

Andersen (1974) demonstrated the cohexistence, in insects (such as the beetle *Tenebrio molitor*), of two distinct mechanisms of cuticle hardening (probably associated to different enzymes), which use the same substrate (dopamine, a derivate of tyrosine) but are active on different parts of the same molecule. One of those enzymes acylates the substrate to N-acetyldopamine (NADA), the other acylates it to N-β-alanyldopamine (NBAD) (Andersen, 2005). Both of the resulting compounds will serve as precursors of the sclerotization process, but the first will produce a pale or colourless cuticle, while the second will sclerotise and at the same time darken it to a dark brown colour (hence the common but misleading use of the term "tanning" to address both to the process of sclerotisation and the process of darkening). The two mechanisms can anyway work simultaneously and the variable ratio observed between their activities can account, at least partly, for the different hues of brown observed on beetle integument.

A second common compound reponsible for the dark colour of the cuticle (in particular, black) is melanin, whose metabolic pathway is almost identic to that of the two mentioned compounds which take part in the hardening process. Melanin, in fact, is produced starting from the same substrate (dopamine, which is oxidated by phenoloxidase) or by its immediate precursor (DOPA), but is supposed to play no role in the hardening process (Andersen, 2005), as suggested by observations in different organisms such as albino mutations in grasshoppers, whose cuticle lack melanin but has the same mechanical properties as the wild type (Malek, 1957).

Among beetles, the independence of darkness from sclerotization can be also inferred from the existence of weakly pigmented (but *not* soft bodied) species, a phenomenon particularly common among species inhabiting caves (e.g. Carabidae Trechinae), or soil (e.g. Staphylinidae Pselaphinae, Scydmaenidae), but also found (although far less commonly) among species with nocturnal free-living habits, such as European beach tenebrionids of the genus *Xanthomus*, whose pale yellow integument is semi transparent. The absence of blackness in species that do not need to protect themselves from sunlight also suggest that the darkening of the cuticle through melanine production is not just a by-product of the sclerotization system, but is an expensive trait which is positively selected and can be disposed of if unnecessary (see also Crowson, 1981).

In conclusion, the link between hardness and darkness of the cuticle appear to be labile and to depend on the involvement of a compound (dopamine) which can syntopically produce three different derivates: one enhancing only darkness (melanine), one enhancing only hardness (NADA), one enhancing both of them (NBAD).

Physical colours

Phenomena leading to the perception of colours in the absence (or regardless) of the presence of pigments are widely distributed in nature and among beetles and other insects as well. Colours observed are usually referred to as "structural

colours" or "physical colours", whereas the anatomical colour-producing devices are described as or as "photonic structures", which may be also defined as "optically active" when they cause polarization of the incoming light.

Basically, a structural colour is produced by the interaction between the light and periodic nanostructures capable to selectively interact with wavelengths in the range of visible light (380-750 nm). The size of the nanostructure period is in the same order of magnitude of that of the wavelength produced.

The existence of physical colours among insects, observed since long time, was regarded as a specific phenomenon, distinct from the pigmentary colours, since Hagen (1883), who introduced an explicit terminology to distinguish colours produced by pigments from colours produced by physical structures. From the nineteenth century to present days, the brilliant and often iridescent colours shown by insects integument have elicited the interest of a number of researchers. In very recent years, physical colours found among beetles have been receiving increasing attention, especially from the structural/architectural point of view (including the search for biomimetic materials, e.g. Lenau & Barfoed, 2008), and some new phenomena were discovered (e.g.: Parker et al., 2003).

In beetles, there are three main classes of mechanisms producing physical colours: multilayer reflectors, three dimensional photonic crystals and diffraction gratings. These correspond to the complete series of mechanisms producing physical colours observed among insects, with the only exception of the Tyndall blue effect, a phenomenon which is known to occur in various insects such as dragonflies (Mason, 1926), grasshoppers (Filshie et al., 1975), lepidopteran larvae and adults (Byers, 1975; Huxley, 1976), but has never been found in beetles. These main mechanisms are here briefly reviewed, together with a discussion of some meaningful examples, study cases, or modifications of the basic mechanisms.

Colours produced by photonic structures of Coleoptera are generally very stable in time (but see *Reversible colour change*, p. 23) and if not exposed to stressful conditions, they may last almost forever (Seago et al., 2008). An exceptionally well preserved fragment of multilayer belonging to a beetle aged 50 million years was studied in detail by Parker and McKenzie (2003), although the assumption of preservation of the original colour cannot be demonstrated. Nevertheless, complete specimens of fossil beetles (such as Chrysomelidae, Buprestidae and Lucanidae) showing well preserved photonic structures which are reliably deemed to show the original colours are common in the limestones of Messel (Germany), about 49 million years old (Lutz, 1992). A much less aged, but uncontrovertable, observation of long-term stability of such a structure is given by Vigneron et al. (2006) and Adachi (2007), who mentioned well preserved elytra of *Chrysochroa* jewel beetles (Buprestidae) used for decorative purposes in a Japanese temple and aged about 1300 years. However, there are evidences that exposition to sunlight for a few years may strongly affect or delete physical colours, possibly as an effect of UV radiations (Seago et al., 2008).

Physical colours: multilayer reflectors

Multilayer reflectors are very common devices producing structural colour in beetles. Their presence is commonly suggested by the observation of bright, saturated colours, often described as “metallic” or “vitreous”, whose appearance is strongly dependent on the angle of observation: an increase of this angle, measured as a shift from the normal to the integument surface, produces a shift of the perceived colour towards shorter wavelengths (e.g., Neville and Caveney, 1969; Berthier, 2007). As a consequence of this “point-of-view dependence”, convex beetles exposed to a directional light (e.g.: direct sunlight) will appear colourful only limited to a small surface, but mostly dark on the rest of the body (fig. 19b, c), while a diffuse illumination will lead to the perception of a polychromous body even if the colour producing device is grossly uniform throughout the entire integument (fig. 4).

A multilayer reflector is made of a series of layers usually alternatively composed of two different materials of lower and higher refractive index. Interaction with light occurs when the spacing between layers approaches one quarter the wavelength of visible light. Under these conditions a constructive interference phenomenon occurs (hence the definition of interference colours for the colours thus produced), with the produced colour being dependent on the thickness and the refractive index of each layer according to the equation W=4IrT (W: colour wavelength, Ir: refractive index, T: layer thickness). Hence, a stack of layers all having the same optical thickness (IrT) will produce a constructive interference for the same wavelength (fig. 6), and their combined action will give rise to a more intense and brighter colour; conversely, a stack of layers of different optical thicknesses (usually with the same Ir but a different thickness, fig. 7) will produce different wavelengths and thus a less pure colour (see also *Broadband reflectors*, p. 18).

Less commonly, interference colours can arise from particular multilayer reflectors, the so-called “Bouligand structures” (cfr. Lenau and Barfoed, 2008), whose particularity is that these are made of the same chitinous material, rather than of two alternating substances. Within each layer, chitin fibrils lie parallel, but the arrangement of fibrils in each layer is twisted with regard to the fibrils of the adjacent layers. Twisting occurs in such a regular way that, given a stack of layers, the arrangement of the fibrils along the vertical axis becomes helicoidal. As a result of this structure, light is circularly polarized, a phenomenon of selective reflection occur and hence interference colours are produced. The largest reflectivity for a given colour is obtained when the optical thickness of half a period is equal to the wavelength of the colour (Lenau and Barfoed, 2008). The existence of polarization phenomena in the beetle cuticle was observed by Michelson (1911) and treated by Gaubert (1924), but the first detailed study on the structures responsible for these phenomena was produced much later by Neville and Caveney (1969). To date, despite helicoidal arrangement of chitin fibrils being very common among insects optically active stacks of chitine layers were observed only among the Scarabaeoidea, with several species scattered across different families such as Melolonthidae, Rutelidae, Cetoniidae, and

Scarabaeidae (Goldstein, 2006; Seago et al., 2008); it is also interesting to observe that in the same families interference colours may arise both from ordinary multilayer reflectors and from Bouligand structures (Neville and Caveney, 1969).

Both kinds of multilayered structures described here extend parallel to the surface of the elytron (the periodicity thus develop perpendicularly to this surface) and may be located in different cuticular layers. Most commonly, they are found in the epicuticle (Chrysomelidae, Carabidae, Meloidae), but are typically observed in the exocuticle among the Scarabaeoidea (Neville & Caveney, 1969), and occasionally in the endocuticle among the Chrysomelidae (Seago et al., 2008, see also *Reversible colour change*, p. 23). Some works dealing with the ultrastructure of multilayer reflectors seem to make unwarranted assumptions about the anatomical placement of their subject of research, such as the recent works on *Chrysochroa* (Buprestidae), where the multilayer is assumed to be exocuticular despite being placed in the outer 2 μm of the elytron thickness (Vigneron et al., 2006; Adachi, 2007). The distinction is relevant, since chemical composition of the two layers is different.

In general, knowledge about the precise chemical composition of the layers forming cuticular photonic structures is rather limited. However, while chitin is regarded as the main component of exocuticular laminations - even though relevant amounts of uric acid have been detected in Rutelidae (Caveney, 1971) - it is completely absent from epicuticle (Richards, 1951). To date, although these are very widespread photonic structures, epicuticolar multilayers have a poorly known chemistry, due to the complexity of the epicuticle itself (which is composed, in addition, of other different layers) and the extreme thinness of the complex (a stack of layers is usually in the range of 1 μm), which makes difficult to investigate on it (Richards, 1951). Epicuticle laminations were interpreted as alternating layers of proteins and lipids by Neville (1975), while the presence of melanoproteins within the laminations of tiger-beetles epicuticle was inferred by Schultz and Rankin (1985) and, with the same method, by Liu et al. (2008) in *Chlorophila* Tenebrionidae, but an unquestionable demonstration has never been produced. Vigneron et al. (2006) suggested the presence of thin air films separating epicuticular layers in *Chrysochroa vittata*, but Adachi (2007) and Noyes et al. (2007), respectively working on the related *Ch. fulgidissima* and *Ch. rajah*, did not provide any evidence for this hypothesis, demonstrating however the presence, within the multilayer reflectors, of pores with a radius of 0.25-0.30 nm and capable of adsorbing fluids (Adachi, 2007) and the existence of two kind of layers with a refractive index different from that of the air. Despite the poor chemical knowledge outlined, from a strictly mechanicistic point of view, to perform an analysis on the optical properties of the discussed multilayers only requires an adequate knowledge of the thickness of the layers and of their refractive index, for determining which a reliable method was recently developed by Noyes et al. (2007).

Multilayer reflectors are the most common mechanism producing structural colours in beetles (Seago et al., 2008) and are widespread across several families of different suborders. In particular, they are extremely common among

Carabidae, Buprestidae, Tenebrionidae and the superfamilies Scarabaeoidea and Chrysomeloidea.

Broadband reflectors

Among the chromatic phenomena produced by multilayer reflectors are some light-coloured integuments with a strong metallic appearance, notably those belonging to the Rutelidae of the genera *Chrysina*, *Plusiotis* and *Anoplognathus*, which may show a stunning resemblance with pieces of polished silver or gold (fig. 5). Such an exceptional appearance is achieved by the reflection of most (virtually, all) wavelengths. This effect will happen when the multilayer is composed by a high number of layers of different thickness (fig. 7), producing each a constructive interference for a peak wavelength different from the others. Although this optical phenomenon will happen regardless of the distribution of the layers' thicknesses (see Parker et al., 1998), among beetles only chirped multilayers are known, that is, a stack of layers whose thickness gradually increases (e.g. Parker et al., 1998 for Chrysomelidae Cassidinae and Rutelidae) or decreases (e.g.: Vigneron et al., 2007 for Chrysomelidae Cassidinae) approaching to the surface. Conversely, among the beetles stacks with chaotic thickness such as those observed in the skin of trichiurid scaleless fishes (McKenzie et al., 1995) are not known.

"Pointillistic" colour mixing

Although interference colours appear most often bright and conspicuous even from a macroscopic point of view, they can also serve as a source to produce a dull appearance. This phenomenon, usually referred to as "pointillistic colour mixing" is mainly known for its occurrence in a large number of Cicindelinae tiger beetles (such as *Cicindela* and allied genera, figs. 10-12) and was recently described in *Chlorophila* (Tenebrionidae Lagriinae) (Liu et al., 2008), beside being known also for butterflies. The occurrence of this phenomenon is surely uncommon among beetles, however I can add (pers. obs.) a further example of it on the integument of some *Elaphrus* species (Carabidae), notably on those of *E. riparius* (figs. 13-15), which, interestingly converge with cicindelids also in the general habitus and in their behaviour of day-active sight-hunters.

In these beetles the cuticle surface is densely microsculptured, being covered with small hexagonal pits (diameter in the range of 10-15 μm both in Cicindelinae and in *Chlorophila*, as large as 20-50 μm in *Elaphrus*) which modify the architecture of the epicuticle. Thus, the thickness of the multilayer responsible for the colour is not uniform across the surface: at the bottom of each pit the strata are thinner than in the ridges between. As a consequence, each of these two areas reflect a different peak wavelength, and, under magnification, it will appear of a distinct, bright colour. However, under the naked eye, small portions with different colours blend in a single, much duller colour. The production of different wavelengths which get mixed in a single macroscopic colour is further enhanced

by the sculpture itself, which breaks the surface into differently oriented plans and makes the perceived colour less dramatically sensitive to the angle of observation. In Cicindelinae, in addition, small patches of alveoli (40-80 μm across) with colouration different from that of the neighbouring ones may be observed (Schultz and Bernard, 1989), strongly enhancing the production of mixed colours in small discrete areas. The small size of the differently coloured points is determinant to achieve the merging effect. Similar combinations of sculpture and colouration, in fact, may be retrieved also in the integument of other beetles, such as the Neotropical species genus *Omocerus* (Chrysomelidae Cassidinae) (figs. 16-18), where however pits and ridges are much larger (diameter ca. 500 μm), and thus perceived as distinct elements (pers. obs).

Dull colours produced by pointillistic colour mixing are usually interpreted as a camouflage device, a reasonable hypothesis further supported by the observation that the elytral colouration of some tiger beetle species varies geographically, matching the local soil colour (Schultz, 1986).

Physical colours: diffraction gratings

This kind of optical systems is notably less common than the multilayer reflectors and produces a structural colour far less conspicuous in appearance. The presence of a diffraction grating is usually recognisable because of the silky shine shown by the integument surface, associated to the production of a rainbow-like reflectance which moves with changing the angle of observation. Diffraction colours produced this way are usually quite faint, in particular if compared with the bright structural colours produced by other mechanisms.

From the structural point of view, a diffraction grating is a nanoscale array of parallel ridges, grooves, rows of denticles or other similar high-density linear structures having a typical density around 1000 lines/mm (Anderson and Richards, 1942) and thus capable to diffract light in its constituent wavelengths, reproducing the rainbow spectrum. In this case, the plane with periodicity is parallel (or, better, coincident) with the surface of the elytron. Iridescence arising from these structures appears in the form of one or more ordered spectra directed perpendicularly to the direction of ridges, with additional spectra being less bright and possibly lacking longest wavelengths.

A peculiar diffraction grating was described by Seago et al. (2008) for Neotropical Nitidulidae of the genus *Pallodes*, which have two diffraction gratings intersecting at a right angle and originating two spectra, one longitudinal and the other transversal.

An internal diffraction grating system was assumed to be responsible for the colour of some metallic beetles scales by old authors (cfr. Onslow, 1921), however this hypothesis, already questioned by Onslow (l.c.) appears now to lack foundation in the light of recent acquisitions on the ultrastructure of beetle scales (see *Physical colours: photonic crystal structures and scales*, p. 20).

This optical phenomenon, although uncommon, is scattered among few beetles families and seems to be particularly common in Melolonthidae Sericini

(with ultrastructure studied by Kim and Kim, 2003) and Phalacridae (Hinton et al., 1969), however its contribution to the macroscopic appearance of the insects is rather poor and its presence may easily go unnoticed.

Among other insects diffraction gratings is generally rare and, beside beetles, was until now recognised only in mutillid wasps (Hinton et al., 1969; Vukusic and Sambles, 2003).

Quasi-ordered scattering

This phenomenon arises when identically sized nanoscale light-scattering structures are evenly spaced on the surface of the elytron or embedded within a transparent matrix, but are not ordered in regular rows as in an ordinary diffraction grating. The colour produced is a non iridescent diffuse bluish-green. This phenomenon is fairly uncommon among beetles and has been reported only very recently (Seago et al., 2008).

Physical colours: photonic crystal structure and scales

This kind of structural colour producing device, already known for butterflies (Ghirardella, 1989; Argyros, 2002), was discovered in the beetles only in very recent years by Parker et al. (2003), who recognised this phenomenon in the elytral scales of the entimine weevil *Metapocyrtus* sp., originally misidentified as *Pachyrrhynchus argus* (Seago et al., 2008). From the macroscopic point of view, the presence of this photonic structure is suggested by bright saturated colours lacking iridescence (at least on a macroscopic scale), i.e. the perceived colour is independent from the angle of observation and illumination (fig. 19). This peculiar condition was already regarded as unusual by earlier authors, such as Onslow (1921), who first deemed worth of explanation the peculiar appearance of scale-covered curculionids of the genus *Cyphus* and *Eupholus*.

To date, only few other observations of photonic crystals among beetles were produced, all belonging to the family Curculionidae (*Eupholus*: Vukusic, 2007; *Pachyrrhynchus* spp.: Seago et al. 2008; *Cyphus*: Berthier, 2007; *Lamprocyphus*: Galusha et al., 2008). The scarce number of examples, however, is surely due to lack of research and this kind of ultrastructure will probably turn out to be much more widespread.

In all known cases, the structures which are responsible for colour production are not located within the integument, but within the covering scales: each scale contains a three-dimensional, highly ordered lattice of nanoscale spheres, whose spatial arrangement is analogue to that of regular mineral crystals: the observed arrangement can be hexagonal (as in the opal), or cubic (as in the diamond). Colour is produced by constructive interference, i.e. by the same phenomenon exploited by the multilayer reflectors previously described. However, the macroscopic iridescence is here strongly reduced by the optical device being active in three dimensions, thus producing a considerably smaller

shift of the perceived colour (in *Metapocyrtus* a shift as large as 70° in the angle of observation leads to a 140 nm shift in the observed peak wavelength).

In addition, the macroscopic reduction of the iridescence appears to be strongly enhanced by the chaotic structure observed in each scale at a higher level of organization: the scale, in fact, is not composed of a single crystal, but contains several closely packed crystalline domains with different orientation, that is, several pieces of highly ordered structures each oriented randomly and independently from the others. As a consequence of this architecture, from a given angle of observation each scale will produce a mix of different wavelengths, which blend together into an average colour at lower magnification. This unordered structure is probably a positively selected condition, being useful to enhance a diffuse reflection device which produces a single macroscopic colour independent of the viewing angle (Vukusic, 2003; Vukusic and Sambles, 2003; Galusha et al., 2008).

The characteristics of crystalline domains are still poorly investigated, but they are reported to be usually few microns in diameter (Vukusic, 2007; Galusha et al., 2008), a condition which surely fits with the production of a diffuse uniform colour. However, preliminary observations on a wider range of subjects suggest the existence of a wider range of sizes even within closely related species, leading to different output in macroscopic appearance. Scales of *Eupholus* curculionids, where photonic crystal have already been observed (Vukusic, 2007), may show abrupt internal discontinuities in reflectance (referrable to different crystalline domains) of size notably different across species, as exemplified by the comparison between *E. schoenherri* (fig. 20) and *E. chevrolati* (fig. 21), the latter showing much larger domains giving a gem-like appearance to its scales (pers. obs.).

The presence of several multiple crystalline domains does probably apply also to the polychromous scales of *Entimus imperialis* (Curculionidae), whose differently coloured sectors were first addressed by Dimmock (1883). Indeed, the sparkling appearance of *E. imperialis* has been the centre of a debate and source of fame for this Amazonian weevil since centuries: it was the first beetle to elicit interest for the colour of covering scales, as it was mentioned by old authors such as Drury (1773) and Lindenberg (1777, 1780; cfr. Dimmock, l.c.). Dimmock's contribution is of historical interest as this author demonstrated that the observed colours are of physical origin, and produced (unconsciously) the first account of multiple crystalline domains within a beetle scale. *Entimus* scales were later investigated by Michelson (1911), who referred the bright colours to a diffraction grating contained within the scales, and by Onslow (1921), who deemed Michelson's theory unsatisfactory. No recent studies have been produced to propose a definitive explanation for the colours of the scales of this species, and the polychromous condition described (correctly) long ago is still today unresolved matter. Anyway, in the light of observations available for other weevils, it seems a reasonable hypothesis that the discontinuities observed within each scale have to be referred to multiple crystalline domains (or, at least, to multiple domains of photonic structures). In this case, the size of each domain

would be remarkable, as each scale (about 160-180 μm long) would contain only 2-6 domains (figs. 22-23), each of size in the order of 100 μm (pers. obs.), against tens or hundreds of smaller domains observed in each scale of *Eupholus* and other weevil species. These large units are thus responsible for the exceptionally shining appearance of *E. imperialis* (also called "Brazilian diamond beetle"), which is well perceivable at close inspection with the naked eye (fig. 22). In addition, the wide range of colours produced by a single scale is also noteworthy, since scales of other investigated beetles usually produce an almost monochromous reflection. Consequently, it seems likely that each domain within the scales of *E. imperialis* may have geometric properties of its own and independent from those of the neighbouring ones, contrarily to *Lamprocyphus augustus* where different crystalline domains were demonstrated to have the same structure (Galusha et al., 2008).

Apart from structural characteristics of the scales, it should be noted that the chaotic scattering of microscopic polychromous areas tightly packed together leads to a phenomenon analogous to the pointillistic mixing of colours described for the multilayer reflectors of Cicindelinae and *Chlorophila* tenebrionids. The perception of the "mixing" effect is dependent on the size of the single coloured elements, being more effective in species with small domains.

Tridimensional non-crystalline structures and whites

Although only few recent studies are available on the ultrastructure of photonic structures contained within the scales of beetles, an interesting variety of internal arrangements responsible for different structural colours has been unveiled, apart from the tridimensional highly ordered lattices previously mentioned.

Hoplia coerulea is a Western European beetle whose males are covered with scales of a brilliant light blue colour. This highly unusual appearance called since long for the researchers' attention and the first structural observations were produced over a century ago by Dimmock (1883), who demonstrated that the colour is not due to pigments and provided a first rough description of the internal structure of these scales. The ultrastructure and the physics of *Hoplia coerulea* scales were later analyzed with modern microscopy techniques by Vigneron et. al. (2005), who provided a detailed description of the ultrastructure and an explanation of the physical phenomena involved. Each scale contains about 20 chitinous layers, each of them covered with thick longitudinal rods having a similar orientation across the different layers. This system mainly works as a photonic multilayered structure (peak wavelength about 448 nm, responsible for blue), but rods produce a tridimensional contribution, accounting for the scarce variability of the colour under non-zenithal view, and thus quite similar to a tridimensional lattice in the output.

Particular arrangements of the chitinous structures contained within the scales may lead to the production of white colours, sometimes particularly brilliant or pearlescent, basically as a result of the random scattering of all wavelengths in a way similar to that described for the chirped multilayers of silver

beetles. This phenomenon was observed in the longhorn beetle *Prosopocera lactator* (Lamiinae) (Seago et al. 2008), with scales containing a network composed of irregular ball-and-stick structure, and was studied in detail in a species of *Cyphochilus* (Melolonthidae). In the latter, the scales are filled with a completely random network of filaments producing a white of exceptional strength and purity (figs. 8-9), having a saturation as low as 6.2% on the dominant wavelength (Vukusic et al. 2007).

White markings on beetle integument are commonly produced by scale covering or pilosity (Dimmock, 1883) which may be sometimes inconspicuous due to the exceedingly small size and very dense arrangement (e.g., members of Cetoniidae Goliathinae such as *Goliathus* and *Ranzania*); among the exceptions, a notable one is represented by the Cicindelinae, whose whitish markings on elytra are not produced by phaneres.

Tower (1903) listed some North African beetles ("*Arthia punctata*, *Graphypterus serrator* and *Scaeitus polyphemus"*) whose white scales are supposed to owe their colour to a "white substance" contained within; however, this statement is not referenced and seems to be unwarranted and not supported by other authors. To date, the white colour of insect scales is commonly referred to optical phenomena, as demonstrated by data gathered about species investigated. White pigments (leucopterine and isoxanthopterine) are indeed known to occur among insects, but they are rare and mostly found among Pieridae butterflies (Berthier, 2007).

Reversible colour change

The capability to reversibly change the colour of the integument is a rare occurrence in insects. Among the beetles, it was first observed in Cassidinae leaf beetles by Mason (1929) and later in *Dynastes hercules* by Beebe (1947). To date, this unusual characteristic has been further recorded only for a few other species of Cassidinae, reviewed by Jolivet (1994), but it is not known to occur in beetle groups other than the mentioned ones.

Reversible colour change in *Dynastes hercules* is dependent on the humidity level of the hair: its increase beyond 75% up to 100% will progressively turn into black the yellowish colour observed in drier condition (Rassart et al., 2008). This phenomenon can be observed on live as well on dead specimens, and even on a detached elytra and on a limited area of the elytral surface when exposed to a humidity level different from that of the surrounding area. In addition, the colour does not seem to be affected by environmental stimuli conveyed to the living beetle (Hinton and Jarman, 1973), thus appearing to be a completely passive phenomenon.

Conversely, colour switch in Cassidinae leaf beetles appears to be active or, at least, elicited by a stimulus conveyed to the alive animal and not dependent from any physical parameter of the environment (Jolivet, 1994). A detailed case study has been recently proposed by Vigneron et al. (2007) for the Panamian species *Charidotella egregia*. *In vivo* experiments demostrated that this species

can switch its colour from gold (resting state) to red (excited state) in about two minutes as a response to nearly any sort of disturbance (touch, blow, etc.), including "stressful" events such as a rainstorm and copulation. Cases of beetles spontaneously and continuously changing their colour even in the absence of external stimuli have also been observed (Jolivet, 1994).

The switching colour mechanism was elucidated in detail and is similar in both groups: the responsible structure is a thick porous cuticular layer, overlaying a pigmented layer. The colour state perceived by the observer is determinated by the "wetness" of the porous structure, whose variations deeply modify its optical properties. In *Dynastes* the porous structure is quite like a sponge and, when the lacunae are filled with air, a strong reflectance is produced, due to the difference between the refractive index of air and chitin; conversely, when it is filled with water the two refractive indices are more similar, the reflectance is much lower and the "sponge" looks transparent, allowing to see the underlying black pigment (Hinton and Jarman, 1973; Rassart et al., 2008). In *Charidotella* the mechanism is similar but for the fact that the multilayer becomes capable to interact with the light when filled with fluid and becomes inactive when dry; however, the colour change occur without swelling or shrinking of the integument, as believed or reported by previous authors (e.g.: Mason, 1929; Srinivasarao, 1999; Berthier, 2003).

Along with the described switching capability, several Cassidinae beetles are known to show physical colours fading with death but not capable to switch in life (Jolivet, 1994), in a way similar to what happens with the members of the Australian Chrysomelinae tribe Paropsini. Several genera of Paropsini are indeed known for their bright patterned colours, apparently referrable both to pigments and physical phenomena (golden metallic mirror-like patches), which quickly fade after death and can be temporarily restored by soaking the beetle with different media (Selman, 1994). Although no ultrastructural data are known, the described phenomenon is easily referrable to a multilayer or spongy layer whose optical properties vary with the degree of hydratation, as in the Cassidinae. However, although Paropsini are known to change their pigmentary pattern with age, no reversion of the physical colour state has ever been recorded.

The putative adaptive value of the colour switching capability is completely unclear. The thermoregulation and camouflage theories proposed for *Dynastes* by Hinton and Jarman (1972) were reasonably questioned by Rassart et al. (2008), who also stressed that this phenomenon is almost absent in the females, which show switching colour integument only at the tip of the elytra. The switch from gold to red of Cassidinae in response to disturbance is possibly aposematic, however it should be noted that the colour change takes about 1.5-2 min, thus being far too slow to act as an effective warning towards a predator.

PATTERN EVOLUTION AND EVO-DEVO ASPECTS

Anatomy and colour pattern

The relationships between colour pattern and anatomical elements in beetles are a poorly explored field, not only from the developmental or interpretative point of view, but even from the descriptive one. Data on this subject seem to be particularly poor and scattered across literature, and I was unable to find any monographic account, even a short one. Therefore the following paragraphs are proposed as an explorative overview, mainly based on original data, of the most significant phenomena observed.

Muscles insertions and melanization

Relationships between the spatial arrangement of the muscular apparatus and the cuticular colour patterns were first described by Tower (1903), who reported a strong coincidence between black spots on the pronotum and underlying muscular insertions in *Leptinotarsa decemlineata* (Chrysomelidae) and observed that muscular insertions act as the foci of colour patterning also in the cerambycid *Orthosoma brunneum*. According to Tower's description, colour markings on head and pronotum appear in both species during the last days of the pupal stage and then either spread on the remaining integument surface or remain limited to the area of first appearance. Subsequently, a coincidence between muscular insertions and black spotted pattern was observed in *Polistes* wasps (Enteman, 1904). These two authors were cited by Shelford (1917: 411) and, to my knowledge, no other author dealt further with this phenomenon.

Therefore, I performed some original study, thus confirming Towers' observations on fresh specimens of *Leptinotarsa* (figs. 24-27). I could also observe a coincidence between muscular insertions and pronotal dark patterning in dry specimens of some other beetle species belonging to different families (Silphidae, Carabidae, Scarabaeidae, Cetoniidae, Melolonthidae, Lampyridae). The coincidence, however, is not always as precise as in *Leptinotarsa*, and in at least one case it is reversed: in *Daptus vittatus* (Carabidae), pronotal muscles insert in the lighter areas of pronotum, therefore seemingly inhibiting the melanization of the cuticle, as the surrounding integument is indeed dark. Dissections carried out on fresh or properly preserved material will probably allow the recognition of further instances of this correlation, which is likely to turn out as one of the major mechanisms involved in the definition of the pronotal pattern, or in the induction of foci of melanization.

In this sense, the silphid *Oiceoptoma thoracica* may serve as a sensible example, because of its variability in the extension of the dark spots on the pronotum. Their extension is variable, appearing either as single, distinct, elements, or cohalescent in a large elliptical marking covering the whole disc of pronotum. In the former case (fig. 28), a dissection of the specimen will reveal that each dark spot correspond precisely to a muscular insertion, while in the latter

case (fig. 29) the coincidence would be obviously undetectable. However, the existence of specimens with spots of intermediate extension and the agreement of these observations with Tower's indications about the development of the colour in *Orthosoma brunneum* indicate that muscular insertions act as foci of production of the dark markings, whose subsequent extension is however variable. This fact point to the conclusion that muscular insertions are likely to act as foci for the induction of the chromatic pattern also in closely related species, regardless of the final appearance obtained, such as *Xylodrepa quadripunctata* (fig. 30), whose pronotum is invariably black but for the expanded margins. In other words, I suggest here that the developmental mechanism shall be the same in these two species, even though in one of them (*X. quadripunctata*) the foci of pigmentation will invariably expand in such a way that their correlation with muscular insertions cannot be recognised in adults with completely pigmented cuticle.

Within the Scarabaeoidea, more often than the coincidence between muscular insertions and the darkening of the pronotum (figs. 31-34), the coincidence between anatomical elements and colour pattern is commonly verified in respect to an apodeme present in the medial or anterior part of the pronotal side, which is present, as far as I could check, in all Scarabaeoidea families (including Lucanidae and Passalidae). This apodeme is often perceivable from the outside through an impression or a modification of the cuticular surface, and/or by a darkening of the colour, which is obviously perceivable only in species with light-coloured integument (figs. 35-36).

The significance of the coincidence between activation of pigmentation and muscular insertion is not clear, however the "structural" hypothesis vaguely proposed by older authors (pigments would develop wherever rigidity or cuticle strength is necessary) is likely unjustified. More recent acquisitions about cuticle physiology shed light on the relationships between sclerotization and melanization (see *Darkening and sclerotization of the cuticle*, p. 13), which are two independent processes. In addition, if the black spots were positively selected in order to give a solid attachment to the muscles, it would be unclear why so many light coloured insects exists, which have no dark areas corresponding to the areas of muscle attachment. In this respect, it is also meaningful to observe that muscle attachment markings may be present or absent even in very closely related species (e.g.: pattern present in *Leptinotarsa decemlineata*, absent in *L. rubiginosa* and *L. typographica*), which are therefore deemed to have a similar body architecture and an exoskeleton experiencing similarly distributed mechanical stress. Therefore the hypothesis should be considered that black pigment is developed as an occasional by-product of the processes that link the muscle to the exoskeleton.

The elytron: vein patterns, punctuation and sculpture

Wing veins, derived from the tracheal system, are known to have a major role in the determination of the butterfly wing pattern (Nijhout, 1991). The spatial correlation of vein structure with the markings of the pattern is widespread and sometimes almost impressive in its precision and modular regularity (e.g.:

members of Nymphalidae Argynninae). Wing veins are known to represent a landmark necessary to the definition of the wing pattern even among Diptera (observations limited to the genus *Drosophila*, Parchem et al., 2007). However, little is known about the relationships between colour pattern on beetle elytra and veins, as well as with other morphological elements such as integument punctures or striations.

Most beetles, indeed, lack true wing veins in the elytra. The tracheal system of elytra is strongly reduced and most of the remnant tracheae are completely embedded within the thick modified wings (Comstock, 1918). Therefore, any possible correlation between the colour pattern and the vein distribution is much less obvious than in the previously mentioned groups, whose veins are well exposed on the surface.

Exploring the literature, I could only discover a single paper investigating the relationships between vein system and colour distribution in elytra of tiger beetles (Shelford, 1917). This author figured (l.c., plates I-V) a few elytra of Cicindelidae and of some species of Carabidae and Dytiscidae, illustrating, in the first pages of its work, the relationships (or the absence thereof) between the elytral venation and the dark pattern of the integument. Shelford, referring to the same families, also briefly mentioned that the cuticle over the chitinous columns corresponding to the elytral punctures is the last "to lose its pigment", practically indicating that those areas act as foci for the development of the melanized areas of the integument.

To the best of my knowledge, no other author focused its attention on these topics. To date, the ways and the degree to which the vein patterns of the elytra and other morphological characters relate to chromatic pattern remain unanswered questions. Personal observations carried out on a random selection of patterned beetles (mainly belonging to the Palaearctic fauna) (Tab. 1) revealed that all the morphological elements of the elytra can have positional relationship with the elytral pattern. Five apparently different kinds of relation have been observed:

- interaction: the morphological element pass across the chromatic element, modifying its shape by determining a preferential direction of elongation (only observed in some cases where the veins "stretch" the pattern). (fig. 37).
- coincidence: the pattern element and the morphological element are superimposed and coincident in shape and/or size. This suggests an inductive phenomena (from morphology to the colour pattern). (figs. 38-39, 42).
- enhanced expression: the expression of the pattern is enhanced when superimposed to the morphological element, although spread well around the element itself. (fig. 40)
- confinement/alignment: the morphological element seems to act as a boundary to the diffusion of the pattern element on the integument. (fig. 41)
- exclusion: the morphological element excludes the presence of a pattern element. This is opposite to coincidence and is apparently due to a phenomenon of inhibition. (figs. 43-44).

Family	Species	Kind of pattern	Correlation with				Pattern is completely explained by correlation with morphology
			tracheae	sparse punctuation	punct. aligned in striations	striations	
Aphodiidae	*Aphodius distinctus*	melanic	--	no	excl (+)	excl (+)	no
Aphodiidae	*Aphodius obliteratus*	melanic	no	no	?	con / alig (+?)	no
Buprestidae	*Acmaeoderella fasciata*	melanic	--	--	no	no	--
Buprestidae	*Chrysochroa vittata*	physical colours	coin(-)	no	--	--	no
Carabidae	*Badister bipustulatus*	melanic	no	--	enh. expr. (+)	con / alig (-)	no
Carabidae	*Bembidion illigeri*	melanic	--	--	coin (+)	--	no
Carabidae	*Callistus lunatus*	melanic	--	--	no	no	--
Carabidae	*Daptus vittatus*	melanic	no	--	enh. expr. (+)	no	no
Carabidae	*Dromius quadrimaculatus*	melanic	no	--	coin (-)	con / alig (-)	no
Carabidae	*Eurynebria complanata*	melanic	no	--	coin (-)	con / alig (-)	no
Carabidae	*notaphus varium*	melanic	no	--	coin (+)	con / alig (-)	no
Carabidae	*Omophron limbatum*	melanic	--	--	coin (+)	con / alig (+)	no
Cerambycidae	*Acrocinus longimanus*	melanic (hair)	int(+)	no	--	-	no
Cerambycidae	*Leiopus nebulosus*	melanic	--	coin (+)	--	--	no
Cerambycidae	*Leptura maculata*	melanic	no	no	--	--	--
Cerambycidae	*Macrodontia sp.*	melanic	excl (+)	no	--	--	no
Cerambycidae	*Pachytodes cerambyciformis*	melanic	int(-)	no	--	--	no
Cerambycidae	*Pogonocherus hispidus*	melanic	--	no	--	--	--
Cerambycidae	*Rhagium inquisitor*	melanic	coin(-)	no	--	--	no
Cerambycidae	*Stenurella septempunctata*	melanic	no	no	--	--	--
Cetoniidae	*Dyspilophora trivittata*	melanic	no	no	--	--	--
Cetoniidae	*Euselates perraudieri*	melanic	--	no	--	--	--
Cetoniidae	*Pachnoda sp.*	melanic	no	no	--	--	--
Chrysomelidae	*Calligrapha dislocata*	melanic	excl (-)	no	coin(+)	con / alig (+)	no
Chrysomelidae	*Chrysolina bicolor*	physical colours	no	coin	--	--	yes
Chrysomelidae	*Chrysolina cerealis*	physical colours	coin(+/-)*	no	--	--	no/yes
Chrysomelidae	*Chrysolina confluens*	melanic	no	no	coin(-)	no	no
Chrysomelidae	*Coptocephala unifasciata*	melanic	no	no	--	no	--
Chrysomelidae	*Crioceris asparagi*	melanic	con / alig (-)	no	no	--	no
Chrysomelidae	*Crioceris paracenthesis*	melanic	no	no	coin(-)	--	no
Chrysomelidae	*Cryptocephalus bipunctatus*	melanic	no	--	coin? (-)	--	no
Chrysomelidae	*Cryptocephalus connexus*	melanic	--	coin (-)	coin (+)	--	no
Chrysomelidae	*Gonioctena fornicata*	melanic	no	no	no	--	no
Chrysomelidae	*Gonioctena quinquepunctata*	melanic	no	no	no	--	--
Chrysomelidae	*Lachnaia italica*	melanic	no	no	--	--	--
Chrysomelidae	*Leptinotarsa decemlineata*	melanic	no	no	con / alig (+)	--	no
Chrysomelidae	*Oreina speciosa*	physical colours	coin(-)	no	--	--	no
Chrysomelidae	*Pachybrachys hippophaeus*	melanic	no	coin (+)	--	--	no
Chrysomelidae	*Paropsides soriculata*	melanic	no	no	--	--	--
Cleridae	*Trichodes alvearius*	melanic	no	no	--	--	--
Cleridae	*Trichodes apiarius*	melanic	no	no	--	--	--
Coccinellidae	*Coccinella septempunctata*	melanic	no	no	--	--	--
Coccinellidae	*Epilachna chrysomelina*	melanic	no	no	--	--	--
Coccinellidae	*Propylaea 14punctata*	melanic	no	no	--	--	--
Colydiidae	*Bitoma crenata*	melanic	--	--	no	--	--
Elateridae	*Drasterius bimaculatus*	melanic	--	no	?	con / alig (+)	no
Endomychidae	*Ancylopus melanocephalus*	melanic	no	enh. expr. (+)	--	--	no
Endomychidae	*Endomychus coccineus*	melanic	no	no	--	--	--
Glaphyridae	*Eulasia vittata*	melanic (hair)	no	no	--	--	--
Glaphyridae	*Eulasia vittata*	melanic	excl (+)	--	--	--	si?
Haliplidae	*Haliplus caesus*	melanic	no	--	coin (+)	--	no
Haliplidae	*Haliplus sp.*	melanic	--	coin(+)	coin(+)	--	yes
Hydrophilidae	*Berosus spinosus*	melanic	--	coin (+)	coin (+)	con / alig (+)	no
Laemophloeidae	*Laemophloeus monilis*	melanic	no	no	--	no	--
Meloidae	*Mylabris variabilis*	melanic	int(+)	no	--	--	no
Meloidae	*Tegrodera sp.*	melanic	excl (-)	--	--	--	no
Mycetophagidae	*Mycetophagus quadriguttatus*	melanic	--	no	--	--	--
Nitidulidae	*Glischirochilus quadriguttatus*	melanic	? excl (-)	no	--	--	no
Nitidulidae	*Nitidula carnaria*	melanic	--	no	--	--	--
Nitidulidae	*Stelidota geminata*	melanic	--	--	coin(+)	?	no
Oedemeridae	*Anogcodes rufiventris*	melanic	no	no	--	--	--
Rutelidae	*Anisoplia tempestiva*	melanic	no	no	--	--	--
Rutelidae	*Blitopertha majuscula*	melanic	coin(-)	no	no	no	no
Rutelidae	*Mimela sp. (Thailand)*	physical colours	no	no	--	--	--
Scarabaeidae	*Caccobius schreberi*	melanic	no	excl (+)**	?	excl (+)	no
Scarabaeidae	*Cheironitis irroratus*	melanic	enh. expr. (+)	excl(-)	no	no	no
Scarabaeidae	*Onthophagus lemur*	melanic	--	no	?	excl (+)	no
Scarabaeidae	*Onthophagus vacca*	melanic	--	excl(-)	?	excl (+)	no
Silphidae	*Nicrophorus vespillo*	melanic	con / alig (-)	no	--	--	no
Staphylinidae	*Stenus biguttatus*	melanic	--	no	--	--	--
Tenebrionidae	*Diaperis boleti*	melanic	no	no	no	no	--
Tenebrionidae	*Phaleria bimaculata*	melanic	no	no	coin(+)	?	no

Tab. 1. Correlation between elytral pattern and morphological elements in a sample of beetles. **coin**: coincidence; **con /alig**: confinement/alignment; **enh. expr.**: enhanced expression. **excl**: exclusion; **int**: interaction. See text for explanations.
--: condition not appliable (morphological element absent or not perceivable); **(+)** condition verified on all elements of the considered kind **(-)** condition not verified on all elements of the considered kind . **Notes**: *: (+) or (-) according to the colour form examined; ** faintly perceivable.

The boundaries between these categories are sometimes uncertain, and the co-occurrence of different contiguous morphological elements may cast doubt on their respective roles (this is the case, for example, of punctures aligned in a row within each striation, as in *Drasterius* and *Onthophagus*); nevertheless, this is a first attempt to classify the relationships between the characters taken into account.

Many of the observed species show some degree of correlation between one or more morphological elements and the chromatic pattern. However, as already noted by Shelford (1917: 412) on a smaller and less variated taxon sample and for the vein system only, there is no constant relation between any of the morphological elements and the expression of the pattern. Conversely observing a given morphological element across the species, this relation whenever observed can be either positive (coincidence, enhanced expression) or negative (exclusion).

As indicated in the table, it is often observed that morphological elements, although repeating with an apparently invariated aspect throughout the elytron surface, often do not behave uniformly. This is observed both with veins, whose expected "uniformity" may however be questionable, and with punctuations, which usually appear absolutely constant in shape and size. Particularly evident examples of this "disuniformity" are the South African *Chrysolina confluens*, whose puncture aligned in rows may or may not induce a large, black pigmentary spot (fig. 42b), and *Onthophagus vacca*, whose sparse punctuation may or may not inhibit the expression of the black pigment of the integument (fig. 44b). These two species are also a sensible example of the previously mentioned positive vs. negative relation of similar morphological elements towards the chromatic pattern.

As a consequence, the influence of morphological elements on the pattern is rarely capable to explain on its own the appearance of the definitive pattern, due to the occurrence of elements (punctures, veins) oddly lacking the interaction which is shown by others. In addition, apart from this "disuniformity" in the behaviour of otherwise apparently identical morphological structures, the integument may show, along with the putative morphology-explainable pattern elements, an additional patterning with no relations with any kind of morphological structure. As an example, the pattern of two Haliplidae, *Haliplus* cfr. *obliquus* and *Peltodytes caesus*, can be mentioned, the first showing a pattern completely referrable to the induction of dark spots by the rows of punctures, the second showing, in addition, some vaguely defined dark spots on different areas of the elytra.

Physiological and developmental mechanisms leading to the observed correlations are not known, however it seems reasonable that some of these differently classed cases may definitely reveal as the different expressions of the same developmental phenomenon. This is possibly true with the conditions descriptively named "coincidence" and "enhanced expression". In both of them, in fact, the pigmentary expression is enhanced by the morphological element with

respect to the background expression, which can be either null or present but at a lower degree.

Interestingly, morphological elements are capable to interact not only with pigmentary expression of the cuticle, as already observed by Shelford (1917), but also with the pigmentary expression of the phaneres pattern, as exemplified by *Eulasia vittata* (fig. 57) and *Acrocinus longimanus* (fig. 37) and, perhaps more notably, with pattern of strictly physical origin, as impressively exemplified by *Chrysolina cerealis* (fig. 79) for the veins and *Ch. americana* (fig. 76) for the punctures. In addition, a relationship of inhibition from surface punctuations towards integument melanization is observed here for the first time, at the moment limited to members of the Scarabaeidae.

Finally, it should be mentioned that a consistent variability in the intraspecific correlation between morphology and pattern can be observed even at a low taxonomic level, as exemplified by the two species of *Onthophagus* taken into account: despite being closely related (they both belong to the subgenus *Trichonthophagus*) they differ sharply in the behaviour of the sparse punctuation.

Secondary sexual characters and colours

The occurrence of secondary sexual characters is widespread and very common among beetles. A few cases, however, show an unusual coincidence between body parts bearing secondary sexual modifications and the alteration of the cuticular colours. The most remarkable example of this phenomenon can be observed among members of the Meloidae tribe Cerocomini, in particular those belonging to *Cerocoma*. Many members of the Meloidae family are known to show minor sexual dimorphism in the morphology of antenna, but *Cerocoma* species are characterised by an extraordinary modification of the male antenna, which is also associated with a strong alteration of the foretibia-foretarsus morphology and of the morphology of the palps. All these parts, along with the morphological modifications, shows in addition an alteration of the colour, which is bright yellow or orange instead of black (figs. 45-46). The correlation, however, is not one-to-one: not all the yellow appendages are morphologically modified, although all the morphologically modified parts are yellow (hence the recognition of the yellow colour as “modified”). The variability in the degree of occurrence of this phenomenon is species-specific, ranging from species showing a remarkably precise coincidence between the two traits, to species with much looser coincidence. As a representative of the first condition can be mentioned *C. festiva* (fig. 47), whose chromatic alterations are observed only in males and only in the part of appendages which are morphologically modified, whereas unmodified appendages of male and female are black. As a representative of species with less strong coincidence *C. schreberi* may be cited, having all appendages of the male, both modified and unmodified, orange, whereas among other members of the genus, as well as in the genus *Teratolytta*, cases can be found where the chromatic alteration is extended to both sexes, regardless of the absence of morphological change in the appendages of females.

A very similar correlation between secondary sexual modifications of morphological nature and chromatic alteration is also observed in a few genera of Malachiidae (such as *Malachius*, *Clanoptilus*, *Ebaeus*, *Cerapheles* and others). The antennal portions which are modified in the male are yellowish, as well as the labrum and the clypeal area, where secondary sexual characters are present (figs-48-49). It is interesting to stress that the yellow colour is not observed on the whole antennal articles involved, but only in the side/portion which is affected by morphological modifications. A chromatic modification related to secondary sexual characters is also observed at the apex of elytra, where a deep, complex impression is present in the males of some groups (figs. 50, 52) As with the previously mentioned genus *Cerocoma*, chromatic modifications, although showing a remarkable coincidence with areas bearing sexual secondary modifications in the male, are not limited to this sex, but are commonly observed also in females (figs. 51, 53) and even in non modified males (fig. 54). However, secondary sexual modification in morphology is always accompanied by alteration in colour.

Less remarkable and/or widespread chromatic modifications of sexually modified parts are scattered in various other groups. Among the Lucanidae, despite their generalized extreme sexual dimorphism, putative example of this phenomenon seem to be unknown, but for the south African *Colophon primosi*, whose unusual orange appendages (legs and male mandible) are associated to hypertrophic mandibles in the male, something exceptional in this genus. Examples are also known for body parts other than the appendages: Glaphyridae members of the genera *Eulasia* and *Pygopleurus* show a morphological alteration of the last abdominal segments which is associated, in males, to a chromatic alteration visible at least on the medial area of the last sternite, which turns red instead of black.

It is interesting to note that in some groups a colour alteration somehow “opposite” in respect to the previously mentioned ones can be observed, in the sense that when dichromism occurs yellow colour is observed in females with unmodified appendages. This is the case of a few species of Palaearctic Lepturini (Cerambycidae), such as *Leptura aurulenta* and *L. quadrifasciata* (antennae are more developed and black in the male, less developed and completely or partly yellow in the female), *Leptura annularis* (tibia are black and modified in the male, yellow and simple in the female). A similar phenomenon is also observed in some *Hoplia* species: legs of females are ordinarily shorter and weaker than those of males and sometimes testaceous instead of black (as happens with the legs of the males).

It should be remarked that in some of the mentioned groups, such as Malachiidae and *Cerocoma*, modified body parts are directly used in courtship behaviour (for *Cerocoma*, see Turco et al., 2003), although not all of them seem directly involved: the foretibiae, for example, are not directly used during the sexual intercourse. In other species, the sexual dichroism is not conspicuous (e.g. the posterior tibia of *Leptura annularis*, the legs of *Hoplia*) or affect areas which

are unlikely to be involved in communication (such as the modest chromatic alteration on the ventral side of *Eulasia* abdomen).

It is proposed here that the (variable) correlation between chromatic and morphological alteration of secondary sexual modifications is, at least in some cases, not by chance but may also have a specific developmental basis, its fixation would be likely obtained via sexual selection mechanism. Under this scenario, the possibility to find, within some groups, modification of colours without morphological alterations, but not vice-versa, may suggest that in these taxa the determination of colour occurs before the determination of morphological modifications, and that the latter requires the former to be explicated. Otherwise, it is possible that morphological modifications and chromatic alterations are elicited, with results of different magnitude, by the same positional marker. In cases where the sensory appendages are involved, it is possible that the alteration of the colour is a by-product of the modification of the integument's ultrastructure, the latter being related or necessary to the development of peculiar sensillar or secretive integumental organs present within the modified integuments, as documented for the genus *Cerocoma* (including foretibia, densely covered with pores) (Turco et al., 2003).

Sexual dichromy is rather rare in Coleoptera generally. Most examples can be found among anthophilous day-active species, such as Buprestidae (many species of the genus *Anthaxia*), various members of the Cerambyicidae Lepturini (such as species of the genera *Stenurella, Leptura, Anastrangalia*) and Lamiinae, a few species of Chrysomelidae Cryptocephalini, rare members of Glaphyridae of the genus *Eulasia*, differing either for the physical colour of the integument (*Eulasia chalybaea*) or the patterning of the hair (an undescribed specis of *Eulasia* from Iran), and, again, various species of the genus *Hoplia* where males are densely covered with scales showing bright physicals colours, while females only show dull brownish scales or have an almost naked inconspicuous integument. The diurnal phenology and the anthophilous behaviour of these species (which are associated with a strong sensitivity to visual stimuli, e.g., Dafni, 1997 for Glaphyridae) suggest that strong sexual dichromism may be related to a visual recognition of partners.

However, uncommon examples of sexually dichromic species can also be found among forest dwelling and/or nocturnal species, such as Dynastidae (*Golofa, Dynastes*) and Rutelidae (*Mimela, Fruhstorferia*), commonly regarding single species scattered among others not showing sexual dichroism (e.g.: *Dynastes hercules*, *Mimela aurata*).

Hair and scales

Unlike butterflies, whose wings and body are densely covered with scales and hairs (from now on, in this thesis, collectively referred to as the phaneres), pattern of beetle integument are most commonly due to cuticular colours, either originated by pigments or by physical structures. The beetle integument is usually covered by sensory setae, but these are often inconspicuous and/or uniformly

coloured and distributed, thus giving no substantial contribution to the shaping of a pattern. Nevertheless, beetle setae are sometimes modified into more conspicuous hairs or scales of various complexity, with shapes going from the simply spatular one to the less common pebble- or feather-like ones (Crowson, 1981), and/or can occur in such an extremely dense arrangement that the underlying integument get completely masked.

Beetle taxa whose colour or pattern is deeply affected by a thick phaneral covering occur at different taxonomic ranks and are scattered across the whole order. A few large groups can be regarded as particularly representative of strongly phaneral patterned beetles, such as Melolonthidae Hopliinae, Cetoniidae, Dermestidae, Cerambycidae Lamiinae, Anthribidae, and Curculionidae (in particular, Entiminae). Along with these families are groups whose integument is typically naked (e.g., Chrysomelidae), or covered with fine, inconspicuous hair not cohoperating to the definition of a pattern (Carabidae); however, exceptions exist: among the Chrysomelidae, densely covered with hair are most Bruchinae and various Eumolpinae (such as members of the European genus *Pachnephorus*, fig. 55), among carabidae patches of coloured hair define the pattern in members of the small subfamilies Graphipterinae and Anthiinae.

As happens for the integument, the colour of the phaneral structures can originate from pigments or from highly complex photonic structures producing physical colours (cf. *Physical colours: photonic crystal structure and scales*, p. 20).

The widespread capability to develop phanera over a thick integument which has its own colour anyway, allow beetles to take advantage of an additional patterning mechanism, which is unavailable to butterflies. The two patterning mechanisms (integument and phaneres) can be present together on the elytra and thus cooperate to the definition of the overall aspect of the beetle. Phaneral structures, in fact, can develop in discrete patches, parted by areas where the integument is completely naked. Taxa having a pattern produced by the discrete distribution of phaneres are very common across the whole order; particularly conspicuous examples can be found within members of the Australian Cetoniidae genus *Trichaulax* (having thick stripes of jellow hairs parted by black integument), or within species of the Old World Buprestidae genus *Julodis* (having patches of dense hairs on bright metallic integument), whose phaneral cover, in addition, may be bicoloured: various species from Southern Africa have generally white-yellowish patches of hair, whereas the sides of the elytra are covered by intensely red hair.

Patterns composed by the partecipation both of phanera and a pigmentary patterned integument are very common among the Cetoniidae, in particular those of the Asiatic genera *Euselates* and *Taeniodera*. In these genera, the integument bears a bicoloured pigmentary pattern red and black, and the surface of the body is more or less extensively covered with spots of dense yellow scales, which provide a fundamental contribution to the overall appearance of the beetle (fig. 56).

More interesting are other examples, involving species whose setation covering the elytra is uniform and complete. The coexistence of superimposed

phaneral and pigmentary patterns sheds some light on the relationships between these two traits, showing that the degree of interdependence between them is highly variable, going from strict to null, and can show remarkable variation even at a low taxonomic level. As an example, among the Cerambycidae Clytinae, members of the genus *Chlorophorus* fit well with Tower's (1903) principle, that in the presence of scales, the underlying integument is uniformly coloured: in this genus, indeed, the elytra are covered with dense patterned hair, but the integument beneath is uniformly dark (fig. 58). However, species belonging to closely related genera, such as *Clytus* and *Xylotrechus*, show a remarkable match between the pattern of the hairs and that of the integument, as exemplified by *Clytus arietis* (fig. 59). It should be noted that this match does not involve the small yellow strip under the humerus: the integument under this strip is uniformly black. In addition, the yellow pubescence of *Clytus arietis* (including hairs of the subhumeral strip) is morphologically different from the black one: hairs are more dense, thicker, more adpressed to the integument and have a slightly different orientation. Conversely hairs of *Chlorophorus* are invariant – colour apart – throughout the surface of the elytron.

A further, noteworthy case in the reciprocal arrangement of the two patterning mechanism, probably much rarer than the previously described ones, is exemplified by *Eulasia vittata* (Glaphyridae). Both the elytral integument and the uniformly distributed phaneral cover are patterned, showing melanized areas well distinct from unpigmented or lighter ones (fig. 57). However, the two patterns, although superimposed, are completely different from each other, thus demonstrating that beetles can evolve the capability to control the pigmentary pattern expressed by the phaneral cover and the pigmentary pattern expressed by the underlying integument independently.

A difference between the phaneric pattern of Coleoptera and that of Lepidoptera should be noted: scales of Lepidoptera are, in general, evenly spaced both in the longitudinal and in the transversal direction (Parchem et al., 2007). That is, they are well ordered in regular rows, like the tiles of a roof or the squares of a chessboard. Scales of Coleoptera, conversely, are not arranged in such a regular way; they appear scattered or, at most, organized in very irregular longitudinal rows. Often hairs or scales of Coleoptera are not as dense and covering as those of butterflies, anyway, they mantain their characteristic irregular arrangement even when they are dense and completely mask the integument, as in *Hoplia* and *Eupholus*. This fact is unlikely to have a deep impact on the general organization of the pattern, however it may affect the finer regulation of pattern elements or the capability to evolve discrete small-size elements. Thus, it will be hard for a beetle producing details such as the fine striations of different brown hues on the lower side of *Nymphalis polychloros* wings, where each line is produced by a single row of well aligned scales (figs. 61-62). Such a fine control may be hard, or impossible, to achieve for a beetle, since it is not just a problem of controlling colour, but also problem of "pixels" alignment or geometric arrangement (fig. 60).

Convergence in colour patterns of sympatrically occurring beetles

The occurrence of convergence of colour pattern among sympatric beetles is an interesting thus little known and poorly understood phenomenon. We shall define it as the occurrence of a similar (or same) colour pattern among different sympatric species, this pattern being variable on a geographical basis but locally uniform across the species involved. Examples of similar patterns are known for few families. It is recorded in Carabinae, for example by Deuve and Li (2000) who dealt with Chinese *Carabus*, and by Okamoto et al. (2001), who investigated in detail this phenomenon among Chilean *Ceroglossus*. This genus ranges over Chile and Argentina with 8 species strongly similar each other, despite their last common ancestor having lived about 30 million years ago. Two or more *Ceroglossus* species occur sympatrically through the largest part of the distribution range, and each species shows a bright metallic appearance with a wide intraspecific variation in body colour (which is of structural origin). An extensive collecting work, carried out into a large part of the *Ceroglossus* range and encompassing 6 species, allowed confirmation of the monophyly of currently recognised species (through phylogenetic analysis of the ND5 gene) and showed that each one of these exhibits a wide range of colours, homogenously varying according to geography. As a result of this phenomenon, in a given area up to four species may be present, all showing the same habitus.

This phenomenon is also known among the Scarabaeoidea: a short record for African Scarabaeidae of the genus *Allogymnopleurus* and *Scarabaeus* is given by Nicolas and Moretto (2002); a more detailed account was produced for the Japanese *Geotrupes auratus* and *G. laevistriatus* by Watanabe et al. (2002a, 2002b), who described a phenomenon similar to that found among Chilean *Ceroglossus*, but with less precise coincidence. A more complex situation, briefly addressed by Montreuil (2006), is found among some Palaearctic Cetoniidae, where the occurrence of circles of "colour races" is well evident.

In the latter case, the species involved are ranked each in a different subgenus of the large genus *Protaetia* (and until recent years even ranked in different genera), namely *Protaetia* (*Potosia*) *cuprea*, *P.* (*Cetonischema*) *speciosa*, and *P.* (*Eupotosia*) *affinis*. *P. cuprea* and *P. affinis* are widespread across Europe with different subspecies or populations of uncertain taxonomic rank, always having the upper side evenly coloured, usually with hues of green, far less commonly with copper or reddish tinge. *P. speciosa* occurs in a less extended distribution range, from Turkey to Iran; however a very similar species (close enough to have this distinction disputed and hybridization allowed), namely *P. aeruginosa*, is widespread across southern Europe. As with the previously mentioned species, the complex *speciosa/aeruginosa* is represented by uniformly coloured specimens in the greatest part of its distribution range.

Interesting facts with the colouration of these beetles occur with the Middle Eastern and Levantine populations. In the southern part of their range, each of these species is found with populations showing a strongly bicoloured body, with red pronotum and green elytra. This pattern, which does not occur

elsewhere, is almost invariant in the involved populations and has lead to the designation of different subspecies: *P. c. ignicollis* (from southern Turkey to Iraq and to Egypt and Libya), *P. a. pyrodera* (from southern Turkey to Lebanon) and *P. s. jousselini* (from southern Turkey to Iraq and to the Golan area) (figs. 63-64). *P. speciosa* and *P. affinis* show additional phenomena of colour convergence in Iran: both of them have a golden-orange subspecies in northern Iran and a blue subspecies in the Zagros range.

A similar, apparently less defined, convergence is observed for Dinaric and Western-Balcanic populations of *Protaetia*, such as *P.* (*Cetonischema*) *aeruginosa* and *P*. (*Netocia*) *angustata*. There, populations of both of these species show a remarkable high occurrence of red to black forms, which are absent or extremely rare elsewhere. For example, a population of *P. angustata* in the Krk Island, surveyed for three years (pers. obs.), showed about 40% of specimens going from red to black (relationships between red and black colour in a *Protaetia* cetonid is later discussed). In addition, red and black specimens of *P. cuprea* have been recorded from Greece (M. Malmusi, pers. comm.), and black specimens of *P. affinis* are known from the north-western Turkey (Tauzin, 2008).

Personal observations also suggest the occurrence of a similar phenomenon among the Chrysomelidae. Observations carried out on the large collections of the Museo di Storia Naturale di Verona provided preliminary evidences for a colour pattern convergence of two species belonging to the highly polymorphic genus *Oreina*, namely *O. cacaliae* and *O. speciosissima*. Syntopic populations occurring in the Slovenian Carst (M. Nevoso/Veliki Sneznik, Selva di Tarnova/Tranovski Gozd and adjacent places), show indeed a pattern with blue background and two longitudinal green stripes. Although striped patterns are widely distributed among other species of the same genus and the capability to produce them was apparently inherited by all species of the subgenus *Chrysochloa*, it absolutely rare (as fare as I know, not observed at all) among other *Oreina cacaliae* populations except those mentioned above. Conversely, *O. speciosissima* has greater variability, ranging from different coloured monochromatic patterns to different coloured striped ones. However, the blue/green striped pattern is rather uncommon, thus suggesting that the syntopic convergence observed is likely not by chance.

Reasons leading to these convergence phenomena are far from be understood, but some comments can be issued. Mimetic chains among invertebrates are usually explained as an exploitation of successful signals, hence involving a matter of communication among individuals either of the same species or of different specis.

In the discussed cases, the intraspecific communication hypothesis is unlikely to be true: ground beetles are nocturnal animals, consequently it would be unlikely for them to exploit a communication system requiring a defective source of signal (light). Even for day-active species, a use of body colour in intraspecific communication seems unlikely, at least as a sexual signal: perfectly assortative mating within polymorphic populations was observed in the genus *Chrysolina*, closely related to *Oreina* (Fujiyama and Arimoto, 1988). Even in

cetoniids, the colour of adults does not seem to be involved in partner recognition or to affect the reproductive behaviour. Adult behaviour suggests that intraspecific recognition among cetoniids is reached, in part at least, via chemical signals, while there are no observations pointing to the involvement of body colour. Captive specimens of the *P. speciosa*/*aeruginosa* complex, for example, readily mate even when belonging to different colour forms or even to different species/subspecies (pers. obs.), even when very different colours were involved (as in the pair black x green, *jousselini* x black, etc.). Moreover, if the colour was involved in intraspecific recognition, the syntopic presence of similar species with similar colour would make no sense.

A geographically coordinate variation in the colour of different species fits better with the hypothesis of an interspecific signal (i.e. a Batesian mimicry chain involving aposematic colours), however there are some facts detracting from this explanations. Again, brightly coloured ground beetles are active in scarce light conditions, so there is no ground to support a hypothesis involving any kind of communication. Cetoniids have a chemical defense against predators (distasteful fluids can be emitted from the anus), nevertheless they mainly show (at least for the Palaearctic species) a uniform and inconspicuous green or copper metallic colour, usually giving quite a good camouflage among vegetation (for canopy dwelling species) or still resulting in a not-shocking look for flower dwellers ones. There is no evidence that mentioned cetoniids use visual stimuli to advertise their defence system, as done by other insects having a similar lifestyle but bearing much more striking patterns (e.g.: *Trichodes* among the Cleridae, *Leptura* and *Clytus* among the Cerambycidae, *Mylabris* among the Meloidae etc.). The significance of a striped pattern among *Oreina* and *Chrysolina* (which are chemically protected) is often described as having an aposematic role (e.g. Hsiao & Pasteels, 1999), however no evidence has ever been proposed for this hypothesis, nor can be easily explained the common observation of striped (aposematic?) individuals mixed with monochromous (inconspicuous) ones. As a final comment, applying to all of the mentioned examples, it should be said that it is unclear why such an hypothetic signal should vary between adjacent areas, where landscape and beetle behaviour does not seem to have significant variation: aposematic colours, instead, are usually very conservative on a wide geographical range (if not worldwide).

Finally, it should be considered that colours expressed by the integuments of highly polymorphic species (including species shoving sympatric colour convergence), may be under poor direct selection, and behave mainly as a by-product of different morphogenetic processes. In this case, the coincidence observed among colours and geographical areas should be referred to a pleiotropic genetic system whose phenotypic traits are exposed to an uneven selective pressure: colours, despite being a most evident phenotypic output, would experience a comparatively mild selective pressure, the latter being stronger on less directly evident characters or developmental processes connected with the colour determination.

It is important to stress that sympatric convergence of colour patterns in beetles is so far only known for species with physical colours, whose variation is achieved through tiny modifications of integument morphology. Since a single morphological trait (the thickness of the integumental multilayer) has to be modified in order to achieve very different colourations, it is possible that the polycromy is achieved and/or mantained by a relatively simple genetic system.

Determination of colour forms among polymorphic beetles owing their colour to photonic structures is little known but, as far as known, it is chiefly of genetic origin. Polymorphism among a two-forms population of *Chrysolina aurichalcea* (a species close to *Oreina*, cfr. text fig. 1) was shown to be dependent on two alleles and to strictly follow Mendelian laws (Fujiyama and Arimoto, 1988). Weaker evidences on colour control system are known also for the *Protaetia* complex, where colour forms seems to be under the control of genetic factors. Breeding *ex situ* of various colour forms of *Protaetia* species always produces the expected "natural"/parental colour, in spite of the different environmental conditions experienced and of the feeding substrate used (pers. obs.). In addition, explorative crossing experiments involving red colour forms of *P. aeruginosa* also shed some light over the colour determination system (Dutto and Malmusi, 2006; pers. obs.), again pointing to a genetic determination system of colour. Published data are very poor, but it is interesting to note that crossing F1 red phenotypes produced three different forms with ratios corresponding to those of a Mendelian system with two alleles with incomplete dominance (25% green form, 25% black form, 50% red form). However, the different output coming from the crossing of wild red adults (no black specimens were obtained in F1) and the occasional emergence of specimens with unexpected colours indicate a more complex colour determination system. Crossing experiments carried out between bicoloured forms of *Protaetia speciosa* (ssp. *jousselini*), and red or black forms of *P. aeruginosa* produced F1 hybrids with colour intermediate between that of parents (pers. obs.).

The Chrysomelidae: a taxonomic and phylogenetic outline

With an estimated 35.000 extant species (Farrell, 1998), the Chrysomelidae account for about 10% of known beetles and are the third largest families in the whole order Coleoptera after Curculionidae s.l. and Staphylinidae.

From the phylogenetic point of view, the Chrysomelidae (including Bruchinae) are recognised as a monophyletic clade (Farrell, 1998; Gómez-Zurita et al., 2007, until recent treated as a separate family Bruchidae), and are the sister group of the Cerambycidae lineage, with which they form the superfamily Chrysomeloidea. The latter, in turn, is the sister group of the megadiverse Curculionoidea clade and these two taxa are the only components of the huge clade Phytophaga, represented by about 135.000 described species.

The vast majority of Chrysomelidae (also known as leaf-beetles) are phytophagous insects with free-living larvae. A notable exception to phytophagy is represented by the feeding habits of Camptosomata (Clytrinae, Cryptocephalinae, and allied groups), whose larvae are myrmecophilous and often at least in part myrmecophagous (Erber, 1988; Jolivet, 1992, 1995). Exceptions to the free-living habit are more widespread: Sagrinae, Donaciinae and Bruchinae are a monophyletic clade whose larvae are primarily endophagous within stems of foodplants or within seeds (the latter apply to Bruchinae, long regarded as an autonomous family) and endophytic larval behaviour arose also among members of Zeugophorinae, Criocerinae, Hispinae, and Alticinae (Jolivet, 1995). Other representatives typically feed on the green parts of plants and are usually oligophagous on a narrow range of plant species or genera. With the exceptions of very species-poor ancient groups feeding on gymnosperms, the great majority of Chrysomelidae depend on angiosperms, cases of shift back to gymnosperms being notably rare (examples for the Palaearctic fauna are few representatives of *Cryptocephalus* and *Calomicrus* feeding on *Abies* and *Picea*).

The family Chrysomelidae is currently subdivided in about 12 subfamilies, whose phylogenetic relationships have been investigated recently and are known in a rather satisfactory way. Modern cladistic analysis revealed that most of the traditionally recognised groups which are ranked around the subfamily level are true natural groups, with only few of them being paraphyletic (e.g.: Hispinae is nested within Cassidinae, Megascelinae within Eumolpinae, Chlamysinae within Cryptocephalinae) or still ambiguously placed (e.g. Synetinae, either nested within or sister to Eumolpinae) (Gómez-Zurita et al., 2005, 2007).

Among the traditional subfamilies doubtfully supported by modern phylogeny are the Chrysomelinae, which are possibly paraphyletic, although with low support (Gómez-Zurita et al., 2007), with respect to Galerucinae as long as the Timarchini and Phaedonini are included in the former (as with the traditional concept of Chrysomelinae).

Taxonomy of the Chrysomelinae and of Chrysolina *(s.l.)*

The Chrysomelinae, according to the traditional concept, are a large and well defined subfamily diffused worldwide, comprising about 2000 species arranged in about 130 to over 170 genera, according to authors (Daccordi, 1994). Generic and suprageneric arrangement of the Chrysomelinae is actually a matter of debate among the specialists: the classification of taxa above the species or group-of-species level is often highly uncertain, let aside strictly nomenclatorial problems originating from the plethora of genus-group names proposed along the years. The last generic catalogue, proposed by Daccordi (1994), lists 134 valid genera and hundreds of valid subgenera.

Within the subfamily Chrysomelinae, one of the largest group is the genus *Chrysolina*, which in its current circumscription includes about 470 species and over 250 subspecies arranged in 64 subgenera (Bieńkowski, 2001, 2007) (tab. 2). *Chrysolina* is widespread especially in the Palaearctic region, where it reaches its maximum diversity, but a significant number of representatives are found in the tropical areas of South Western Asia and throughout the whole African continent including Southern Africa. A small number of representatives is also found in North America (composed both of native and introduced species from Europe) and in Oceania, where two European and one South African species were introduced as a biological agent to control *Hypericum* and *Chrysanthemoides monilifera* weeds respectively.

The diversity of the genus, although heavily explored by the several specialists active in the last decades, is not completely known. As a matter of fact, in the few years between 2001 and 2007, as much as 22 new species were described (about 5% of the total) and at least as many are awaiting description (M. Daccordi, com. pers.). Although the greatest part of new species come from poorly explored areas of Central Asia (chiefly from China), new taxa are occasionally discovered also in otherwise well known areas (e.g.: *Ch. bourdonnei* from Southern Italy; Daccordi and Ruffo, 2004). Anyway, the alpha-taxonomy of the genus may be considered on the whole well-extablished and satisfactory, except for a limited part of the distributional range.

However, despite the huge taxonomic effort undertaken in the last years, the supraspecific taxonomy of the genus *Chrysolina* is still unsatisfactory. Alongside with several well-defined and clearly homogeneous subgenera, others exist which are strongly heterogeneous (e.g., *Pezocrosita*), whose autonomy is debated, or to which species of uncertain position are traditionally (but doubtfully) referred (e.g.: *Ch. stachydis*, doubtfully assigned to the subgenus *Taeniosticha*). Still worse, to date it is impossible to circumscribe *Chrysolina* by a comparative diagnosis capable to set it apart from the closely related genera. The recent synopsis proposed by Bieńkowski (2007) does not provide a comparative diagnosis either. In particular, the distinction between *Chrysolina* and *Oreina* is highly problematic. The main traditionally accepted distinctive character (the ratio between the length of the metasternum and the length of the first abdominal sternite) turned out to be inconsistent at a closer analysis and failed to offer a sharp division between the two genera, as outlined by Bieńkowski (2007). This is

Subgenus included in phylogeny	Subgenus colour coded in tab. 3	Subgenus	nr. of species	nr. of species + subsp.	Type species	DISTRIBUTION
TS	X	*Allochrysolina*	4	10	*fuliginosa*	Mediterranean Area, Central Europe
X	X	*Allohypericia*	15	27	*lobicollis*	Central Asia, E. Asia, Canada, USA
	X	*Altailina*	2	3	*dudkoi*	Kazakhstan, Altai
TS	X	*Anopachys*	10	12	*asclepiadis*	Eurasia (escl. Eur. Centro-occ.), Taiwan.
TS	X	*Apterosoma*	3	3	*angusticollis*	Far East, N.E. China, Japan
	X	*Arctolina*	18	18	*birulai [subsulcata]*	Central and Arctic Asia, N. America
X	X	*Atechna*	36	37	*striata*	South Africa, Congo, Angola
	X	*Atlasiana*	1	1	*seriatipora*	Algeria
1	X	*Bechynea*	2	5	*kabakovi*	China, Korea, Amur, Sakhalin, Kurili
TS	X	*Bechynia*	5	5	*platypoda*	S. France to Greece to Altai
	X	*Bittotaenia*	8	11	*salviae*	Europe, Caucaso, Middle East, Asia Minor
TS	X	*Camerounia**	8	8	*ornata*	Central and S. Africa
	X	*Cecchiniola*	1	1	*platyscelidina*	Crimea
TS	X	*Centoptera*	1	1	*regalis [bicolor]*	Mediterranean Basin
TS	X	*Chalcoidea*	30	60	*marginata*	from Europe and N. Africa to Central Asia, India, USA
TS	X	*Chrysocrosita*	5	7	*spectabilis*	China, central Asia
TS	X	*Chrysolina*	5	8	*staphylaea*	Holarctic
TS	X	*Chrysolinopsis*	1	1	*gemina*	Canary Islands
TS	X	*Chrysomorpha*	1	5	*cerealis*	Europe to Siberia
TS	X	*Colaphodes*	2	5	*hottentota [haemoptera]*	Europe to Middle East
TS	X	*Colaphoptera*	16	43	*hemisphaerica*	France to Asia Minor, Middle East
TS	X	*Colaphosoma*	1	3	*goettinngensis [sturmi]*	Europe to Siberia
TS	X	*Craspeda*	3	6	*besseri [limbata]*	Morocco, Alps, Eur. Russia to Mongolia
TS	X	*Crositops*	3	3	*pedestris*	Central Asia, Siberia
TS	X	*Diachalcoidea*	3	5	*sacarum*	N. Africa, Middle East, Central Asia
TS	X	*Erythrochrysa*	1	3	*polita*	Palearctic
TS	X	*Euchrysolina*	2	8	*graminis*	Europe to Japan
TS	X	*Fastuolina*	1	5	*fastuosa*	Europe to Siberia
1	X	*Ghesquiereita*	13	13	*spiloptera*	Central Africa
1	X	*Heliostola*	5	11	*islandica*	Alps to Siberia
TS, 2	X	*Hypericia*	14	22	*hyperici*	Palaeardtic, Australia, USA
	X	*Jacobsonia*	1	1	*pudica*	China
	X	*Lithocrosita*	1	1	*rugulosa*	Central Asia
TS	X	*Lithopteroides*	2	4	*musiva [exanthematica]*	Siberia, India, China, Vietnam, Japan, Taiwan
TS	X	*Maenadochrysa*	12	33	*femoralis*	Mediterranean Countries
TS	X	*Melasomoptera*	3	7	*grossa*	W. Mediterranean
	X	*Mimophaedon*	1	1	*pourtoyi*	Atlantic Pyrenees
TS	X	*Naluhia*	4	5	*confluens*	E.,C., S. Africa
2	X	*Ovosoma*	10	23	*vernalis*	Mediterranaean Countries to Caucasus
TS	X	*Ovostoma*	3	10	*coerulea [olivieri]*	S. E. Europe to Caucasus
TS	X	*Palaeosticta*	5	6	*diluta*	S.W. Europe, Morocco, Lybia, Middle East
	X	*Paracrosita*	1	1	*armeniaca*	Caucasus, Afghanista, Middle East
TS	X	*Paradiachalcoidea*	4	5	*vignai*	Ethiopia, Middle East, Turkey
	X	*Paraheliostola*	1	1	*soiota*	Sayan Mts.
	X	*Paramenthastriella*	1	1	*beatricis*	E. Africa
	X	*Pezocrosita*	48	51	*sahlbergiana*	Central Asia, to Mongolia and Siberia
TS	X	*Pierryvettia*	25	29	*stictica*	China, Vietnam, India, Java. Indochina, Philippines?
	X	*Pleurosticha*	7	9	*sylvatica*	Central Asia, Alaska, Hokkaido, Urals,
		Pseudocrosita	1	1	*bactriana*	Central Asia
		Pseudolithoptera	1	1	*interlucea*	Korea
TS	X	*Pseudotaeniochryse*	2	5	*superba*	Central Africa
		Pseudotimarchomim	1	1	*luminosa*	Tanzania
TS	X	*Rhyssoloma*	1	1	*fragariae*	Madeira
		Sibiriella	2	2	*paradoxa*	Altai
TS	X	*Sphaeromela*	1	3	*varians*	Europe, Siberia
1	X	*Stichoptera*	12	29	*sanguinolenta*	Europe, Turkey, Primorski, China,
TS	X	*Sulcicollis*	4	5	*chalcites*	Europe, Middle East
TS	X	*Synerga*	4	16	*bella [coerulans bella]*	Europe, Middle East, China, Siberia
TS	X	*Taeniochrysea*	1	1	*americana*	S. Europe
TS	X	*Taeniosticha*	10	22	*lurida*	Europe, middle east, Central Asia, Tien Shan
TS	X	*Threnosoma*	20	30	*helopioides*	Central Europe, Mediterranean countries
		Timarchomela	3	3	*- not designated*	China (Yunnan)
TS	X	*Timarcholina*	9	9	*templetoni*	India, Sril Lanka, Myanma
TS	X	*Timarchoptera*	1	1	*haemochlora*	Central-E.asia
TS	X	*Vittatochrysa*	1	1	*nigrovittata*	Central Asia, N.W. China

Tab. 2.
Overview of the subgenera of *Chrysolina* according to Bieńkowski (2001).
TS: type species included in phylogeny; **1**: one species included in phylogeny; **2**: two species included in phylogeny.
Notes. *****: considered as separate genus by Bienkowski (2007).

confirmed by the morphological recognition carried out in the present thesis. The distinction of *Chrysolina* from *Oreina*, however, is a matter of debate since long time (es. Garin et al., 1999), and the inclusion of the latter genus within the former one has been proposed by various authors. In addition, the boundaries of the genus are uncertain in regard to the inclusion of tropical taxa, such as *Camerounia*, grouping species from Central Africa (Bieńkowski, 2001, 2007), and is sometimes splitted within its most traditional boundaries by authors such as Bourdonnè (2005), who elevated to the generic rank the subgenus *Craspeda* including within of it *Taeniosticha* and *Palaeosticha*, a proposal which seem to be rejected by Bieńkowski (2007).

Currently, a huge revision work is being carried out by the latter author; of the planned 6 volumes the first was published in 2007; however the treatment is that of traditional taxonomy and no cladistic evaluation of the groups is performed.

Compared to *Chrysolina*, the genus *Oreina* is much smaller, including 28 species. Most of them are highly polytypic and currently about 75 subspecies are recognised as valid. The distribution range is fragmented and less extended than that of *Chrysolina*. The great majority of these taxa inhabit the European mountains, from the Pyrenees to the Balkans; a few populations are found in lowlands of Central Europe. In addition, two species are endemic of the Russian Far East. The highest diversity is reached across the Alpine range. The genus is divided into seven subgenera, some of them particularly distinctive, i.e. *Protorina* for the unusual colour and *Frigidorina* for the notably small size. On the whole, the supraspecific taxonomic assessment is well extablished.

Chrysolina *and* Oreina *phylogenetics*

Phylogenetic investigations on *Chrysolina* are very scarce and always limited to a small subset of taxa. The first phylogenetic attempt was published by Bourdonné and Doguet (1991), who proposed an rough evolutionary hypothesis for 10 groups of Palaearctic species; however, rather than using a modern cladistic approach, authors based their evolutionary tree on a subjective estimation of the evolution of two traits, the chromosomic number and the choice of the foodplant. Later, two cladistic studies of the genus *Chrysolina* were independently produced in 1999, both attempting to reconstruct the evolution of host plant affiliation. In one of these studies (Garin et al., 1999) a phylogenetic analysis was performed based on mitochondrial DNA sequences [16S rDNA and cytochrome oxidase subunit I gene (COI)]. The ingroup included 30 *Chrysolina* and 2 *Oreina* species, representing a total of 22 subgenera. The maximum parsimony trees produced for the two sets of data had a quite poor resolution, however the authors succeeded in confirming the monophyly of the subgenera that were represented by more than one species. The position of the two *Oreina* species resulted puzzling, since they appeared to be only distantly related. However, it is interesting to note that they both fall within the *Chrysolina* radiation, supporting the hypothesis of non-distinction between the two genera, as already outlined by previous authors. The

other study (Hsiao and Pasteels, 1999), was based on 12S and 16S mtDNA and CO1 sequences and applied to 30 species of the *Chrysolina-Oreina* complex (16 *Chrysolina* species belonging to 14 subgenera, 14 *Oreina* species belonging to 7 subgenera). The strict consensus tree had a poor resolution of the basal nodes, but was well resolved in the distal nodes, allowing to recognise some well supported natural groups. *Oreina* species were gathered in a strongly supported monophyletic clade, however within this clade was deeply nested *Chrysolina fastuosa*. The trees proposed by these two studies are poorly comparable, due to the scarce overlapping between the two ingroups and the poor resolution of basal nodes. Both of them agree in the close relationship between the subgenera *Synerga* and *Melasomoptera*, and the between the subgenera *Hypericia* and *Sphaeromela*, however significant differences are observed in the reciprocal placement of the subgenera *Taeniochrysea* and *Colaphodes*.

As for *Oreina*, two independent phylogenetic analyses exist, one presented by Dobler et al. (1996) and based on genetic distances of 18 allozyme loci and taking into account 12 species, and the other by Hsiao and Pasteels (1999), previously mentioned, taking into account 14 species together with several *Chrysolina*. Both trees are quite well resolved, however they differ in several aspects. In particular the allozyme tree (Dobler et al., 1996) confirm the monophyly of all of the subgenera, even though only two of them are represented by more than one species. Conversely in the tree by Hsiao and Pasteel (1999) the subgenus *Chrysochloa* turns out paraphyletic and even the monophyly of the genus *Oreina* is questioned, since *Chrysolina fastuosa* seem to fall within its radiation, its position being supported by a good bootstrap value (83). Nevertheless, the authors were strongly reluctant to accept this results, mentioning the existence “strong morphological evidences” which would contradict this hypothesis.

Chrysolina phylogeny

A single most parsimonious tree was found (l=986.79, text fig. 1). Unfortunately, despite the prolonged effort with alternative selections and coding of characters, measures of support are not comfortable: CI= 0.17, RI=2.3. Different resampling techniques applied to the most parsimonious tree produced low values for most of the branches, being as low as 0 for most of the basal nodes.

A few considerations can be issued, enhancing confidence for some of the most apical nodes. The confidence of basal nodes, conversely, has to be considered very cautiously.

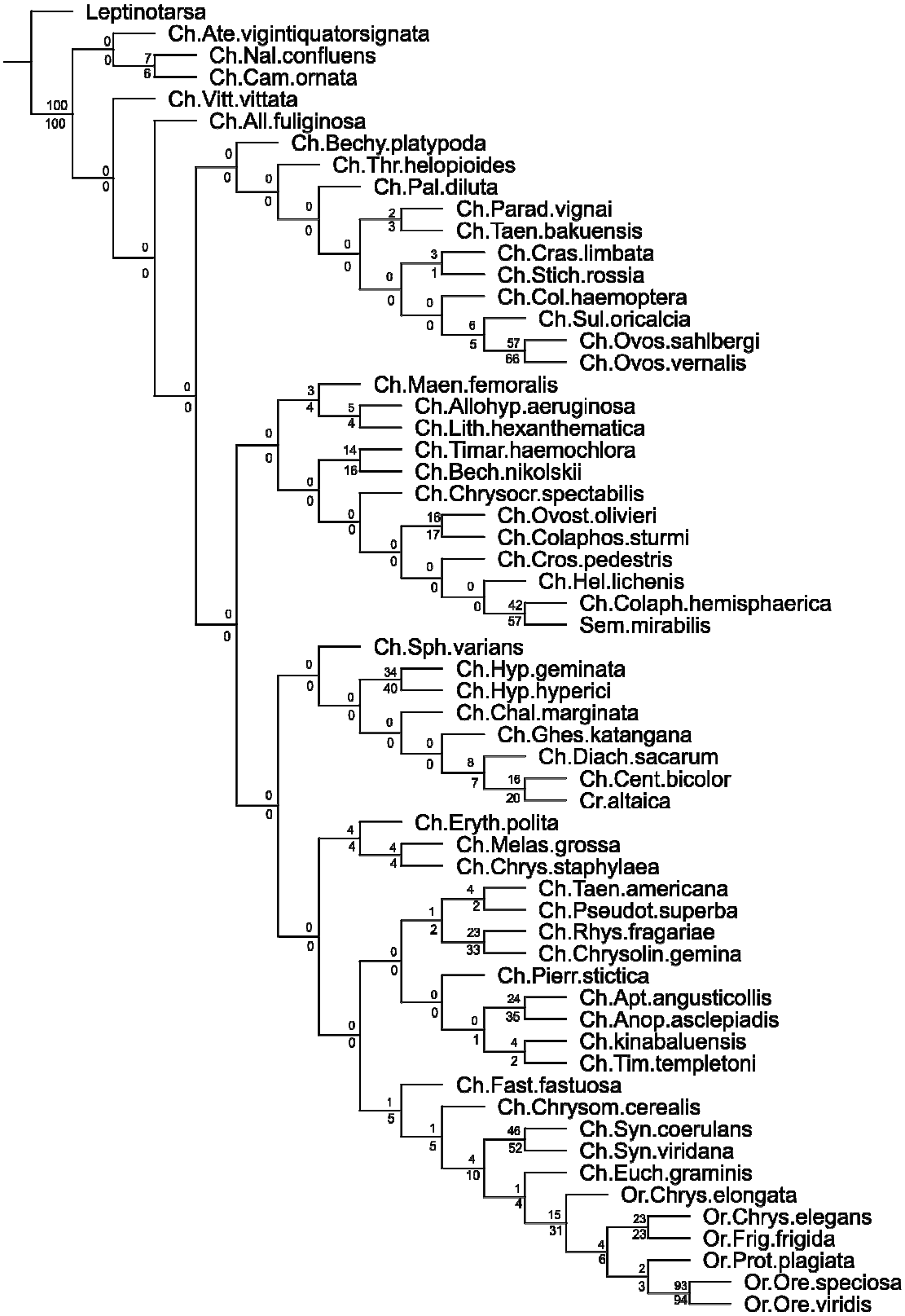
The five pairs of species traditionally referred to as many subgenera which were included in the phylogeny, branch closest to one another, in a sister-group relation, their placement thus resulting in agreement with their current taxonomic arrangement. The only exception is that of subgenus *Chrysochloa*, which turned out to be paraphyletic, thus being in agreement with results obtained by Hsiao and Pasteels (1999). In addition, the present phylogeny agrees with the traditional

taxonomy also confirmating the monophyly of the genus *Oreina*, a view which was supported also by Hsiao and Pasteels (1999).

Moreover, the arrangement of the clade *Ch. fastuosa-Oreina* is quite agreement with the phylogenetic hypothesis proposed by Hsiao and Pasteels (1999). Actually, the reciprocal arrangement of the contained taxa (*Fastuolina*, *Chrysomorpha*, *Synerga*, *Euchrysolina* and *Oreina* s.l) is different between the two trees taken into account, however they both indicate the mentioned subgenera as close relatives, together forming a monophylum. The only remarkable difference in respect to Hsiao and Pasteels (1999) is their inclusion within this group of the clade *Erythrochrysa-Melasomoptera*, which conversely fell out of it (although not far) in the present phylogeny. Another condition of agreement with all the previous studies (Bourdonné and Doguet, 1991; Garin et al., 1999; Hsiao and Pasteels, 1999) is the sister-group relation between the subgenus *Sphaeromela* (represented only by *Ch. varians*) and the subgenus *Hypericia*.

Beside confirming some of the groupments retrieved in previous phylogenies, the present tree proposes a few not obvious clades whose identity makes sense on the account of characters not considered in the phylogeny: the *Taeniochrysea-Pseudotaeniochrysea* clade includes two subgenera which have a well disjointed distribution (Mediterranean vs. Central Africa), but are notably similar in appearance and share peculiar chromatic conditions. The *Rhyssoloma-Chrysolinopsis* clade, conversely, groups two species looking rather different, but sharing (allopatric) Macaronesian distribution. The already mentioned *Fastuolina-Oreina* clade is characterised by the presence of an unique chromatic pattern (see the *fastuosa-like pattern* discussed below), and even the basal clade grouping the subgenera *Naluhia*, *Atechna* and *Camerounia*, all from tropical Africa, is characterised by the shared presence of orange/testaceous integuments carrying various pigmentary patterns that find no equal among other groups.

With reference to the latter clade, it should be said that *Atechna* + *Chrysolina* (s.l.) turned out to be paraphyletic with respect to *Leptinotarsa* according to the Chrysomelinae phylogeny of Gómez-Zurita et al. (2007). However, running a phylogeny with the exclusion of these subgenera from the data set did not produced any change within the phylogenetic output: the tree recalculated in the absence of *Atechna*, *Naluhia* and *Camerounia* (l=933.280, C.I=0.l8, R.I.=2.11) showed the very same relations between the remaining taxa, in such a way that the African clade seemed just cut off from the remainder of the tree.



Text fig. 1. Maximum parsimony phylogenetic tree of the *Chrysolina* and allied genera. Figures above nodes are bootstrap resampling values with 1000 replications, figures under nodes are jacknife resampling values with 1000 replications.

Chromatic patterns in *Chrysolina*

The results of the chromatic survey are summarized in tab. 3 (pages 48-49).
The different chromatic conditions observed on the dorsal side of investigated specimens are described and briefly discussed in the following paragraphs, with reference to names and numbers used in tab. 3. I will arrange them according to four main criteria:

1) main colour
2) elytra/forebody relations
3) physical patterns
4) pigmentary patterns

For each pattern or condition which discussed here, a descriptive name will be introduced. Within the text, these names are spelled in *italics* for the sake of clarity. For the same reason, the adjective "metallic" has been commonly used instead of the would be more appropriate term "of physical origin", since colours of physical origin are commonly and shortly referred as such.

Main colour

1-2. Black elytra/Black pronotum (figs. 70, 83-84, 86-87)
This category encompasses beetles with fundamentally black colouration and not showing any obvious coloured shine as can be perceived by human eye. Strongly dark specimens, but showing a even faint coloured reflection are classed as *metallic*. In this class are also counted beetles whose fundamental black colouration is replaced in small part by a coloured pattern, such as members of the genus *Taeniosticha* showing a *red elytral margin* pattern (fig. 82). Black coloured forms are well distributed across the investigated group and several of the subgenera where this condition was not found are the ones where the available sample was poor and/or the number of included species is particularly low (1-3) (cfr. tab. 3).

Nevertheless, subgenera where black forms are present as a common condition are rare; rather, they appear as an individual aberration (or a form within an intraspecific polymorphism) of otherwise metallic species. The relationships between black forms and conspecific metallic coloured ones will be further discussed in the chapter *The origin of physical colours and the evolution of the black phenotypes* (p. 59).

3-4. *Metallic elytra/Metallic pronotum* (figs. 72-78, 79-82)
In this class are counted all species whose dorsal side has a colour with at least a faint metallic colour, deemed to be of physical origin. Among others, I include here also dark forms with at least perceivable coloured reflections (but therefore indicating anyway the existence of a photonic structure capable to interact with light) and forms with patterned integument.

This condition is the most common one and is usually associated to a strong intraspecific polymorphism, often encompassing frankly black forms (cf. column *Black/metallic transition* in tab. 3). TEM microscopy observations carried out on some representative species revealed the existence of a photonic structure responsible for the metallic coloured effect, as will be discussed in *The origin of physical colours and the evolution of the black phenotypes* (p. 59).

5. *Rufous integument* (figs. 71-72, 86).
Within this group are included forms whose integuments have a orange, testaceous or red colouration. Among others, were included cases where the main colour of the integument is orange/red (fig. 71), even in the presence of dark pigmentary pattern (e.g.: *Ch. vittata, Ch. bruneli*, fig. 86). Forms where the orange/red parts are poorly extended (e.g., fig. 83-84) were not included.

Rufous integument are mainly associated to the *Forebody dark, elytra rufous* pattern (later discussed), being otherwise rare and scattered across various subgenera, where they mostly appear as the product of a poor/failed melanisation of the integument, sometimes as a condition species specific or at least common within a species (e.g.: *Ch. staphylaea*).

Elytra/forebody relations

6-7. Homocromy/heterocromy
I treat as homochromous those chromatic forms where the colour of the forebody (head and pronotum) and the colour of elytra are the same. Conversely, in heterochromous forms the colour of the forebody is different from that of the elytra.

Heterochromy may depend on different situations: different pigmentary colours (e.g.: *Taeniosticha, Craspeda,* fig. 86), different physical colours (e.g.: *Rhyssoloma, Chrysocrosita,* fig. 73), or a combination of physical and pigmentary colours (e.g.: *Melasomoptera,* fig. 72). The degree of heterochromy, which depends on the difference between two colours, obviously vary along a continuum; nevertheless I meant to explore through a qualitative classification, although approximative, the occurrence of strong heterochromy, i.e., of forms whose colour of forebody is heavily different from that of the elytra. The greatest part of these strongly heterochromic patterns were found to be associated to a peculiar species-specific pigmentary condition (*forebody dark, elytra rufous*), while they are notably rarer among metallic species. Nevertheless, among these, they can either appear as individual aberration (e.g.: *Oreina speciosa*) or species-specific pattern (e.g.: *Chrysolina spectabilis,* fig. 73).

Conversely, forms with low heterochromy (figs. 68-69), were found to be widespread. In fact, although most of the metallic species are commonly described as “unicoloured” or “monochromatic”, this survey revealed that this is acceptable for a general description (e.g., aiming to allow species identification), but cannot be regarded as a rule for most of the metallic-coloured subgenera. For

					MAIN COLOUR					BLACK/METALLIC transition		ELYTRA/FOREBODY RELATIONS			
					1	2	3	4	5	-	-	6	7	-	8
Genus	Subgenus	Number of species	Number of studied species	% of studied species	Black elytra	Black pronotum	Metallic elytra	Metallic pronotum	Rufous integument	Elytral transition black/metallic verified in the same sp.	Pronotal transition black/metallic verified in the same sp.	Homochr.	Heterochr.	Maximum degree of heterochr.	Forebody dark, elytra rufous
Chrysolina	*Allochrysolina*	4	4	100					-	YES	YES			low	-
Chrysolina	*Allohypericia*	15	4	27						YES	YES			HIGH	
Chrysolina	*Altailina*	2	1	50	-	-			-	/	/			low	-
Chrysolina	*Anopachys*	10	9	90					-	YES	YES			HIGH	-
Chrysolina	**Apterosoma*	3	3	100	-	-			-	/	/			HIGH	-
Chrysolina	*Arctolina*	18	11	61					-	YES	YES			HIGH	-
Chrysolina	**Atechna*	36	21	58						YES	YES			HIGH	
Chrysolina	*Atlasiana*	1	1	100	-	-			-	/	/		-	-	-
Chrysolina	*Bechynea*	2	1	50					-	YES	YES			low	-
Chrysolina	*Bechynia*	5	4	80					-	no	no			low	-
Chrysolina	*Bittotaenia*	8	5	63						YES	YES			low	
Chrysolina	*Camerounia*	8	8	100						YES	YES	?		HIGH	
Chrysolina	*Cecchiniola*	1	1	100					-	YES	YES		-	-	-
Chrysolina	*Centoptera*	1	1	100					-	YES	YES			HIGH	-
Chrysolina	*Chalcoidea*	30	30	100						YES	YES			HIGH	
Chrysolina	**Chrysocrosita*	5	2	40	-	-			-	/	/			HIGH	-
Chrysolina	*Chrysolina*	5	4	80	-					/	YES			low	-
Chrysolina	*Chrysolinopsis*	1	1	100	-	-			-	/	/			low	-
Chrysolina	*Chrysomorpha*	1	1	100	-	-			-	/	/		-	-	-
Chrysolina	*Colaphodes*	2	2	100					-	YES	YES			low	-
Chrysolina	*Colaphoptera*	16	16	100						YES	no			low	-
Chrysolina	*Colaphosoma*	1	1	100					-	YES	YES			low	-
Chrysolina	*Craspeda*	3	3	100			-		-	/	YES		-	-	-
Chrysolina	**Crositops*	3	2	67	-	-			-	/	/			low	-
Chrysolina	*Diachalcoidea*	3	3	100					-	?	?			low	-
Chrysolina	*Erythrochrysa*	1	1	100		-				YES	/	-		HIGH	
Chrysolina	*Euchrysolina*	2	2	100					-	YES	YES			low	-
Chrysolina	*Fastuolina*	1	1	100	?	?			-	/	/			low	-
Chrysolina	**Ghesquiereita*	13	9	69					-	YES	YES	?		low	-
Chrysolina	*Heliostola*	5	5	100					-	YES	YES			low	-
Chrysolina	*Hypericia*	14	14	100					-	YES	YES			low	-
Chrysolina	*Jacobsonia*	1	1	100					-	YES	YES		-	-	-
Chrysolina	*Lithocrosita*	1	1	100					-	YES	YES		-	-	-
Chrysolina	*Lithopteroides*	2	2	100						YES	YES			HIGH	
Chrysolina	*Maenadochrysa*	12	6	50					-	YES	YES		-	-	-
Chrysolina	*Melasomoptera*	3	3	100	-		-			/	YES	-		HIGH	
Chrysolina	**Mimophaedon*	1	1	100	-	-			-	/	/		-	-	-
Chrysolina	*Naluhia*	4	4	100	-	-				/	/	?		HIGH	-
Chrysolina	*Ovosoma*	10	10	100					-	YES	YES			HIGH	-
Chrysolina	*Ovostoma*	3	3	100					-	YES	YES			low	-
Chrysolina	*Palaeosticta*	5	5	100	-		-	-		/	/	-		HIGH	
Chrysolina	**Paracrosita*	1	1	100					-	YES	YES		-	-	-
Chrysolina	*Paradiachalcoidea*	4	2	50	-					/	/	?		HIGH	?
Chrysolina	**Paraheliostola*	1	1	100	-	-			-	/	/		?	?	-
Chrysolina	**Paramenthastriella*	1	1	100	-	-			-	/	/			HIGH	-
Chrysolina	*Pezocrosita*	48	29	60					-	YES	YES			HIGH	-
Chrysolina	*Pierryvettia*	25	25	100						YES	YES			HIGH	
Chrysolina	*Pleurosticha*	7	7	100					-	YES	YES			low	-
Chrysolina	*Pseudotaeniochr.*	2	4	200	-					/	YES			HIGH	?
Chrysolina	**Rhyssoloma*	1	1	100	-	-			-	/	/	?		low	-
Chrysolina	*Sphaeromela*	1	1	100	?				-	YES	YES			HIGH	-
Chrysolina	***Stichoptera*	12	12	100			-			/	YES			low	
Chrysolina	*Sulcicollis*	4	4	100		-			-	YES	YES			low	-
Chrysolina	*Synerga*	4	4	100					-	/	/			low	-
Chrysolina	*Taeniochrysea*	1	1	100	-	-			-	/	/		-	-	-
Chrysolina	*Taeniosticha*	10	7	70	-		-	-		/	/	-		HIGH	
Chrysolina	*Threnosoma*	20	20	100						YES	YES			low	-
Chrysolina	*Timarcholina*	9	8	89	-					/	YES	?		HIGH	
Chrysolina	**Timarchoptera*	1	1	100	-		-			/	YES	-		HIGH	
Chrysolina	*Vittatochrysa*	1	1	100	-	-	-	-		/	/		-	-	-
Semenovia	-	6	6	100	-	-			-	/	/			low	-
Crosita	-	9	8	89					-	/	/		-	HIGH	-
Oreina	*Allorina*	4	4	100					-	YES	YES			low	-
Oreina	*Chrysochloa*	5	5	100					-	YES	YES			low	-
Oreina	*Frigidorina*	1	1	100					-	YES	YES			low	-
Oreina	*Intricatorina*	1	1	100					-	YES	YES			low	-
Oreina	*Oreina*	9	9	100					-	YES	YES			HIGH	-
Oreina	*Protorina*	7	5	71			-	-		/	/		-	-	-
Oreina	*Virgulatorina*	1	1	100					-	YES	YES			low	-

Tab. 3. Colour conditions and colour patterns in the subgenera of *Chrysolina* and allied genera. / : coding unappliable; **-**: condition absent;**?**: condition doubtful. Notes. *****: poor sampling; ******: *Ch. stachydis* excluded due to doubts on placement. (follows in next p.)

		PHYSICAL PATTERNS							PIGMENTARY PATTERNS				
		9	10	11	12	13	14	15	16	17	18	19	20
Genus	**Subgenus**	**Areolated punctures**	**Striped patterns**	**Puncture produced stripes**	**Fastuosa-like pattern**	**Crosita-like pattern**	**Patterned pronotum, central symm.**	**Patterned pronotum, bilateral symm.**	**Red elytral margin**	**Red elytral base**	**dark punctures/ stripes/ spots**	**Vittata-like pattern**	**Rufous elytral apex**
Chrysolina	*Allochrysolina*	-	-	-	-	-	-	-	-	-	-	-	-
Chrysolina	*Allohypericia*	-	-	-	-	-	-	-	-	-	-	-	
Chrysolina	*Altailina*	-	-	-	-	-	-	-	-	-	-	-	-
Chrysolina	*Anopachys*	-		-	-	-	-	?	-	-	-	-	-
Chrysolina	**Apterosoma*	-		-	-	-	-	-	-	-	-	-	-
Chrysolina	*Arctolina*	-	-	-	-	-	-	-	-	-	-	-	-
Chrysolina	**Atechna*	-	-	-	-	-	-	-		-		-	-
Chrysolina	*Atlasiana*	-	-	-	-	-	-	-	-	-	-	-	-
Chrysolina	*Bechynea*	-	-	-	-	-	-	-	-	-	-	-	-
Chrysolina	*Bechynia*	-	-	-	-	-	-	-	-	-	-	-	-
Chrysolina	*Bittotaenia*		-	-	-	-	-	-	-	-	-	-	-
Chrysolina	*Camerounia*	-	-	-	-	-	-	-	-	-		-	-
Chrysolina	*Cecchiniola*	-	-	-	-	-	-	-	-	-	-	-	-
Chrysolina	*Centoptera*		-	-	-	-	-	-	-	-	-	-	-
Chrysolina	*Chalcoidea*	-	-	-	-	-	-	-			-	-	-
Chrysolina	**Chrysocrosita*	-	-	-	-			-	-	-	-	-	-
Chrysolina	*Chrysolina*	-	-	-	-	-	-	-	-	-	-	-	-
Chrysolina	*Chrysolinopsis*	-	-	-	-	-	-	-	-	-	-	-	-
Chrysolina	*Chrysomorpha*	-		-		-	-		-	-	-	-	-
Chrysolina	*Colaphodes*	-	-	-	-	-	-	-	-	-	-	-	-
Chrysolina	*Colaphoptera*	-	-	-	-	-	-	-	-	-	-	-	-
Chrysolina	*Colaphosoma*	-	-	-	-	-	-	-	-	-	-	-	-
Chrysolina	*Craspeda*	-	-	-	-	-	-	-					-
Chrysolina	**Crositops*	-	-	-	-	-	-	-	-	-	-	-	-
Chrysolina	*Diachalcoidea*	-	-	-	-	-	-	-		-	-	-	-
Chrysolina	*Erythrochrysa*	-	-	-	-	-	-	-	-	-	-	-	-
Chrysolina	*Euchrysolina*	-		-		-	-		-	-	-		-
Chrysolina	*Fastuolina*	-		-		-	-		-	-	-	-	-
Chrysolina	**Ghesquiereita*				-	-	-	-	-	-	-	-	-
Chrysolina	*Heliostola*	-	-	-	-	-	-	-	-	-	-	-	-
Chrysolina	*Hypericia*		-	-	-	-	-	-	-	-	-	-	-
Chrysolina	*Jacobsonia*	-	-	-	-	-	-	-	-	-	-	-	-
Chrysolina	*Lithocrosita*	-	-	-	-	-	-	-	-	-	-	-	-
Chrysolina	*Lithopteroides*	-	-	-	-	-	-	-				-	-
Chrysolina	*Maenadochrysa*	-	-	-	-	-	-	-	-	-	-	-	-
Chrysolina	*Melasomoptera*	-	-	-	-	-	-	-	-	-	-	-	-
Chrysolina	**Mimophaedon*	-	-	-	-	-	-	-	-	-	-	-	-
Chrysolina	*Naluhia*	-	-	-	-	-	-	-				-	-
Chrysolina	*Ovosoma*		-	-	-	-	-	-	-	-	-	-	-
Chrysolina	*Ovostoma*	-	-	-	-	-	-	-	-	-	-	-	-
Chrysolina	*Palaeosticta*	-	-	-	-	-	-	-			-	-	-
Chrysolina	**Paracrosita*	-	-	-	-	-	-	-	-	-	-	-	-
Chrysolina	*Paradiachalcoidea*	-	-	-	-	-	-	-		-	-	-	-
Chrysolina	**Paraheliostola*	-	-	-	-	-	-	-	-	-	-	-	-
Chrysolina	**Paramenthastriella*	-	-	-	-	-	-	-	-	-	-	-	-
Chrysolina	*Pezocrosita*		-	-	-	-	-	-	-	-	-	-	-
Chrysolina	*Pierryvettia*	-	-	-	-	-	-	-	-	-	-		-
Chrysolina	*Pleurosticha*	-	-	-	-	-	-	-	-	-	-	-	-
Chrysolina	*Pseudotaeniochr.*				-	-	-		-	-	-	-	-
Chrysolina	**Rhyssoloma*	-	-	-	-	-	-	-	-	-	-	-	-
Chrysolina	*Sphaeromela*	-	-	-	-	-	-	-	-	-	-	-	-
Chrysolina	*Stichoptera***	-	-	-	-	-	-	-			-	-	-
Chrysolina	*Sulcicollis*		-	-	-	-	-	-	-	-	-	-	-
Chrysolina	*Synerga*	-		-		-	-		-	-	-	-	-
Chrysolina	*Taeniochrysea*				-	-	-		-	-	-	-	-
Chrysolina	*Taeniosticha*	-	-	-	-	-	-	-					-
Chrysolina	*Threnosoma*	-	-	-	-	-	-	-		-	-	-	-
Chrysolina	*Timarcholina*	-	-	-	-	-	-	-	-	-	-	-	-
Chrysolina	**Timarchoptera*	-	-	-	-	-	-	-	-	-	-	-	-
Chrysolina	*Vittatochrysa*	-	-	-	-	-	-	-	-	-			-
Semenovia	-	-		-	-	-	-		-	-	-	-	-
Crosita	-		-	-	-			-	-	-	-	-	-
Oreina	*Allorina*	-	-	-	-	-	-	-	-	-	-	-	-
Oreina	*Chrysochloa*	-		-		-	-		-	-	-	-	-
Oreina	*Frigidorina*	-	-	-	-	-	-	-	-	-	-	-	-
Oreina	*Intricatorina*	-	-	-	-	-	-	-	-	-	-	-	-
Oreina	*Oreina*	-		-		-	-		-	-	-	-	-
Oreina	*Protorina*	-	-	-	-	-	-	-	-	-		-	-
Oreina	*Virgulatorina*	-		-		-	-		-	-	-	-	-

(follows from previous page)

occurs as aberration in single specimens (atypical colour form)

occurs regularly, but uncommon and/or distributed among few species

regularly occurs as a common/typical condition

the greatest part of these, in fact, a careful examination of several specimens under uniform, diffused light, revealed the existence of specimens showing at least a faint discrepancy between the colour of pronotum and that of elytra.

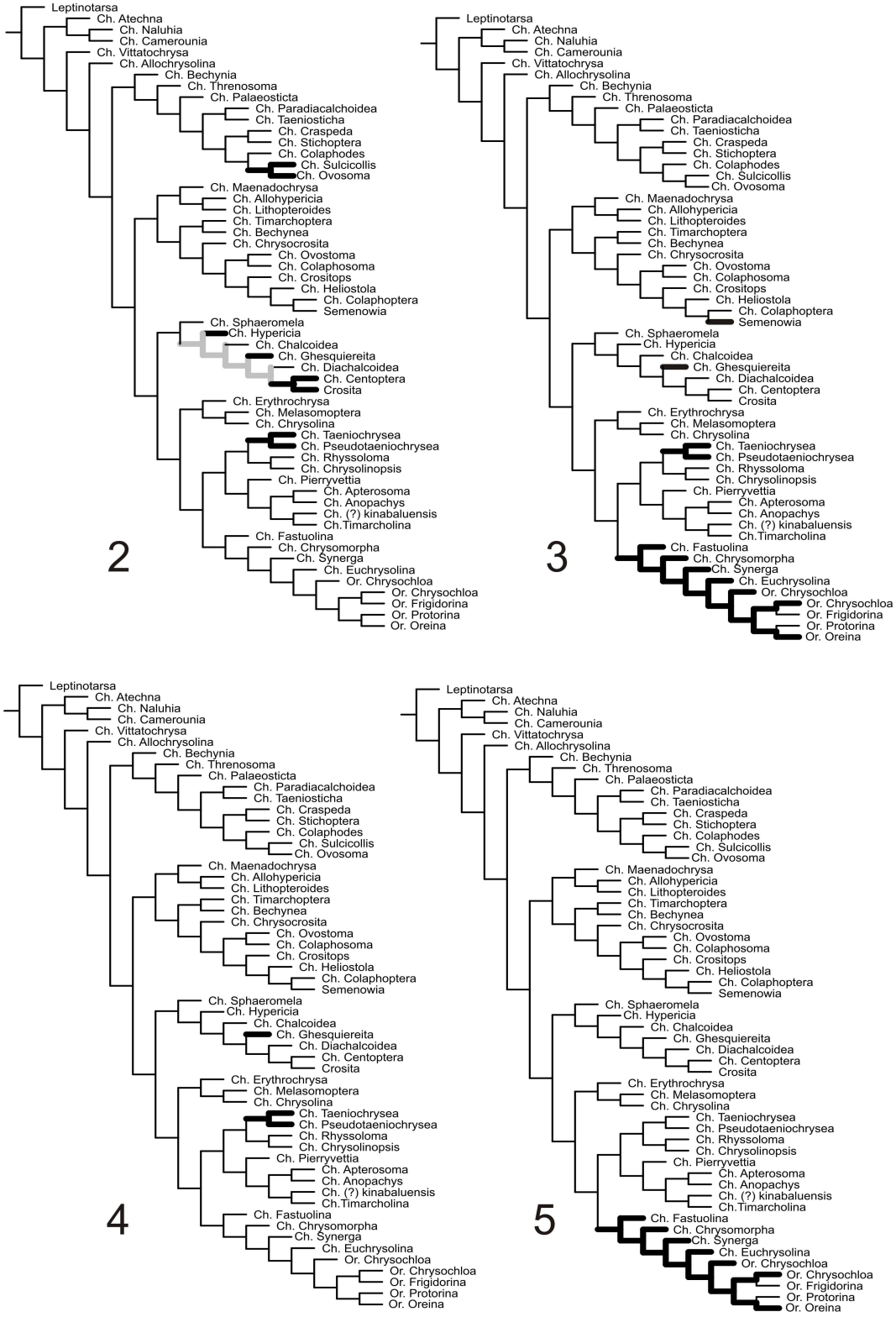
Such specimens with inconspicuous heterochromy are usually scattered among large series of actually homochromous ones (or at least so for the human perception, figs. 66-67); however, their presence is relevant since it indicates that the two parts of the body (forebody and elytra) can be controlled independently, and that this capability is commonly (perhaps always?) mantained also in clades or species having a substantially homochromous body pattern.

8. Forebody dark, elytra rufous (figs. 72, 86)
Extremely heterochromic pattern, characterised by rufous elytra and black or dark metallic pronotum. This pattern occurs quite scattered among several, where it is commonly found as the typical condition of one or more species. It normally do not appear in form of aberration and, where present, it is poorly subject to individual variations. Doubts in the attribution of specimens to this form may come from the presence of a coloured metallic shine, which in some species may be well perceivable above a light-coloured reddish background. Aberrations are rare too, although black coloured specimens are recorded at least in the ordinarily red *Ch. polita* (Porta, 1934).

Physical patterns

9. Areolated punctures (figs. 74-76; text fig. 2)
Patterns produced by the presence of coloured circles scattered more or less uniformly on the elytra. These metallic circles are invariantly associated to sharp impressions (punctures) of the integument (fig. 39, see also *The elytron: vein patterns, punctuation and sculpture,* p. 26), and in particular to those which are classed as "second order punctuation of the second order" in the list of characters compiled for the phylogenetic study (cf. *Appendix 2*, p. 101). Usually, punctures characterizing this pattern are larger than the first order punctures, which are always present together and are not associated to a colour alteration; however, exceptions exist and demonstrate that a comparatively larger size is not necessary and not sufficient for the puncture to be associated to the presence of a coloured areola. In fact, large punctures on metallic integuments lacking an areola are found among members of the subgenus *Diachalcoidea*, while second order punctures associated to a coloured areola but not different in size from the first order punctures are observed in specimens of *Ch. ruandana*. However, in specimens where punctures *do* associate to a coloured areola, all the second order punctures of the elytra are invariably involved – no exceptions allowed.

This pattern is widely distributed among *Chrysolina* species: it is found among 10 subgenera and is considered as common or most common condition among 6 of them; its presence is normally species-specific. According to the evolutionary tree proposed here, this pattern arose independently at least five times



Text figs. 2-5.
2. Occurrence of *areolated punctures*. **3**. Occurrence of *striped pattern*. **4**. Occurence of *puncture-produced stripes*. **5**. Occurrence of *fastuosa-like pattern*.

in the evolution of the investigated taxa, but it must be taken into account that two subgenera where it occurs are not included in the phylogeny.

A peculiar condition produced by areolated punctures is discussed later as a pattern of its own, under *puncture-produced stripes*.

10. Striped patterns (figs. 76-79; text fig. 3)

In general, chromatic patterns characterised by the presence of longitudinal stripes having colour different from that of the background. This class is heterogeneous, and the chromatic patterns of its members can be referred to two distinct phenomena, corresponding to at least two different control systems which can be alternatively used to produce patterns with similar appearance. Thus, longitudinal coloured stripes can be produced by the cohalescence of metallic circles densely aligned in longitudinal rows (see *areolated punctures*), or may be associated to the elytral veins, in this case each stripe being itself the most elementary pattern unit.

In both cases, despite the outlined differences in the basic components, both patterns owe their longitudinal shape and position to the veins, since these act as landmarks also for the alignment of punctuations.

All species with a longitudinally striped pattern either show *puncture produced stripes*, or a *fastuosa-like pattern*, two conditions which are separately treated in the following paragraphs. The only putative exceptions are *Ch.* (*Semenowia*) *mirabilis*, whose condition was impossible to evaluate since its elytra were too thick to be properly observed in transmitted light, and striped members of the clade *Anopachys-Apterosoma* (e.g. *A. lineigera*), which were unavailable to direct study but whose stripes, based on descriptive literature available, are certainly referrable to a vein-associated pattern, and therefore similar to the *fastuosa-like pattern*, although less conspicuous.

11. Puncture-produced stripes (fig. 76; text fig. 4)

A pattern produced by the longitudinal alignment of tegumentary impressions (punctures) surrounded by a coloured areola (see *areolated punctures*), which cohalesce in a longitudinal stripe.

This condition was found in three subgenera only, namely *Taeniochrysea* and *Pseudotaeniochrysea*, where its occurrence is ordinary and verified in all species, and *Ghesquiereita*, where its occurrence is occasional and limited to some populations of *Ch. spiloptera* only (currently named as the infrasubspecif form *upembae* Jolivet, 1952 but possibly belonging to a distinct taxon; Daccordi, 1982 and pers. comm.). The first subgenus has Southern European-Mediterranean distribution, while the other two are tropical taxa distributed in subsaharian Africa. Despite this distribution, phylogenetic analysis revealed a close and well supported relationship between the first two genera, which apparently inherited the pattern from a common ancestor. *Ghesquiereita* (fig. 75), conversely, appear to be quite distant from this clade; it belongs to branch to which other taxa with pattern characterised by the presence of discrete metallic areolae can be referred, although it is not clear if they shared a common ancestor. However, this situation

is in agreement with the occasional occurrence only of the discussed pattern within *Ghesquiereita* itself, where it arises from an increased density of otherwise scattered punctures, thus leading to a phenomenon of convergence towards the typical pattern of *Taeniochrysea* and *Pseudotaeniochrysea*.

12. Fastuosa-like pattern (figs. 77-79; text fig. 5).
The definition of this elytral pattern is based on the appearance of *Chrysolina fastuosa* (fig. 78), whose pattern can be more or less marked (contrasted) but, when visible, is invariable in its structure. Basically, on a blue to green background there are two longitudinal stripes, fused at the base and convergent at the apex, whose colour wavelength is longer than that of the background, going from green to red. These stripes occupy the submarginal internal and external areas of the elytra, while the background colour is preserved along the margins and along the midline. A few variations are observed, ranging from longitudinal stripes being more or less faded (specimens of *Ch. cacaliae*), or, conversely, being very wide and leaving only a narrow background area along the midline (such as in *Oreina gloriosa*).

This pattern is observed, with a few variations discussed later, within 16 species only, traditionally referred to the genera *Chrysolina* and *Oreina* and distributed among eight different subgenera. In spite of traditional taxonomy, phylogenetic analysis indicates that all these forms belong to a monophyletic clade with support values different from zero, mostly in agreement with previously produced molecular phylogenies.

This result is particularly relevant, since it suggest that this pattern has most probably appeared only once in the evolutionary history of the treated group. This is obviously reflected in the remarkable chromatic uniformity of species traditionally regarded as distantly related, such as *Oreina virgulata*, *Chrysolina (Euchrysolina) graminis* and *Chrysolina (Fastuolina) fastousa* (the latter until recent attributed to a genus of its own, *Dlochrysa*). The hypothesis of a single origin for this pattern is also well consistent with its absolute uniqueness: in spite of its relatively simple geometrical architecture, this model of colouration is almost unique among beetles. Metallic integuments carrying well-defined striped pattern are rare: few examples may be cited among the Meloidae (*Lytta*), Chrysomelidae (*Chrysochroa*), Rutelidae (*Mimela*).

13. Crosita-like pattern (figs. 80-81)
The definition of this elytral pattern is based on a few *Crosita* species, such as *C. altaica* (fig. 81). Each elytron is occupied by a chromatic gradient with radial symmetry, with longest wavelength in the discal area and shorter wavelenegth along margins (base and apex included).
This pattern, which can be observed on the pronotum as well (see *patterned pronotum*), was observed only in two genus-level taxa, *Crosita* and *Chrysocrosita* subgenus of *Chrysolina* (fig. 80), as already mentioned by Mikhailov (2008). Phylogeny seems to confirm the partition between these two taxa, therefore suggesting that this chromatic condition arose two times, independently. The poor

The *fastuosa –like pattern* and its variations
(figs. 88-93)

A synoptic table describing the occurrence of the *fastuosa-like pattern* within all the species of the *Fastuosa-Oreina* clade is given in the table (next page). All species of *Oreina* displaying physical colours are listed, including those belonging to subgenera not taken into account in the present phylogeny but reliably belonging to a monophyletic *Oreina* clade according to the literature (Hsiao and Pasteels, 1999; Dobler et al., 1999). Apart from the subgenus *Protorina*, where physical colours have been completely lost and the criterion of presence/absence of the physical pattern cannot therefore be applied, we see that the capability to produce the *fastuosa-like pattern* has been retained by most of the members of the clade. This capability has been repeatedly lost: once in two out of three members of the *Synerga* clade (*Ch. herbacea* and *Ch. viridana*, closely related each other and to the patterned *Ch. coerulans*, cfr. text fig. 1), and at least once in members of the *Oreina* (s.l.) clade. Actually, *Oreina* members missing the striped pattern belong to as many as four subgenera, which seem not to belong to a monophyletic clade according to the available phylogenies. Therefore, the loss has quite likely occurred four or more times in the genus *Oreina* itself.
This repeated loss of the *fastuosa-like pattern* is not surprising, since its presence is strongly variable at a very low taxonomic level. In fact, in all species displaying the pattern there are also monochromatic forms, occurring either as individual specimens more or less frequently scattered among others (e.g.: *Oreina speciosa*) or with a defined geographical trend (e.g.: *Ch. coerulans*, whose striped forms are mainly typical of the eastern populations/subspecies). The only putative case of absence of a monochromatic form is found in *O. genei* but, due to its rarity, I could only study a small number of specimens, which is not sufficient for a firm statement.

The *fastuosa-like pattern* is rather conservative in its general shape. Normally, at least two major longitudinal elements, with colour shifting toward the red end of the spectrum, are recognized; one superimposed to the innermost vein (Cu), the other superimposed to the most external vein (Sc), in a topological relationship suggesting induction of the pattern from the veins. The behaviour of the two other veins M and Rs (comprised between Cu and Sc), as well as the extension of the two main longitudinal elements is variable, according to a distinct species-specific trend and to minor individual variation. In *Chrysolina cerealis* (see fig. 88) M and Rs often behave as the other two veins (although inducing less wide stripes), the whole pattern appearing as composed of four distinct elements parted from each other by a blue “background” stripe. This pattern is only known to occur in this species. However, specimens with a pattern with more or less confluent stripes occur quite commonly too, the background being partially “obliterated” by the red-shifting pattern. The complete obliteration of the intervein spaces Sc-Rs and M-Cu is, instead, mostly a rule in *Oreina gloriosa* (fig. 89), where the striae are usually green, and never reaching the red colour. In this species, all veins undertake a induction-like relationship with the colour pattern, but the two major elements (corresponding to Sc and Cu) are extremely wide and fuse with the thinner elements produced by the two minor veins, thus leaving a single free intervein space in the form of a narrow blue stripe. The would-be inductive behaviour of M and Rs is often missing: each one of these can be completely “inactive” (in other words, showing no relation with the colour pattern), or correspond only to a weak/incomplete longitudinal stripe, the latter case appearing usually scattered among individuals where

the same trachea seems has no relation with the colour pattern at all. Lack of pattern elements corresponding to the trachea Rs is typical of *O. speciosissima* (fig. 90), whereas an only partial correspondance with M may be observed in *O. alpestris* (fig 91). The latter species often shows lack of correspondance with colour both for M and Rs, its pattern being related to the two main veins (Cu and Sc) only. This condition is also typical of *Ch. fastuosa*, where the two coloured stripes are usually expressed with unsharp definition.

However, all the mentioned patterns are referrable to the same "architecture", only differing in the expression of the constitutive modules.

Apart from these, two major deviations are found, each in a single species, namely *O. genei* and *O. liturata*. In the first one (fig.92), a single red stripe is found exactly between the veins M and Rs. These two veins do not even look as being laterally well superimposed to the pattern: they rather appear to act like a boundary between the red area and the green area. The pattern of *O liturata* (fig.93), look rather like the background (blue-black) and the stripes (green to gold) were inverted. However, despite the sharp definition of the stripes, there is no precise coincidence nor a boundary relation between any of the colour stripes and the vein system. These two pattern, despite the "striped" appearance, are fundamentally diverse from the normal condition of the *fastuosa-like pattern*. However, since they are nested within a clade characterized by that pattern, their are likely to have evolved from it, and to share a similar morphogenetic process.

Species	Striped form	Not striped form	Background colour shortest wavelenght	Background colour longest wavelenght	Stripes colour shortest wavelenght	Stripes colour longest wavelenght	Black form
Oreina (Allorina) bidentata	NO	**YES**	dark blue	orange	-	-	**YES**
Oreina (Allorina) caerulea	NO	**YES**	dark blue	green	-	-	**YES**
Oreina (Allorina) canavesei	NO	**YES**	dark blue	green	-	-	**YES**
Oreina (Allorina) collucens	NO	**YES**	dark blue	blue	-	-	NO
Oreina (Chrysochloa) cacaliae	**YES**	**YES**	dark blue	green	light blue	gold	**YES**
Oreina (Chrysochloa) elongata	**YES**	**YES**	dark blue	green	gold	gold	**YES**
Oreina (Chrysochloa) fairmairiana	?	**YES**	dark blue	green	-	-	**YES**
Oreina (Chrysochloa) genei	**YES***	NO	green	green	orange	red	**YES**
Oreina (Chrysochloa) speciosissima	**YES**	**YES**	dark blue	green	light blue	red	**YES**
Oreina (Frigidorina) frigida	NO	**YES**	dark blue	red/bronze	-	-	**YES**
Oreina (Intricatorina) intricata	NO	**YES**	dark blue	green	-	-	**YES**
Oreina (Oreina) alpestris	**YES**	**YES**	dark blue	green	light blue	dark red	**YES**
Oreina (Oreina) bifrons	**YES**	**YES**	dark blue	red/bronze	light blue	gold	**YES**
Oreina (Oreina) gloriosa	**YES**	**YES**	dark blue	blue	light blue	orange	**YES**
Oreina (Oreina) liturata	**YES***	**YES**	dark blue	blue	green	gold	**YES**
Oreina (Oreina) speciosa	**YES**	**YES**	dark blue	green	light blue	dark red	**YES**
Oreina (Oreina) redikortzevi	NO	**YES**	green	bronze	?	?	**YES**
Oreina (Oreina) sulcata	**YES**	**YES**	dark blue	purple/red	light blue	red	**YES**
Oreina (Oreina) viridis	**YES**	**YES**	dark blue	purple/red	gold	gold	**YES**
Oreina (Virgulatorina) virgulata	**YES**	**YES**	dark blue	green	light blue	red	**YES**
Chrysolina (Fastuolina) fastuosa	**YES**	**YES**	dark blue	green	light blue	red	?**
Chrysolina (Euchrysolina) graminis	**YES**	**YES**	dark blue	green	green	red	**YES**
Chrysolina (Euchrysolina) virgata	**YES**	?	dark blue	red	green	red	?
Chrysolina (Synerga) coerulans	**YES**	**YES**	dark blue	blue	light blue	dark red	NO
Chrysolina (Synerga) herbacea	NO	**YES**	dark blue	red	-	-	NO
Chrysolina (Synerga) viridana	NO	**YES**	green	red	-	-	**YES**
Chrysolina (Chrysomorpha) cerealis	**YES**	**YES**	dark blue	green	light blue	dark red	**YES**

Occurrence and characteristics of the *fastuosa-like pattern* in the species of the *Fastuolina-Oreina* clade, with exclusion of the subgenus *Protorina* (lacking metallic colours), and of *O. ganglbaueri*, unavailable for study. Data mainly directly observed and partly retrieved from Binaghi (1973), Mallet (1933), Mikhailov (2001, 2008) and Porta (1934).

-: condition not appliable; ?: condition doubtful due to poor material available.

* strongly modified *fastuosa-like* pattern (see details in text).

** very specimens dark of *Ch. fastuosa* are known, but none was described as completely black.

support of the phylogenetic tree obtained requires to be cautious, but a comment can be issued: according to the presently proposed phylogeny, Crosita seem to have originated from a metallic-coloured *Chrysolina* group including forms with *areolated punctures* pattern, and to be sister of *Chrysolina bicolor*, which has a strongly different pattern (similar to *Ch. vernalis*, fig. 74). In addition, their overall look is quite different from each other as well, and it is therefore possible that the position of *Crosita* on this phylogeny will reveal not consistent. Possibly, *Crosita* and *Ch. bicolor* were put together on the basis of convergent traits, such as the large body size and their unusual bare foot.

14-15. Patterned pronotum (*central or bilateral symmetry)* (figs. 76-81)
Patterns characterised by a polychromous patterned pronotum. Both alternatives are strictly associated (apparently, at the individual level) to the presence of pattern on elytra.

The pattern with central symmetry has the same taxonomic distribution as, and is always found in association with, the *Crosita-like pattern* of elytra, to which it is geometrically identic (figs. 80-81). However, the presence of the *Crosita*-like pattern does not imply the occurrence of a patterned pronotum, as demonstrated by *Ch. spectabilis*, its elytra are ordinarily patterned in red and green (*Crosita-like pattern*), while its pronotum is uniformly blue (fig. 73).

The pattern with bilateral symmetry (figs. 76-79) occurs only, and in all groups showing a striped pattern, either due to "true" stripes, or to puncture-produced stripes. The only exceptions are represented by *Ghesquiereita* (fig. 74) (which is not closely related to any other striped taxon) and possibly by *Anopachys*, whose patterned species were not available to study. As with the previous one, the expression of this pattern is always linked, at the individual level, to the presence of an elytral pattern; conversely, and again as for the previous one, the presence of an elytral pattern does not necessarily imply the presence of the patterned pronotum (at least in some taxa).

In fact, the relation between the pattern of elytra and the symmetrical pattern of pronotum varies according to the group taken into account: a biunivocal relation is observed in the *Taeniochrysea-Pseudotaeniochrysea* clade, in *Ch. cerealis* and *Ch. coerulans*, the latter two showing, in addition, a strict uniformity in the intensity of pattern expression across the two different body parts (cfr. fig. 79). However, in other groups such as the subgenus *Oreina* (*Oreina*), the pronotal pattern expression is always low (and sometimes missing), even in the presence of strongly patterned elytra (cfr. fig. 77).

The appearance of the symmetrical pattern is rather variable (intensity of expression apart), it shape being variable across the different taxa. In all clades except *Oreina*, it shows a shift from the blue end of spectrum towards the red one on two areas on each side, a para-medial area and the marginal area, which often are cohalescent along the anterior border and the pronotum midline. The sharpest expression is observed in *Ch. cerealis*. In *Oreina*, the expression is less defined and slightly different: it can be observed only along the lateral (inflated) sides, but

the para-medial areas are commonly not perceivable. In some cases (e.g.: *O. cacaliae*) a limited colour change (not going beyond green) is observed on the whole surface of pronotum but for the basal area, which remain blue.

Pigmentary patterns

16-17. Red elytral margin / red elytral base (figs. 83-84; text figs. 6-7)

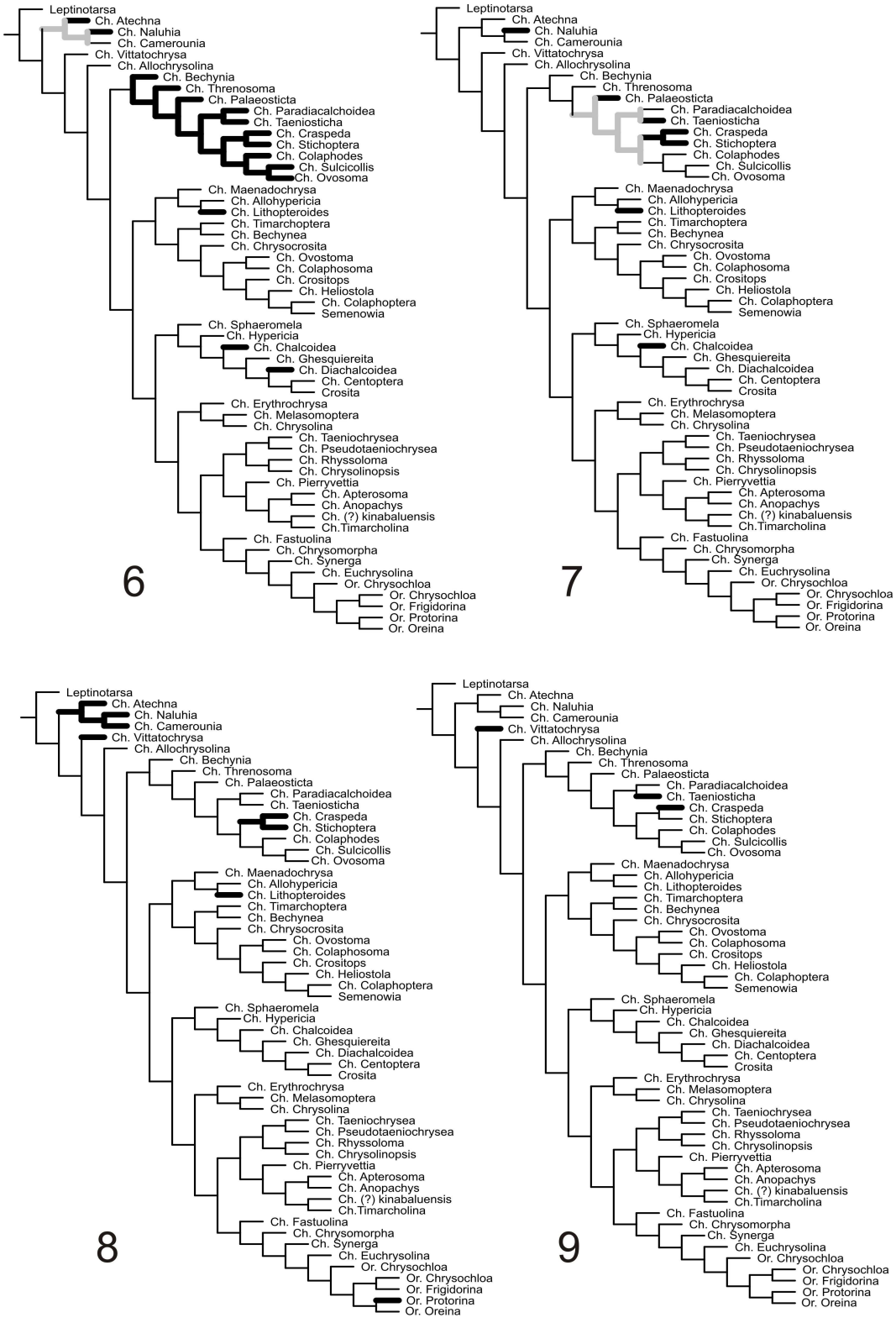
Patterns characterised by the presence of a red stripe running along the lateral edge of elytra (from the humerus to the apex, covering also the elytral epipleura), the rest of the surface being either black or metallic (fig. 84). The "red" stripe can actually be orange, or testaceous, however its precise colour hue has little meaning when observed in dead specimens, as mostly available for this study, since the original life colour often fades when exposed to solvants (such as the ethyle acetate, commonly used to kill beetles) and/or during drying process.

This pattern is widespread in several groups and, according to the present phylogeny, it seems to have arisen independently at least 5 times. Although the poor support of this phylogeny imposes caution, the hypothesis of a multiple origin is in agreement with the common occurrence of this pattern among other genera of Chrysomelinae, such as *Hydrothassa* (palaearctic) and *Microtheca* (neotropical). Within the ingroup itself, this pattern can be produced by two distinct "architectures" converging in a similar output: one characterised by the pattern "as a whole", and not composed by discrete subunits (e.g., members of *Craspeda*), the other (observed only in *Naluhia*) where the pattern is composed by the cohalescence of melanic spots originating around punctuations, which are lacking on the most external side of the elytron, therefore appearing as a reddish-orange lateral stripe.

The *red elytral base* pattern (fig. 85) is observed across most of the groups with the red elytral margin, and is always associated to the presence of the red margin itself, of which it seem to be a continuation. I was unable to observe any specimen having a red elytral base but lacking red elytral margin.

18. Dark punctures/stripes/spots (figs. 85-86; text fig. 8)

This is a heterogeneous class of patterns, defined by the presence of dark elements over rufous elytra. These pattern are very poorly distributed in the investigated group; their rarity and their reciprocal difformity justifies the treatment as a whole (apart from the *vittata-like pattern*, discussed later). Pigmentary patterned elytra are mostly found among the basal African clade composed by the subgenera *Atechna*, *Naluhia* and *Camerounia*, where they occur in a variety of shapes and undertake different relations to the internal structure of the elytra. Patterns observed among these species have no equal in other *Chrysolina*. Among these African species, a case where large dark spots are associated to (induced by?) punctuations (*Ch. confluens*) has been illustrated and briefly discussed in *The elytron: vein patterns, punctuation and sculpture*, p. 26, while a case where several dark spots cohalesce and produce a *red elytral margin* pattern (e.g.: *Ch. simonsi* "form C") was discussed under *Red elytral margin* / *red elytral base*.



Text figs. 6-9.
6. Occurrence of *red elytral margin* pattern. **7**. Occurrence of *red elytral base* pattern. **8**. Occurence of *Dark punctures/stripes/spots*. **9**. Occurrence of *vittata-like* pattern.

Among other cases, a mention is deserved for the Mediterranean *Chrysolina variolosa*, whose aspect is unique among *Chrysolina* other than the discussed African clade. The pattern of. *Ch. variolosa* is identical, for the structure and the aspect, to the *areolated punctures* pattern, but for the fact that integument punctuations are associated to pigmentary (melanic) areolae instead of physical coloured areolae.

19. Vittata-like pattern (fig. 86; text fig. 9)
A pattern characterised by a rufous elytral background, with an elongated black spot in the discal area (without any evident relationship with anatomical characters) and a black sutural stripe.

This pattern is very rare and was observed only in three species, having no strong reciprocal affinities: *Ch.* (*Vittatochrysa*) *vittata*, belonging to a monospecific subgenus, *Ch. bruneli* (fig. 87) a species of uncertain position (Bieńkowski, 2001) temptatively assigned to the subgenus *Craspeda* by various workers (Bourdonné and Daccordi, pers. comm.), and *Ch*. (*Taeniosticha*) *koktumensis*, whose problematic taxonomic placement has recently been addressed by Bieńkowski (2001). This pattern always occur as the typical colour form and seem poorly subject to aberrations. A similar pattern is shown also by other Chrysomelinae, such as members of the Palaearctic genera *Entomoscelis* and *Prasocuris*, which are however only distantly related with the group taken into account in this study.

20. Rufous elytral apex (fig. 87)
This is characterised by a completely black body, with the apical half of elytra turning gradually rufous towards the apex. A pattern only occurring in the subgenus *Allohypericia*, where it was observed in *Ch. aeruginosa*, as an individual form mixed to black and metallic forms. The fact of being characterised by a pigment gradient, and not by the presence of well defined pattern elements suggested a treatment of its own rather than the inclusion within *dark punctures/stripes/spots*.

The origin of physical colours and the evolution of the black phenotypes

One of the aims of this research was to investigate the proximate reason for the "metallic" physical colours which are very common into the investigated group. Cross sections of the elytra of different species of *Oreina* and *Chrysolina* revealed the presence of a cuticular multistratum in the outer 1-1.5 μm of the cuticle, composed of two kinds of alternating layers, one being of electron-lucent the other of electron-dense material (figs. 94-95). The number of alternating layers vary across the different species and, to a lower extent, within different regions of the same elytron (± 2 layers). The lowest number of layers was found in *Chrysolina americana* and *Ch. confluens* (5 layers), the highest in *Oreina alpestris* (12-13 layers).

This multilayer was recognised as the structure responsible for the production of the physical colours, since a) its position, structure and appearance closely match those observed by other authors on other beetles, including the chrisomelid *Plateumaris sericea* (Kurachi et al., 2002); b) their thickness is close to that expected for multilayers producing the expected wavelength; c) no other structure deemed to interaction with light could be observed in the rest of the elytron.

Actually, explorative measurements of individual layers did not allow to retrieve a perfect agreement with the wavelength value expected from the chromatic appearance of the specimen, however this is not a surprise. In fact, the equation mentioned in *Physical colour: multilayer reflectors* (p. 16) can be successfully applied to an ideal multilayer only. But, differently from the latter, actual multilayers are composed of two different media and have layers' thickness irregular and variable from one layer to the other, so that obtaining a reliable measure would require quite an extensive set of measurements over different sections. In addition, deformations during TEM sessions should be taken into account (Neville and Caveney, 1969), as well as the fundamental fact that the application of the equation require the knowledge of the exact refractive indexes of the two media, whose values are actually unknown and often uncritically inferred by authors from the few original data available in the literature. However, as mentioned at point b), measurements do not match the expected values exactly, although very closely. For example, in red elytra of *O. alpestris* I got measurements of 67-80 nm (mean: 75.2; N=12) for dark layers, and 80-102 nm (mean: 86.7; N=12) for light layers, in comparison with 52-92 nm and 72-111 nm respectively in *Chrysochloa* (Noyes et al. 2007), just to mention a very recent and accurate study. Mathematical models applied to the mean values would require, in order to get a wavelength above 600 nm (i.e., orange to red colour), a refractive index above 2 for electron-lucent layers and above 1.75 for electron dense, two values which are about 20% higher than those usually measured.

Investigations on differently coloured specimens of a polymorphic species, such as *Oreina alpestris*, allowed to confirm that the intraspecific chromatic differences are due to small variations in the thickness of the epicuticle layers. The very same mechanism turned out to be responsible for the elytral patterning of polychromous specimens, as demonstrated by the observation of samples from differently coloured areas of a striped specimen (fig. 96). Measurements from samples of different colours vary in agreement to prediction: the layers of the green integument of *O. alpestris* are slightly thinner in comparison to the red integument, with measures of 54-73 nm (mean: 63.8) in dark layers and 54-83 nm (mean: 70) in light layers, to be compared with 50-60 nm and 65-92 nm, respectively, in *Chrysochloa* (Noyes et al. 2007).

These data are in perfect agreement with information available from the literature, which is however notably poor for the whole Chrysomelidae family: beside investigations on the switching reflectors of Cassidinae outlined in *Reversible colour* change (p. 23), the only other data about devices producing physical colours were recently produced by Kurachi et al. (2002; same data

proposed in Hariyama et al., 2002), who investigated the elytral ultrastructure of *Plateumaris sericea*, (subfamily Donaciinae). The *Chrysolina* clade parted from the clade leading to Donaciinae no less than 65 million years ago (cfr. Gómez-Zurita et al., 2007), nevertheless the photonic structure responsible for the physical colours produced has remained the same in the two groups. The mechanism leading to intraspecific colour variation in *Plateumaris* was the same as in *Chrysolina*. Present data are also in agreement with the only other account on the ultrastructure of differently coloured areas belonging to the same individual, which was published during the development of this thesis for the mentioned *Chrysochloa* buprestid (Noyes et al., 2007).

Observations on polymorphic species were also directed towards an understanding of the origin of the black forms, which are common across the whole ingroup, with frequence going from the occasional variation at the individual level to the typical, invariant colour of a species (see also tab. 3, column *Black/metallic transition*)

Black individual forms are particularly frequent among members of the genus *Oreina* (fig. 70), which are otherwise characterised by remarkable bright metallic colours, often patterned by polychromous stripes (*fastuosa-like pattern*). Such black individuals, often referred to as "melanic" or "melanized" specimens, are rather rare, nevertheless they are known among all the *Oreina* subgenera and many - if not all - of the species, where they were often given allusive names such as *O. viridis* f. *lugubris*, or *O. speciosissima* f. *nigrescens*. An extremely rare dark form (f. *carbonaria*) is known even for a species traditionally regarded as chromatically invariable such as *Ch. elegans* (Binaghi, 1973). Occasionally, the black colour may become the rule, as with *O. alpestris nigrina*, an invariantly black subspecies of an otherwise bright coloured species.

From a theoretical point of view, black colour cannot be explained as the product of a multilayer photonic structure, since it is not a reflected colour, but instead the visual effect of the absence of reflected light; therefore it cannot be compared to the other colour morphs. In addition, comparative observations through transmitted light of elytra from different colour morphs suggest that black specimens do not contain additional amounts of melanic pigment. In fact, the testaceous colour observed in transmitted light is absolutely comparable among elytra of different colours morphs (as defined under reflected light), including black specimens (figs. 97-98); therefore the latter cannot be accounted as "more melanised" than the others.

Cross sections of the elytra of different specimens and different species of *Oreina* revealed that black morphs of polymorphic species are invariantly associated to a peculiar and up to date undescribed condition of the epicuticle (figs. 99-102). As shown in the figures, the layers ordinarily composing the multistratum in coloured specimens are replaced by a disordered granular structure. The latter is composed of two different media which quite likely are the same materials which are normally arranged in regular cuticular layers. Apart from the corresponding anatomical localization and size, this view is also

confirmed by the occasional occurrence of a somehow rudimental organization in layers of the dark grains (cf. fig. 99).

Two hypotheses, not mutually exclusive, can be issued to explain the optical effect of this multilayer disorganization and its relation to blackness. As a first, more conservative, hypothesis, the disorganized multilayer would become uncapable to significatively interact with light, therefore it would produce no selective reflection. The black colour would originate from a melanised layer/region underlying the epicuticular photonic structure, which would be invariantly present in all specimens but usually not perceived by the observer, due to the strong coloured reflections of the above-laying multilayer. Black specimens, hence, would owe their appearance to the transparency achieved by the epicuticle, which gives way to the observation of the underlying dark layer. This speculative interpretation is in agreement with observations reported by Neville and Caveney (1969) for *Cetonischema aeruginosa* (for which black specimens are known to occur too, but whose cuticular structure is unknown, see also *Convergence in colour patterns of sympatrically occurring beetles*, p. 35), where a dark melanine layer was observed to lay below the photonic structure contained in the esocuticle. The presence of such dark layer under the photonic structures has a precise functional explanation: it would avoid the reflection of brown colour from the cuticle laying at the bottom of the elytra, therefore avoiding the addition of sparse wavelengths to the colour reflected by the photonic structure, and enhancing the brightness of the colour produced.

As a second, less obvious, hypothesis I suggest that the disordered structure described may actually be not just transparent, but instead co-operate with the melanised background in order to enhance the black colour perceived by the observer. In principle, in fact, it would be possible that the granular region of the epicuticle act as an anti-reflection photonic structure, enhancing the adsorption of light operated by the underlying black pigment. Anti-reflection structures are widespread in biological systems, where they usually serve to assist the transparency of the surface: such devices, having the shape of ordered nipple arrays, are commonly observed protruding from the surface of insect ommatidia and on the transparent wings of some moths (Vukusic and Sambles, 2003). Nevertheless, the first account of a physically assisted blackness by such a structure was only recently described by Vukusic et al. (2004), who retrieved it on the wings of a butterfly. In scales of *Papilio ulysses*, in fact, the same devices which are normally responsible for the production of the physical colours behave in an anti-reflective way, enhancing the transmission of the light towards the inner melanised portion of the scale, and therefore its adsorbtion. Unfortunately, the optical properties of these scales were indirectly demonstrated by filling their empty spaces with a medium with refractive index similar to that of the chitin, an experimental demonstration which cannot be applied to the *Oreina* elytra, where no empty spaces are present. A proper investigation of the case would require a precise measurements of the refractive index of the two media and of the size of the "grains" composing the disorganized multilayer (P. Vukusic, pers. comm.)

Cross sections of elytra of species which *never* have a physical colour, not even a very dark or faint one, did not show any evidence of an epicuticular multilayer, nor of its constitutive substances. I checked this condition both in rufous phenotypes, such as *Ch. grossa* and O. *plagiata*, and in black phenotypes, such as *Ch. rossia*. (figs. 103-105).

CONCLUSIONS

The *Chrysolina* and allied leaf beetles genera are characterized by an impressive variation of colours and colour patterns. The strong dynamism in the evolution of the chromatic traits is well reflected in the frequent occurrence of chromatic variations within a species (polymorphism), which are observed in almost all species groups.

A conspicuous peak of polymorphism is observed in the species of the genus *Oreina*, which are confirmed in this thesis to be a natural group. Members of a single species, and even of a single population (for example, in *O. speciosa*), can show extreme variations, from specimens with a bright, brilliant and uniform colour, to polychromous specimens with conspicuous stripes on the elytra, or the forebody of a colour other than that of the elytra, to that of specimens completely black. In the same clade, just one node above, are branched the members of the subgenus *Protorina*, which show one more derived condition: their dorsal side is completely non-metallic red, with or without dark parts.

The expression of such a noteworthy plasticity, even at a taxonomic level as low as that of the population, is uncommon among beetles, and call for explanations accounting both for its biological significance and the undergoing developmental processes which make it possible.

As for the adaptive significance of colours and colour patterns, conspicuous appearance of most leaf-beetles is usually considered to play an aposematic role, i.e. to advertise the noxious chemicals which these beetles either sequester from the foodplants or syntethize de novo (Pasteels and Rowell-Rahier, 1991) and the bright colours of *Oreina* make no exception (Dobler et al., 1996; Hsiao & Pasteels, 1999). However, this interpretation must be considered, at the moment, as purely speculative. No experimental confirmation has ever been produced, although a research in this sense is now planned by the M. Rahier research group at the Laboratory of Evolutionary Entomology of the University of Neuchâtel (cfr. http://www2.unine.ch/Jahia/site/leae/op/edit/pid/6120, accessed 28.12.2009). Several arguments seems to detract from this hypothesis. First, it is difficult to reconciliate the strong polymorphism with the hypothesis of a warning signal (expected to be constant and standardized across populations and species). Second, some colour morphs actually do not seem to be particularly showy in a grassy landscape: this is true of forms mainly or completely green, which are the most common phenotypes in species such as *Oreina gloriosa*, as well as in other related *Chrysolina*, such as *Ch. graminis* and *Ch. herbacea*. For these phenotypes, the hypothesis of cryptic mimicry would perhaps make more sense, maybe also

taking into account that their shining surface and globous form may recall, to our eyes at least, a large droplet of water on a leaf.

Ecological interpretations for the different frequences of the colour morphs of *Oreina* and *Chrysolina* were suggested by various authors (Fujiyama, 1979; Mikhailov, 2008 and references therein). Among other trends, a correlation between increasing altitude and a colour shift towards the blue was retrieved, a phenomenon explained as an adaptation to a better defence from UV rays, whose intensity increases with altitude. This seem rather reasonable, since UV rays are located beyond the visible blue end of spectrum; however, in addition to not explaining the adaptive value (if any) of the intra-population polymorphism, to the best of my knowledge this hypothesis is not supported by any direct measurement accounting for the putatively different rates of adsorption and reflection. An indirect correlation, with colour morphs to be interpreted as by-products of genes selected for other reasons, cannot therefore be excluded.

Anyway, a strong support for an adaptive value of metallic integuments, whatever its specific nature, is provided by the existence of the *Protorina* clade. These high mountain leaf-beetles, well nested within the *Oreina* clade, are the only *Oreina* which lack metallic colours (and, mostly, also a melanization of the integument) and the only ones which are active only at night, spending the daytime under stones (M. Daccordi, pers. comm; pers. obs.). The coincidence of these two unique traits suggests that in *Protorina* the colour production may have been disposed of as it became unnecessary, and therefore that it has an adaptive value in day-active species.

If the adaptive significance is unclear, literature data from a developmental perspective is also poor. Besides a few elementary informations about the genetic determination of the colour morphs, nothing is known about the processes leading to the production of pigments and structural colours. The present research, however, allowed to gather important informations on the fine anatomy of the photonic structures and on their relationships with other morphological features of the elytra. Three independent components were recognized as the basic components of the definitive colour pattern:

A. the tanned background colour of the cuticle, which is responsible for rufous integuments. The bright red phenotypes (such as *Ch. grossa*, *Taeniosticha* sp. pl. etc.) probably rely on additional red pigmentation, which however does not modify the gross aspect of the beetle to a sizeable extent;
B. a blackish pigmentary layer;
C. a multilayer producing physical colours located above the previous ones, in the most superficial layers of the cuticle.

Eventually this third element turned out to be the key device allowing members of the *Chrysolina* clade to evolve their colouration in a quick, dramatic way, even in an almost an instant phylogenetic time. This feature, in fact, is prone to changes with unusually conspicuous effects, since it can

a. produce all the colours of the light spectrum, just by changing by a few tens nanometers the thickness of its constitutive units;
b. produce different patterns by interfacing with control/induction mechanisms which are independently present in the body architecture;
c. work like a switch, that is, it can inactivate its optical function without the need to physically disappear (i.e.: without the need to be completely removed from the developmental process).

Therefore, the epicuticular multilayer reveals to be a particularly plastic "instrument", which can be easily shaped and modified through likely very modest changes in developmental terms and, presumably, little energetic expenditure if compared with pigments (Parker, 1998). However, a device which is particularly versatile and whose alternative states are easily interchangeable is also likely to experience difficulties in its fine tuning.

Difficulties in the fine tuning of photonic structures would then become evident when different body parts have to be changed in a coordinate manner. Therefore, such a "control difficulty" of the multilayer is the interpretation that I suggest for the widespread occurrence of heterochromic phenotypes, i.e. those characterised by a perceivable difference in the colour of forebody and in that of the elytra. With regard to this matter, it is interesting to note that, within species which are not ordinarily heterochromic, heterochromic individuals do occur, but most frequently exhibit poorly perceivable differences between the colour of the two body regions. This can be read as the outcome of the combined effects of the developmental constraints and of the selective pressure: if we assume that selection drives these beetles toward a uniform body colour, and that, at the same time, they experience problems co-ordinating the two body regions, the output will be likely that actually observed, i.e., forebody and elytra may be different, but the difference is limited enough to be adaptively ininfluent.

The suggested difficulty in colour coordination would be also in agreement with the absence, as far as I could check, of homochromous individuals scattered among ordinarily heterochromous species. This suggests that the processes leading to the co-ordination of body parts are difficult to regain once lost.

As mentioned above, the epicuticular multilayer can co-ordinate with different morphological structures to produce a chromatic pattern. Within the discussed ingroup, the capability of the multilayer to co-ordinate with elytral punctuations and with veins patterns has been discussed above (cf. *The elytron, vein patterns, punctuation and sculpture* p. 26; box, p. 54). Representatives of other genera of Chrysomelinae show that the colour pattern of metallic integuments can co-ordinate also with large elytral impressions (subhumeral impressions in *Ambrostoma quadriimpressum*, fig. 3) and even have poor or no obvious co-ordination at all with other anatomical structures (e.g., *Oreina liturata*, fig. 93).

In general, with reference to beetles of other families, observations about the relationships between colour pattern and anatomy indicate that anatomical

structures play a major role in the determination of colour patterns. In addition to the mentioned interactions with multilayers, both veins and integumental punctures do contribute to the pigmentary pattern (including the aspects due to the phaneres), an interaction which is also extended to muscular insertions, and, to a some extent, to morphological parts undergoing strong sexual modification, or phylogenetically likely to do so. These mechanisms are widespread and varying at a low phylogenetic level, usually with species-specific characteristics. Convergence of morphology-based pattern retrieved across very distant species and sometimes produced by different devices (pigments vs. physical colours; integument vs. phaneres) suggests that these patterns may rely on the same morphogenetic mechanisms widely preserved across the whole Coleoptera, although only occasionally "interpreted" as non-structural prepatterns in the control of a colour pattern. An example is in the convergence of pattern between *Chrysolina bicolor* (metallic) and *Chrysolina variolosa* (pigmentary); a much more remarkable example is the similarity between the striped patterns of *Chrysolina cerealis* and *Eulasia vittata* (Glaphyridae) elytra, both based on the vein geometry although produced one by integumentary physical colours and the other by the spatial distribution of phaneres pigmentation. Their similar outlook is remarkable since these patterns, in addition to a overall similar appearance, both share a similar polymorphism in the "sharpness" of the pattern, and a similar behaviour in the activity of the individual veins (in particular, the Rs-linked pattern is weaker than others) (cf. fig. 106).

None of the different mechanisms of interaction between morphology and colours is known, however it seems likely that, in some instances at least, morphological elements "captured" and enhanced a pattern which would otherwise develop in a different, perhaps less defined way (such as in *Oreina*, where at last *O. liturata* has a pattern not defined by morphological elements). It is fit to observe that in the induction of metallic stripes by the venation (*fastuosa-like pattern*), the vein do not accomplish its role by mere "compression" of the above-laying epicuticle: multilayer strata above the vein are, in fact, thicker than elsewhere.

In other cases, and in accordance to observations in Lepidoptera and Diptera (Nijhout, 1991; True et al., 1999; O'Grady and DeSalle, 2000), it seems likely that the morphological elements actually induce (or repress) pattern production, as observed, for example, for the muscular insertions of *Leptinotarsa*. In this case, it is worthwhile to stress that muscular insertions, beside inducing the pattern, also constrain its evolutionary capabilities. In fact, given that the shape of the muscles is defined by precise biomechanicals requirements, their anatomical structure is very unlikely to change. Thus, as long as the production of spots is dependent on the sites of muscular insertion the evolvabilty of pronotal and abdominal pattern will be almost null. This is actually confirmed by the pronotal pattern found in various members of the genus *Leptinotarsa*: whenever expressed, the black pattern has a very conservative shape.

Finally, beside the existence of several morphology-related patterns, many elytral patterns exist which have no (or not complete) relation with anatomical or morphological structures. The existence of similar spots, stripes, or differently shaped coloured areas on beetle elytra is not surprising, since it has its equivalent in similar, possibly homologous, phenomena in the wings of other holometabolous insects. The existence of a “prepattern” pigmented area, whose development is independent from that of the successively defined vein-dependent pattern, has been demonstated in *Drosophila* (True et al., 1999), and a similar, inexpected phenomenon has been recently observed also in butterflies, where abnormal specimens missing wing veins can nevertheless properly express at least some of their ordinary pattern elements (Reed and Gilbert, 2004).

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Figures 1-9.

1-3: Examples of complex patterns; **1**: Phaneral pattern (*Sternotomis pulchra*, Cerambycidae); **2**: Pigmentary pattern (*Zygogramma chiriquina*, Chrysomelidae); **3**: Physical colour pattern (*Ambrostoma quadriimpressum*, Chrysomelidae).

4. Colour originated by a even spaced multilayer reflector (*Anomala vitis*, Rutelidae).

5. Colour originated by a broadband multilayer reflector (*Chrysina strasseni*, Rutelidae). © B. Strnadova, from http://godofinsects.com.

6. Model of even-spaced multilayer reflector.

7. Model of broadband multilayer reflectors.

8. Example of white colour originated by photonic structures contained within scales (*Cyphochilus* sp., Melolonthidae).

9. SEM image of a fractured edge of a *Cyphochilus* scale. From Vukusic et al., 2007.


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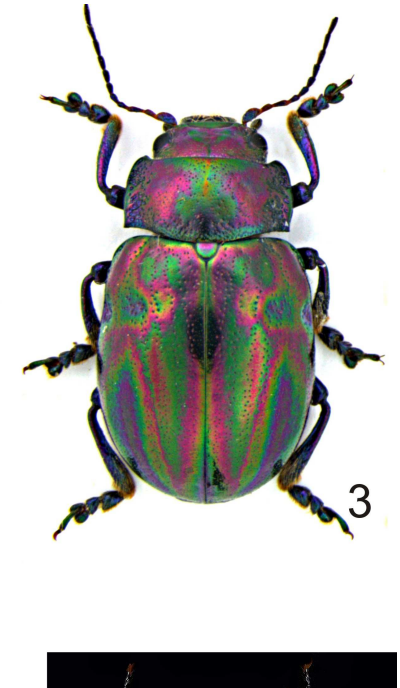
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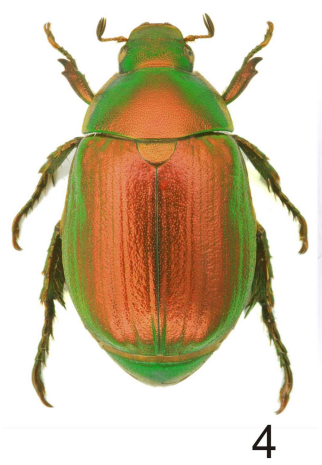
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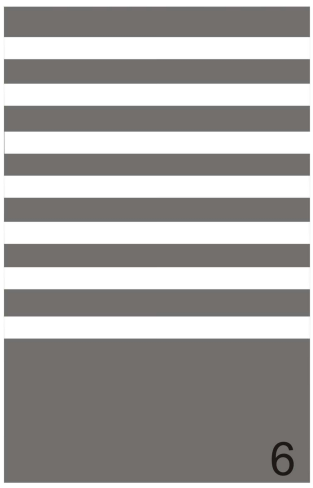
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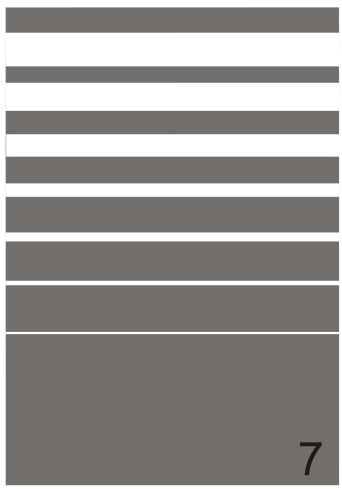
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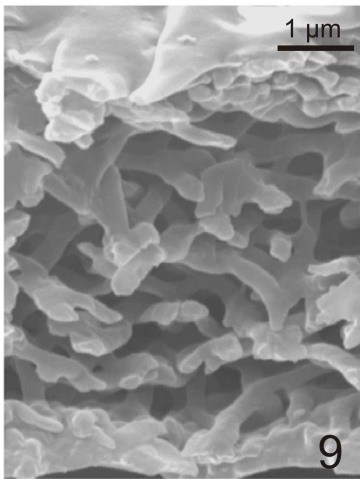
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Figures 10-15.

10-12. Pointillistic diffraction gratings in *Calomera littoralis nemoralis* elytron (Cicindelidae).

13-15: Pointillistic diffraction gratings in *Elaphrus riparius* (Carabidae); **13**: Habitus; **14**: detail of head and pronotum; **15**: detail of elytral sculpture.

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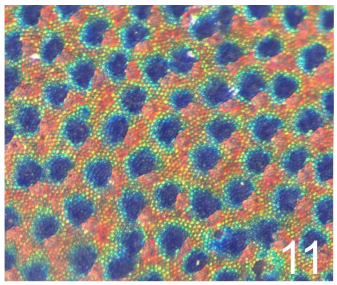

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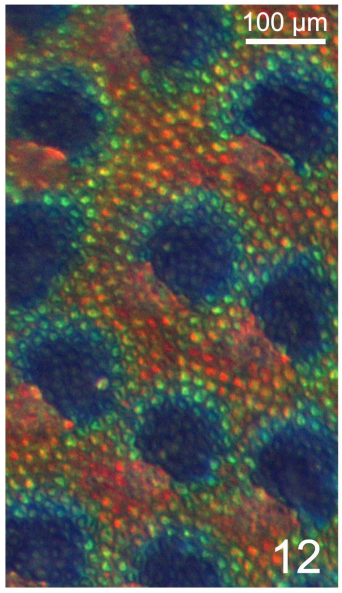

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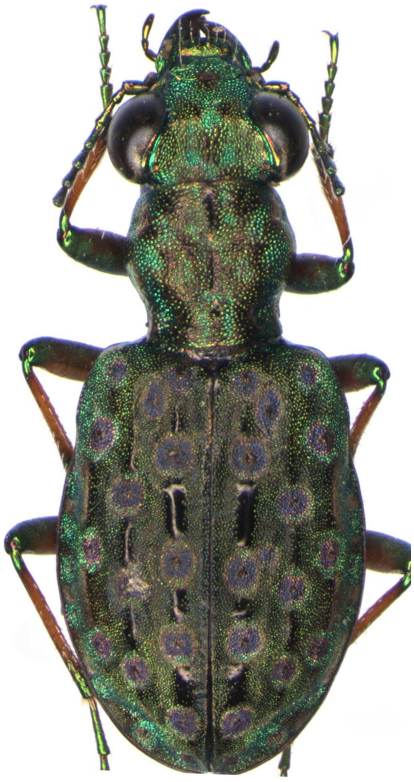

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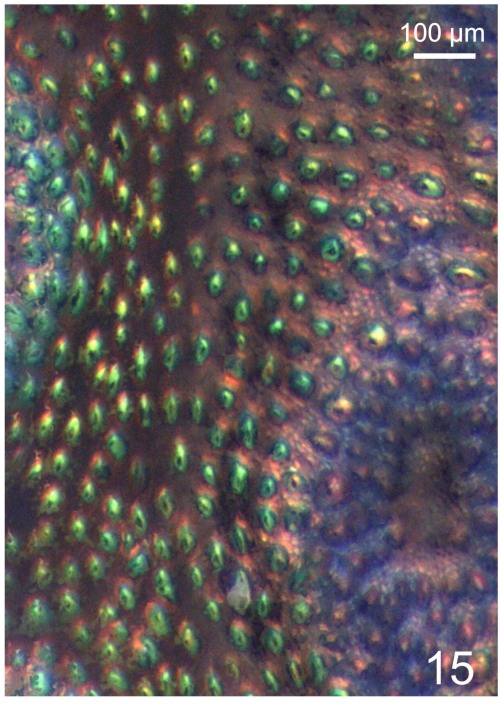

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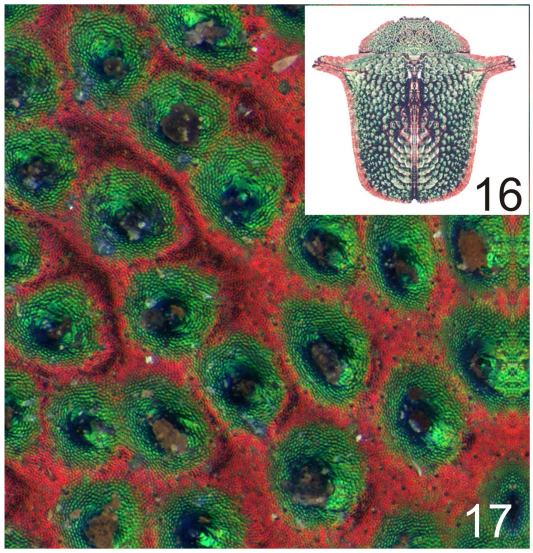
Figures 16-23.

16-18: *Omocerus masoni*; **16**: Habitus (from: http://www.biol.uni.wroc.pl /cassidae/katalog%20internetowy/index.htm); **17-18**: detail of elytral sculpture.

19: Comparison between the diffused reflection of tridimensional photonic structures (a: *Eupholus chevrolati*, Curculionidae) and the directional reflection of multilayer reflectors (b: *Chrysochus* sp., c: *Chrysolina graminis*, Chrysomelidae). Photo taken with a strongly directional flash light.

20-21: Detail of *Eupholus* elytral scales; **20**: E. *chevrolati*; **21**: *E. schoenherri*.

22-23: Detail of *Entimus imperialis* (Curculionidae) elytral scales.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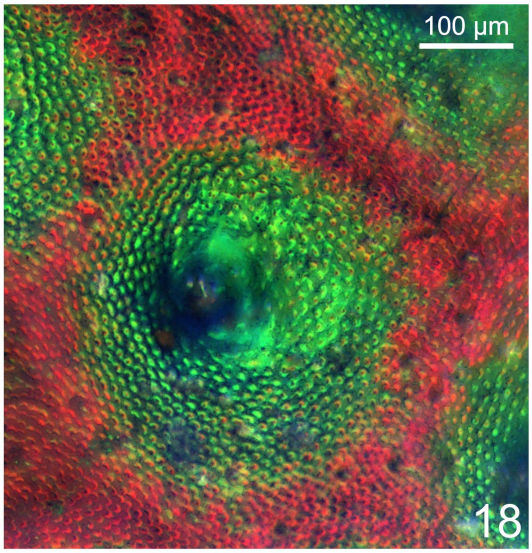
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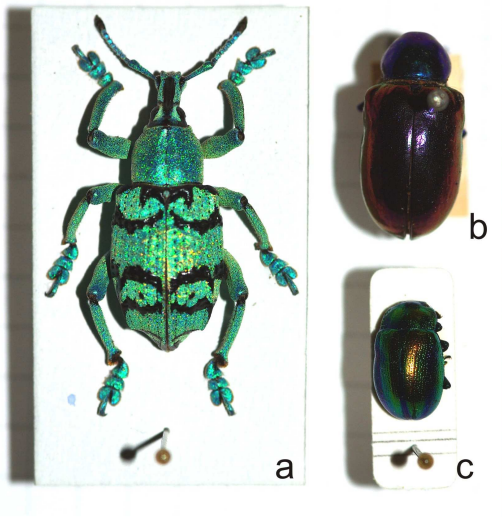
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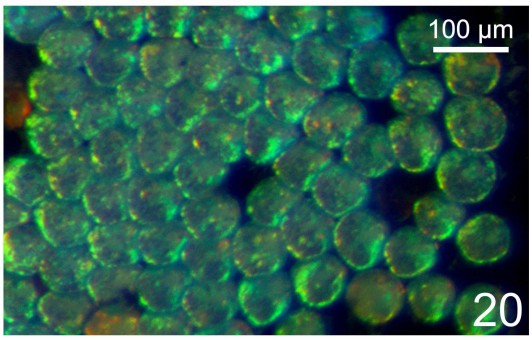
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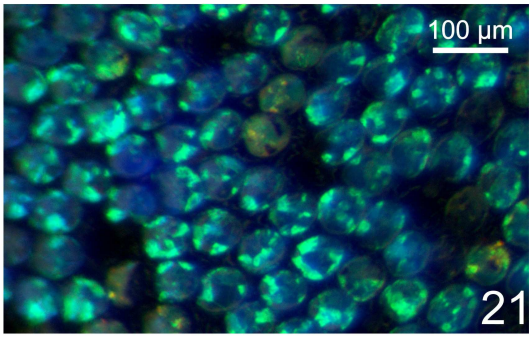
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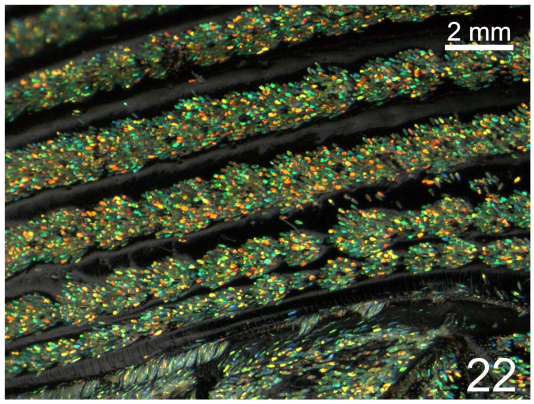
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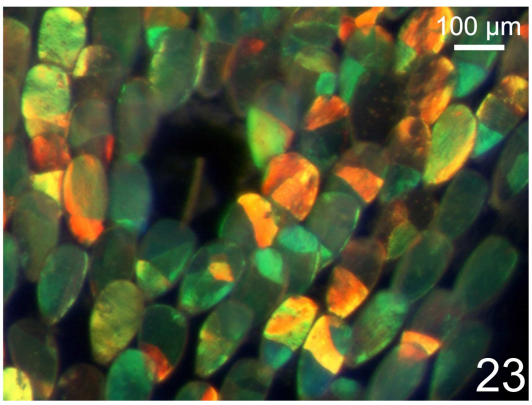
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Figures 24-36.

24-27. *Leptinotarsa decemlineata*; **24**: abdomen, ventral view; **25**: dissected sclerites of the abdomen (internal view), with muscles. **26-27**: dissected sclerites of the abdomen (internal view), with muscles. Coloured with methylene blue.

28-30. Pronotal melanization in Silphidae; **28-29**: *Oiceoptoma thoracica*, different extension of prothoracic melanization, with spots corresponding to muscular insertions in **28**; **30**: *Xylodrepa quadripunctata*.

31-32. *Amphimallon solstitialis* (Melolontidae); **31**: dorsal view; **32**: ventral view of dissected pronotum, with muscles removed on the left side.

33-34. *Dyspilophora trivittata* (Cetoniidae); **33**: dorsal view; **34**: inclined ventral view, with muscles removed on the left side.

35-36. Examples of Scarabaeoidea with dark spots corresponding to the pronotal apodeme (arrows); **35**: *Euoniticellus fulvus* (Scarabaeidae); **36**: *Aphodius* sp. (Aphodiidae).


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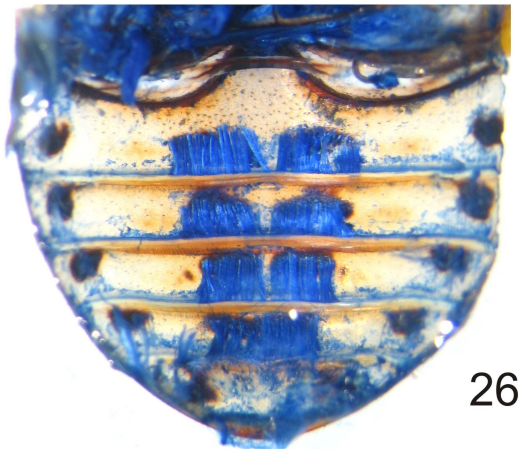
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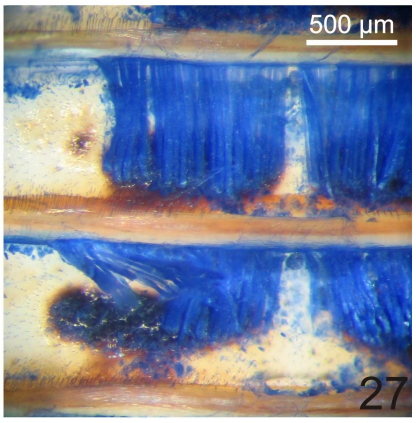
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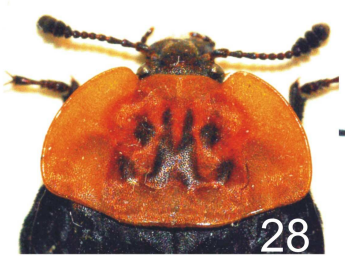
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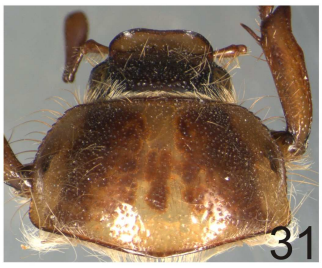
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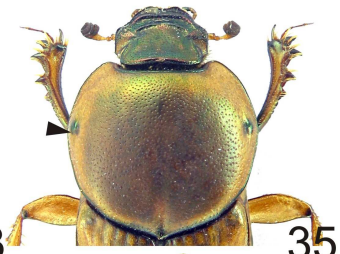
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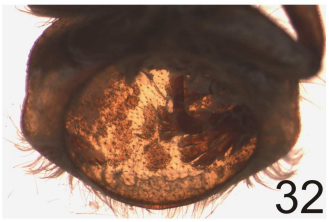
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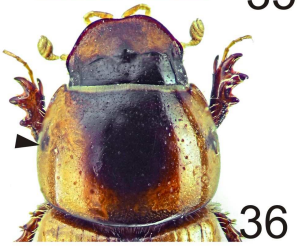
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Figures 37-44.

37. Interaction between elytral tracheae (highlighted in b) and phaneral pattern. (*Acrocinus longimanus*, Cerambycidae).

38: Coincidence between tracheae and pigmentary pattern in *Blitopertha lineolata* (Rutelidae); elytral tracheae highlighted with yellow lines.

39: Coincidence between elytral punctures and physical colour pattern in *Chrysolina superba* (Chrysomelidae).

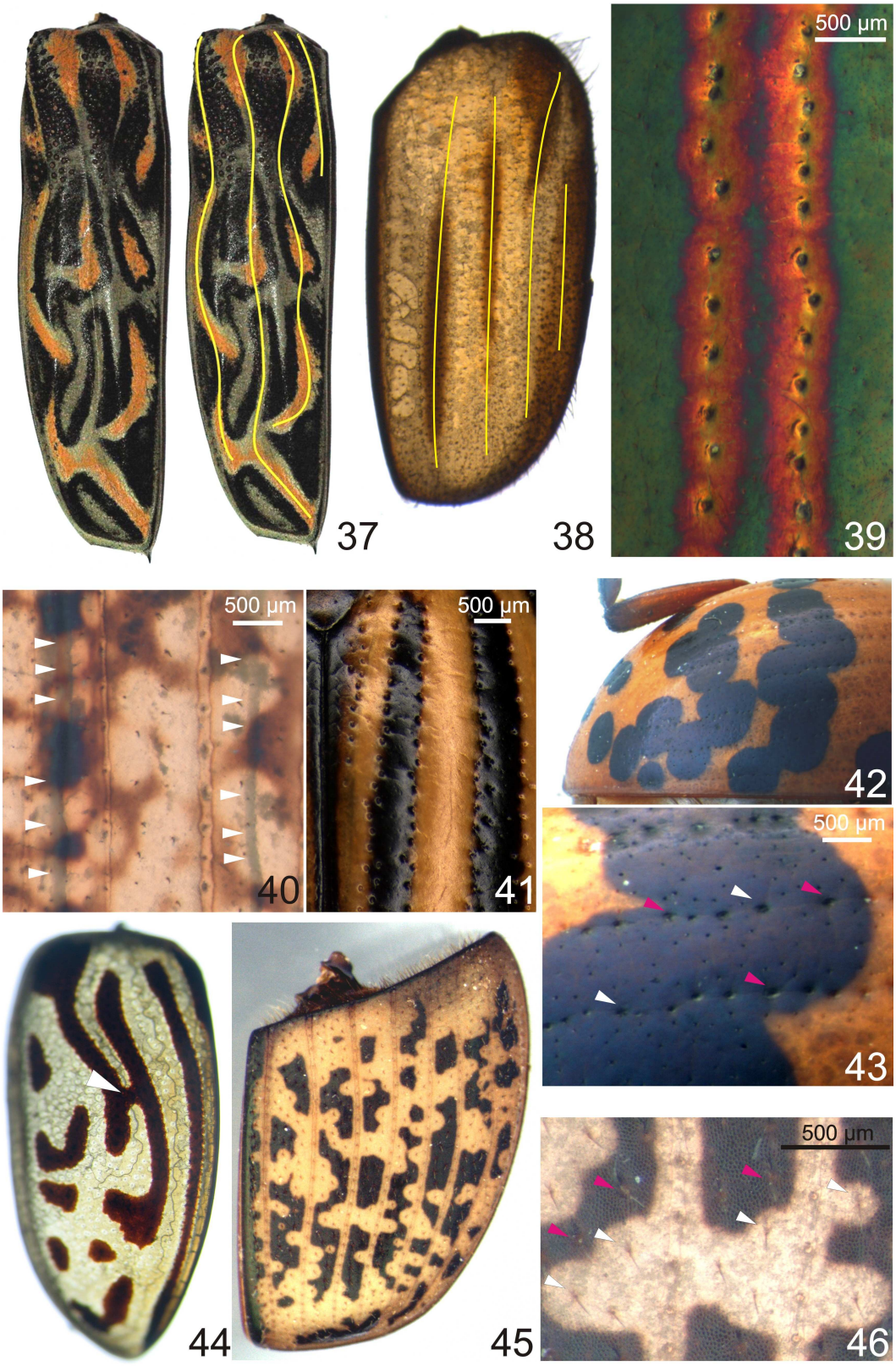
40. Enhancement of pigmentary colour pattern by tracheae in *Cheironitis irroratus* (Scarabaeidae); arrowheads point to tracheae.

41. Relation of confinement/alignment relation between ordinated series of punctures and pigmentary pattern in *Leptinotarsa decemlineata* (Chrysomelidae).

42. Coincidence between elytral punctures and pigmentary colour pattern in *Chrysolina confluens* (Chrysomelidae). b: white arrowheads point to punctures coincident with dark spots, red arrowheads point to similar punctures not coincident with pattern.

43. Relation of exclusion between elytral tracheae and pigmentary pattern in. Arrowhead indicate a point where this relation is not respected.

44. Relation of exclusion between setae-bearing punctures and pigmentary pattern in *Onthophagus vacca*. b: white arrowheads point to punctures excluding pigmentation in their neighbourhood, red arrowheads point to similar punctures not excluding pigment.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Figures 45-54.

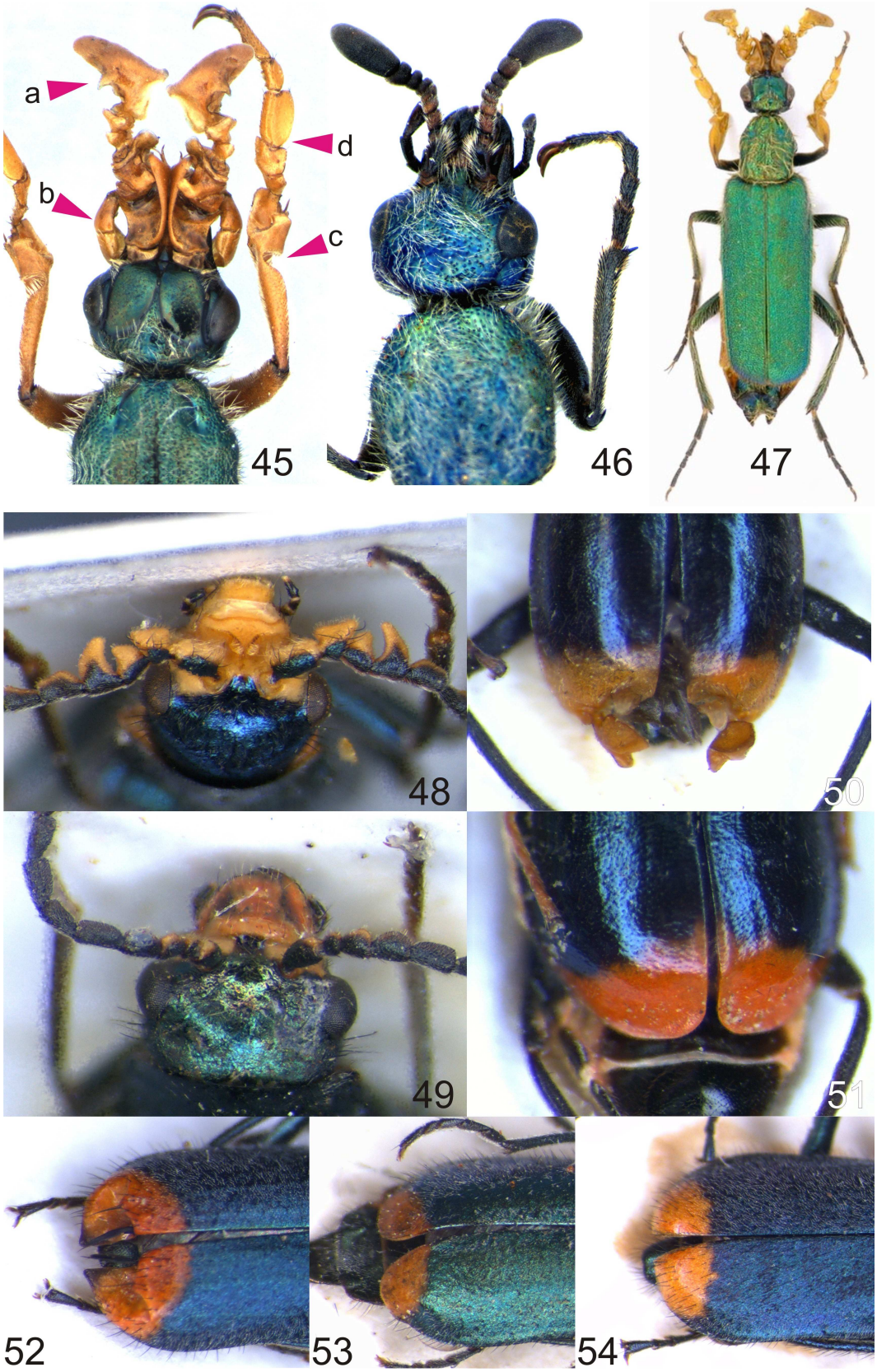
45-46. *Cerocoma schreberi* (Meloidae) (**45**: male, **46**: female); arrowheads point to appendages with alterated colour and shape in male (a: antenna, b: maxillary palp, c: tibia, d: tarsus).

47. Male of *Cerocoma prevezaensisi* (Meloidae), © S. Krejcik, from http://www.meloidae.com/meloidae/displayimage.php?pos=-340

48-49. Head and first antennal articles of *Malachius australis* (Malachiidae) (frontal view); **48**: male; **49**: female.

50-51. Elytral apex of *Ebaeus battonii* (Malachiidae); **50**: male; **51**: female

52-54. Elytral apex of *Malachius* (Malachiidae). **52**: sexually modified apex in male of *M. spinipennis*; **53**: simple apex in female of *M. spinipennis*; **54**: unmodified apex in male of *M. australis*.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Figures 55-62.

55. Example of scale-covered Chrysomelidae: *Pachnephorus tessellatus*. © L. Borowiec, from http://www.biol.uni.wroc.pl/cassidae.

56. Phaneral pattern (yellow patches) superimposed on cuticular pigmentary pattern (red and black areas) on *Euselates perraudieri* (Cetoniidae).

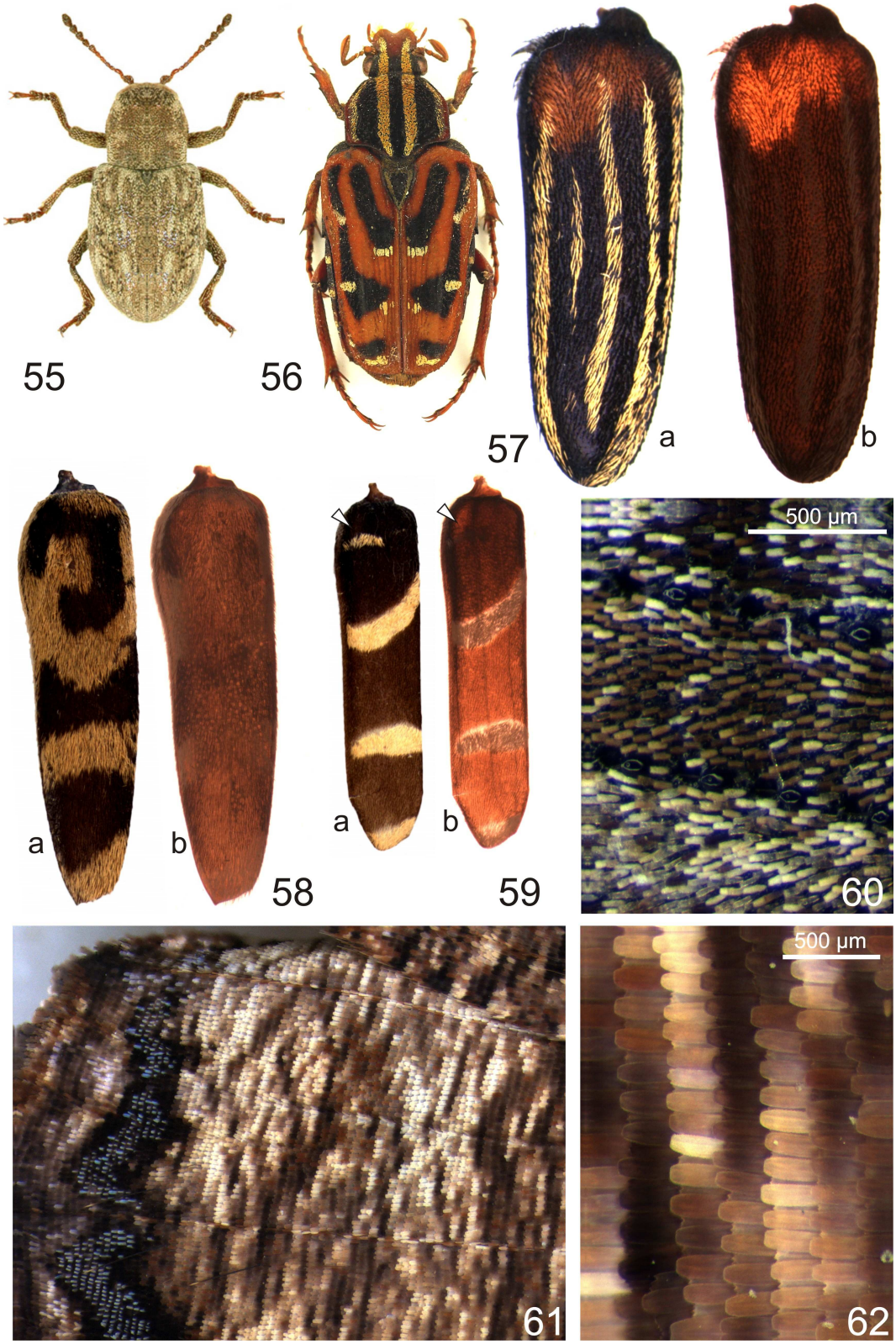
57. Independence of pigmentary pattern of phaneres (a, view in reflected light) from the pigmentary pattern of cuticle (b, view in transmitted light) on an elytron of *Eulasia vittata* (Glaphyridae).

58. Absence of relationship between the phaneral pattern (a) and cuticular colour (b) in *Chlorophorus varius* (Cerambycidae, Clytinae).

59. Presence of relationship between the phaneral pattern (a) and cuticular colour (b) in *Chlorophorus varius* (Cerambycidae, Clytinae). White arrow point to an element of the phaneral pattern which is not mirrored in the integumentary one.

60. Disordered scales on the elytra of an African Anthribidae

61-62. Finely patterned wings and highly ordered wing scales of *Nymphalis polychloros* (Lep. Nymphalidae).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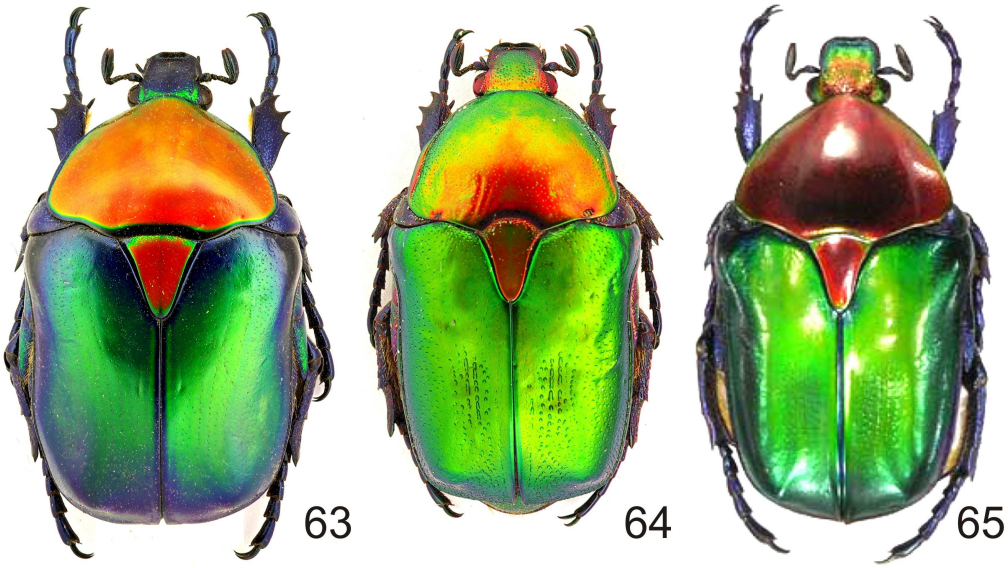
Figures 63-69.

63-65. Sympatric convergence of colour pattern in *Protaetia* (Cetoniidae); **63**: *P*. (*Cetonischema*) *speciosa jousselini*; **64**: *P*. (*Potosia*) *cuprea ignicollis*; **65**: *P*. (*Eupotosia*) *affinis pyrodera* (from Tauzin, 2008).

66-69. Homochromy and heterochromy between pronotum and elytra in *Chrysolina* and *Oreina*; **66-67**: Homochromy in *O. virgulata* (66) and *Ch. schatzmayri*; **68-69**: low degree of heterochromy in *O. tristis* (68) and *Ch. oricalcia* (69).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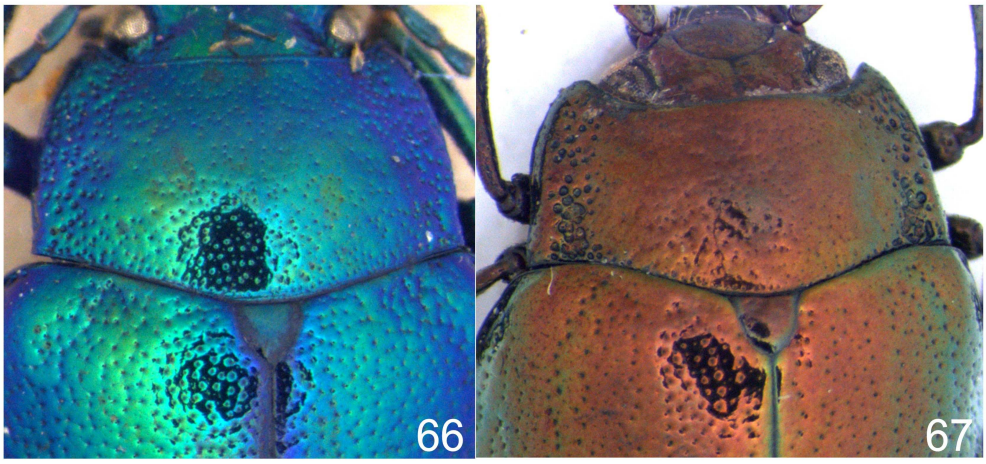
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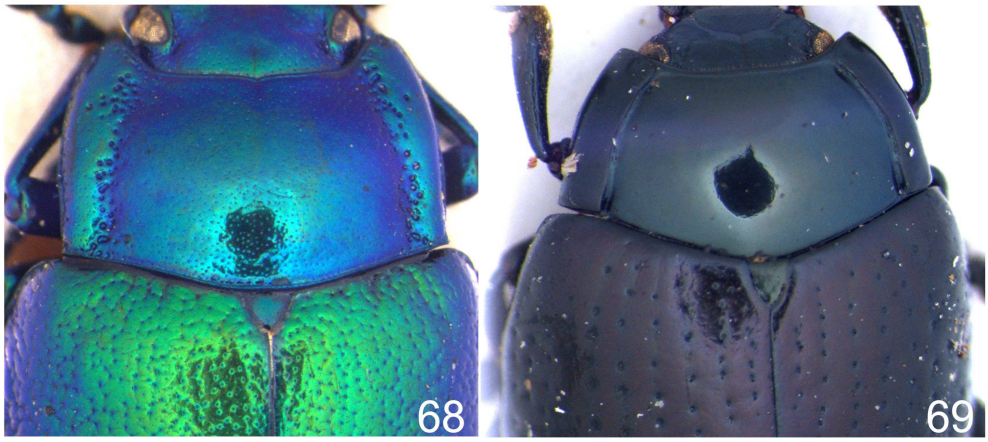
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Figures 70-78.

70. *Oreina* (*Oreina*) *speciosa*, black form.

71. *Chrysolina* (*Colaphoptera*) *blanchei*, © L. Borowiec, from http://www.biol.uni.wroc.pl/cassidae.

72. *Chrysolina* (*Melasomoptera*) *grossa.*

73. *Chrysolina* (*Chrysocrosita*) *spectabilis*, © M.E. Smirnov from http://www.zin.ru/ANIMALIA/Coleoptera/index.htm

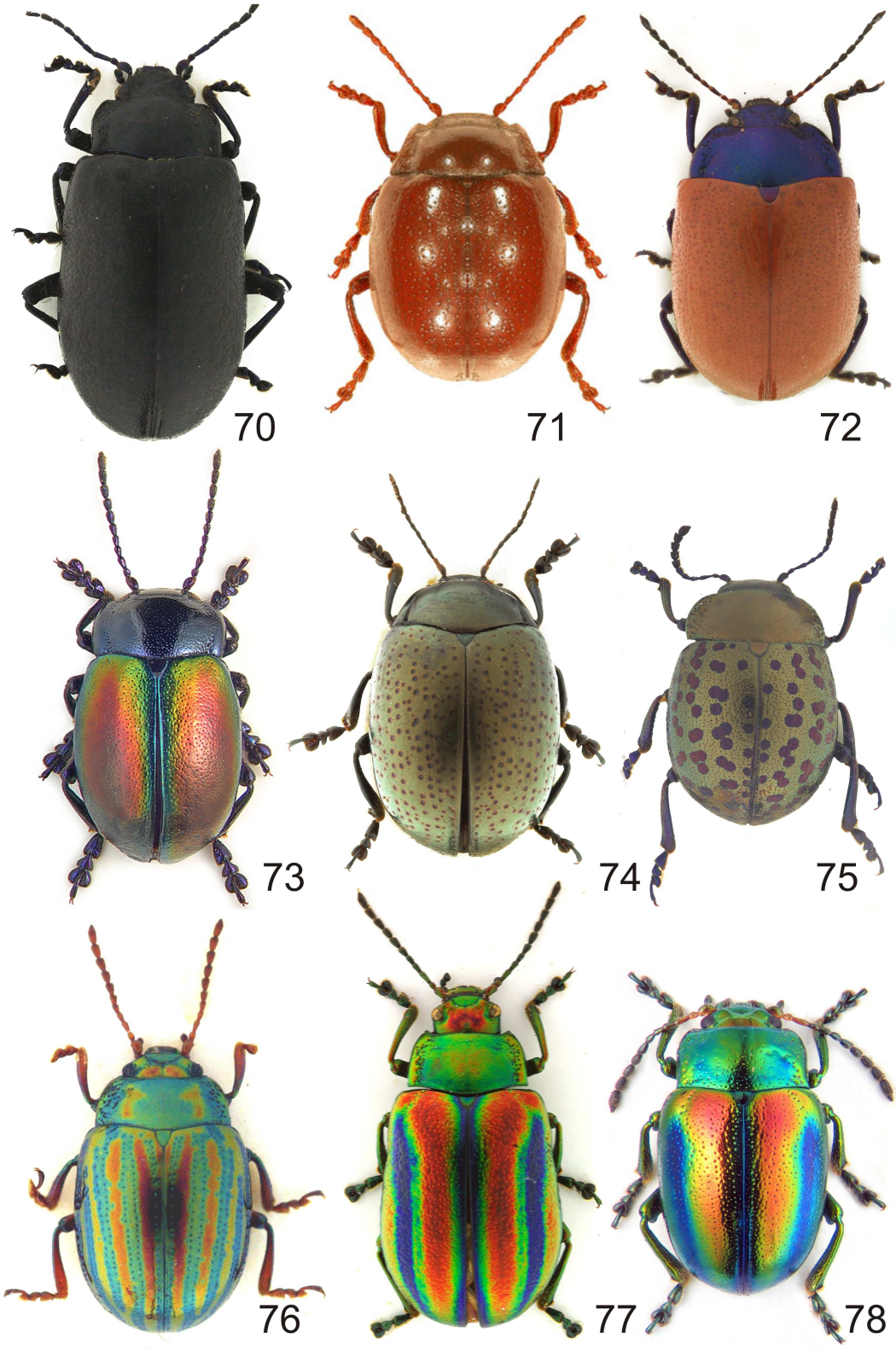
74. *Chrysolina* (*Ovosoma*) *vernalis.*

75. *Chrysolina* (*Ghesquiereita*) n. sp.

76. *Chrysolina* (*Taeniochrysea*) *americana*.

77. *Oreina* (*Oreina*) *speciosa*, striped form (*fastuosa-like pattern*).

78. *Chrysolina* (*Fastuolina*) *fastuosa*, © M.E. Smirnov from http://www.zin.ru/ANIMALIA/Coleoptera/index.htm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Figures 79-87.

79. *Chrysolina (Chrysomorpha) cerealis*.

80. *Chrysolina (Chrysocrosita) jakowlewi.*

81. *Crosita altaica.*

82. *Chrysolina* (*Sulcicollis*) *oricalcia* © M.E. Smirnov from http://www.zin.ru/ANIMALIA/Coleoptera/index.htm

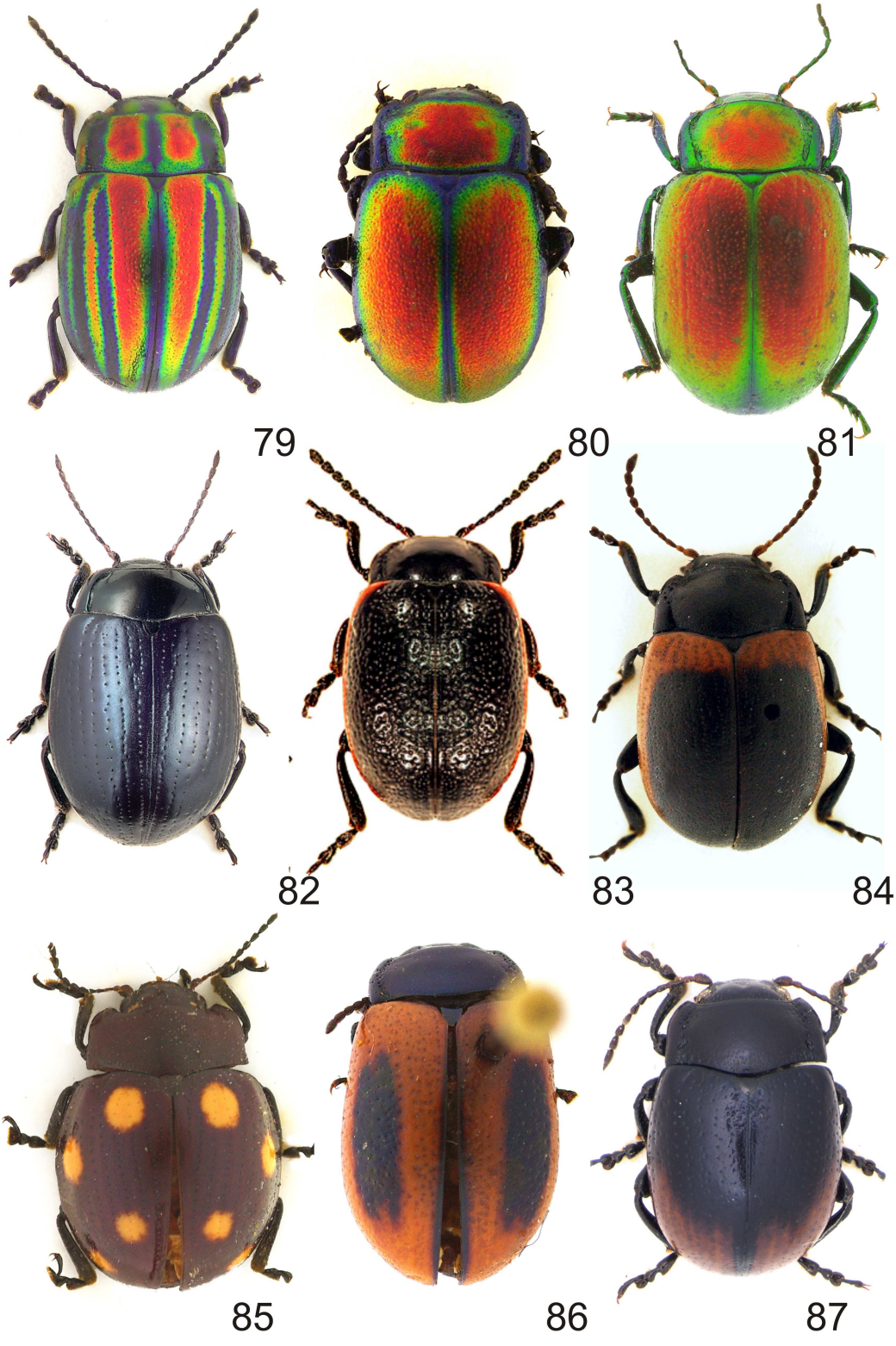
83. *Chrysolina* (*Stichoptera*) *sanguinolenta.*

84. *Chrysolina* (*Craspeda*) *limbata.*

85. *Chrysolina* (*Camerounia*) *elysia.*

86. *Chrysolina* (*Craspeda*) *bruneli.*

87. *Chrysolina* (*Allohypericia*) *aeruginosa.*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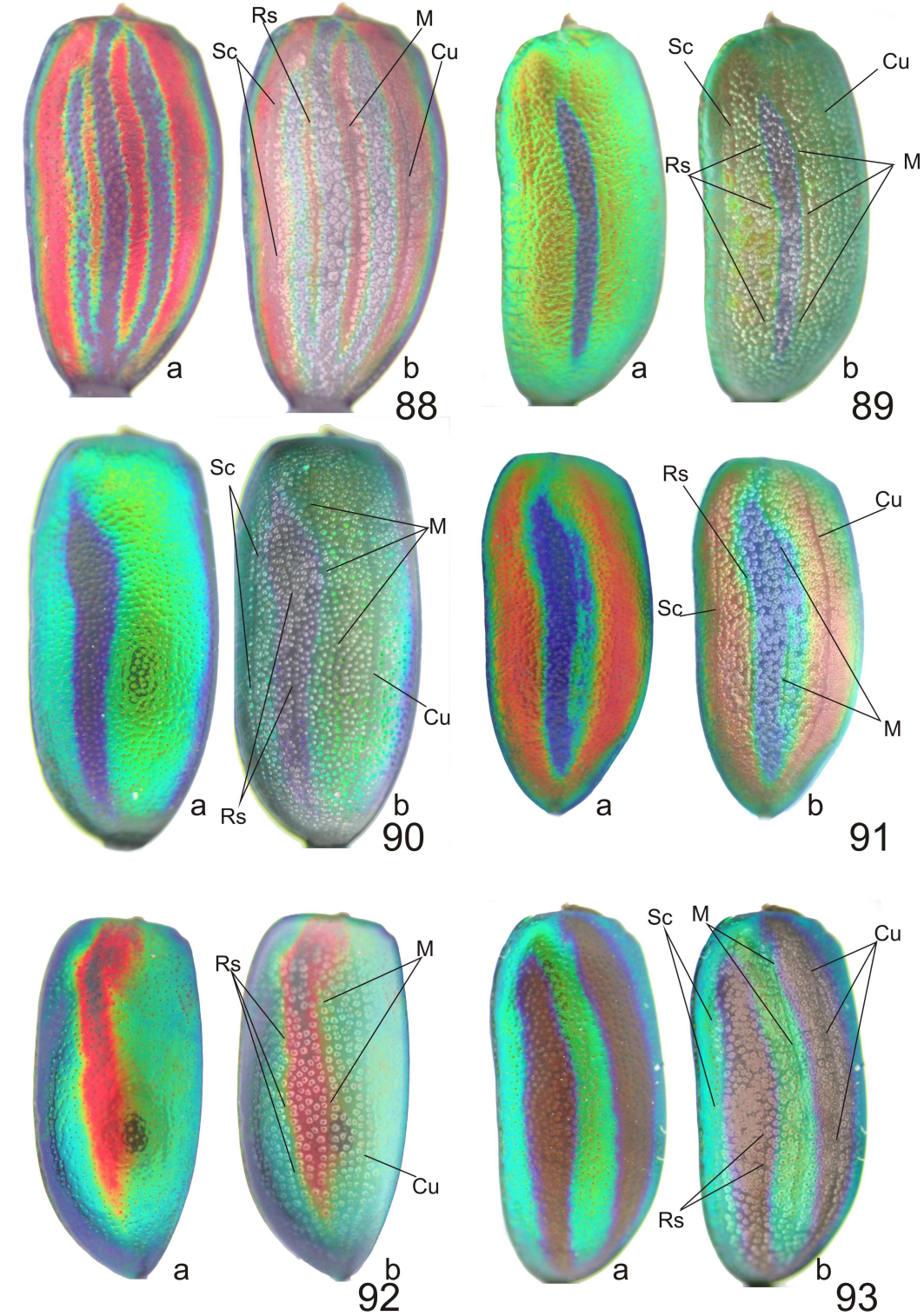
Figures 88-93.

88-93. Left elytron of *Oreina* and *Chrysolina* species; a: reflected light, b. reflected and transmitted light together, with indications of tracheae:
88. *Ch. cerealis*
89. *O. gloriosa*
90. *O. speciosissima*
91. *O. alpestris*
92. *O. genei*
93. *O. riturata*.


Rs
M
Sc
Cu
a
b
88
Sc
Cu
Rs
M
a
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89
Sc
M
Cu
a
Rs
b
90
Rs
Cu
Sc
M
a
b
91
Rs
M
Cu
a
b
92
Sc
M
Cu
Rs
a
b
93

Figures 94-99.

94. Cross section of elytron of *Oreina alpestris* (aniline blue colouration).

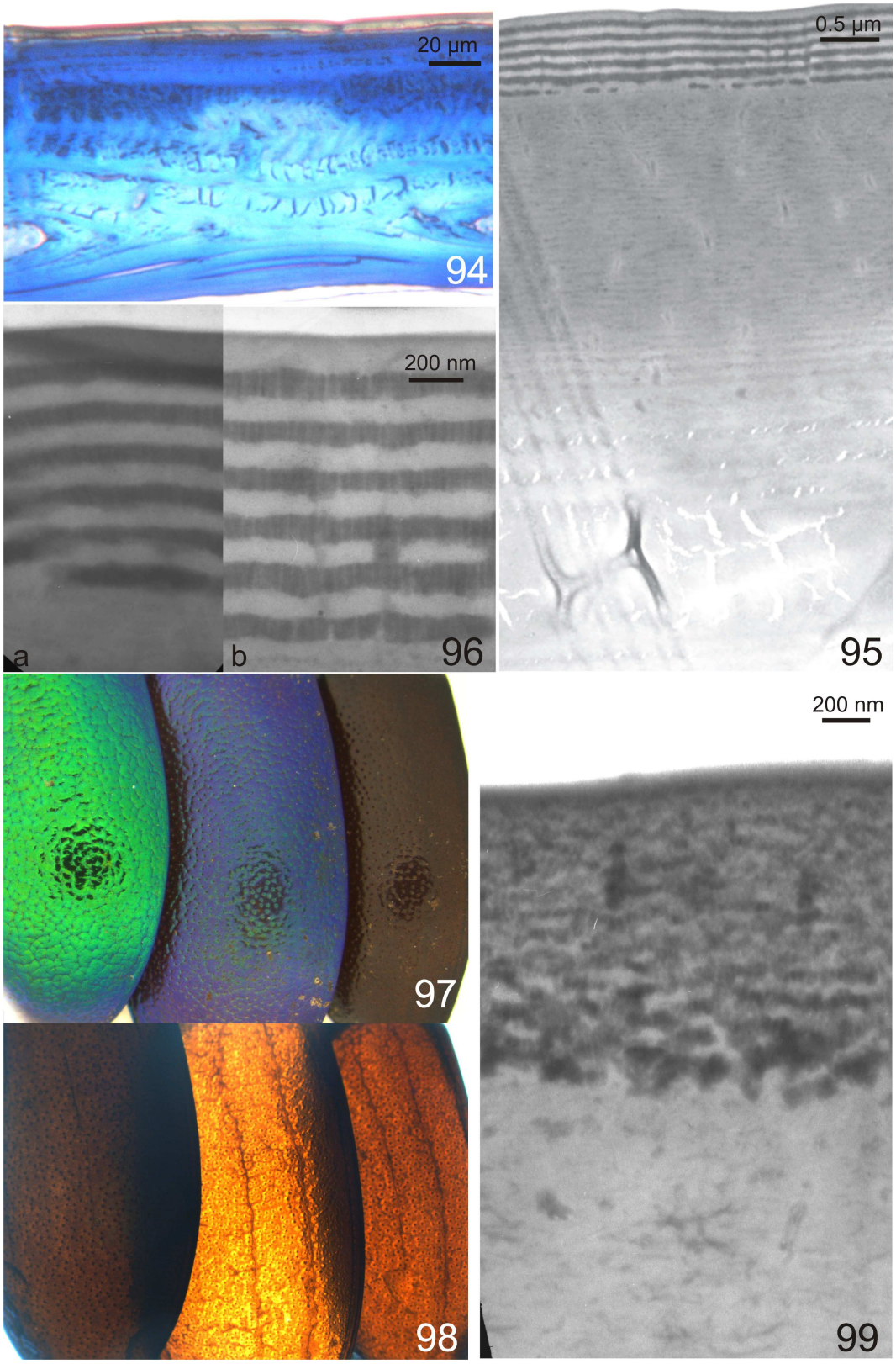
95. TEM cross section of the surface (epicuticle) of elytron of *Oreina alpestris* (metallic specimen)

96. Comparison between the epicuticular multilayers from different regions of a same elytron of *Oreina alpestris* (metallic, striped specimen). a: green area, b: red area.

97. Comparison between elytra of *Oreina speciosa*, different chromatic forms, reflected light.

98. Same as fig. 97, in transmitted light.

99. TEM cross section of the surface (epicuticle) of the a black specimen of *Oreina speciosa*.


20 µm
94
0.5 µm
200 nm
a
b
96
95
200 nm
97
98
99

Figures 100-107.

100. Cross section of black elytron of *Oreina viridis*.

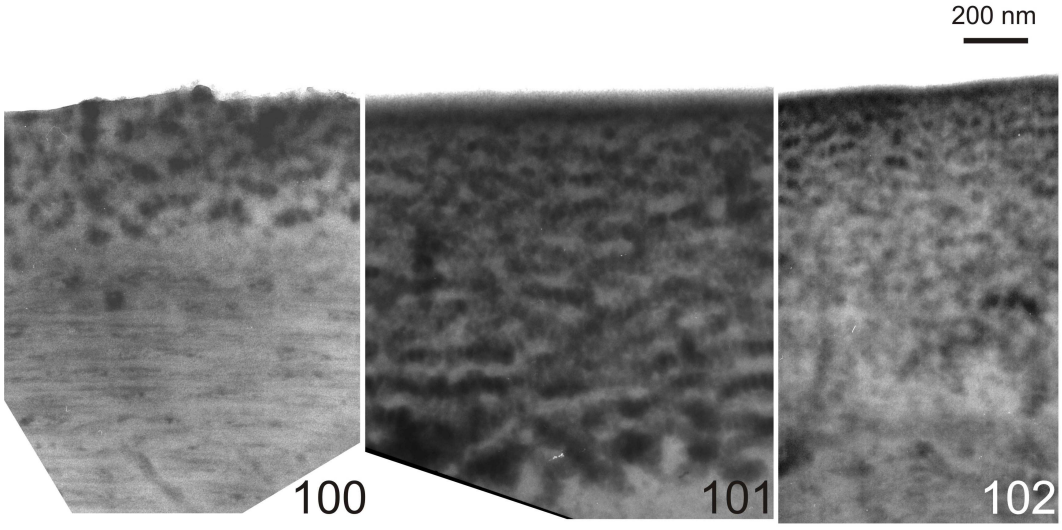
101. Cross section of black elytron of *Oreina speciosa*.

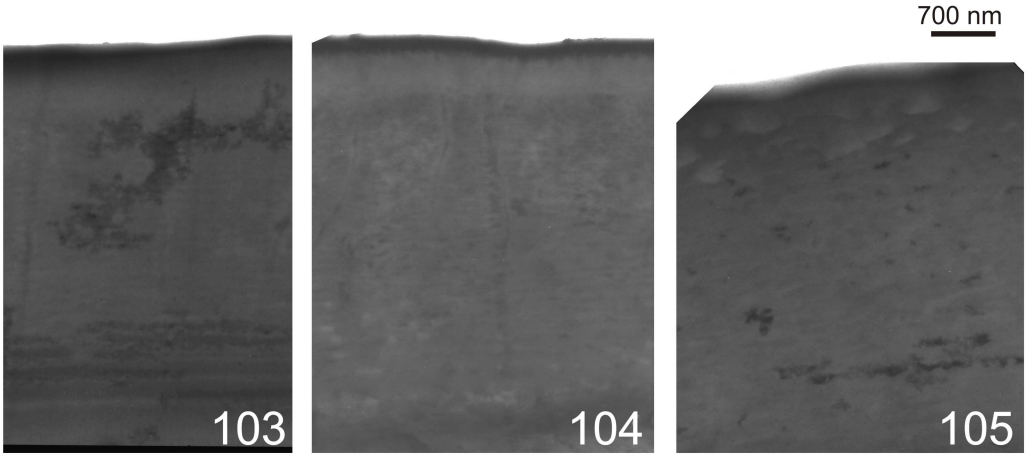
102. Cross section of black *Oreina alpestris nigrina* elytron.

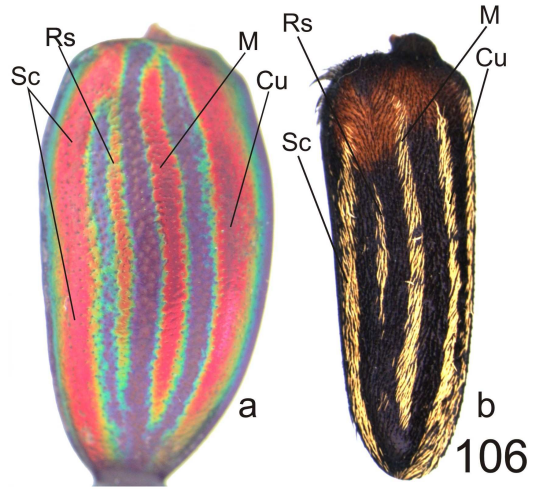
103-105. Cross section of elytra not showing any epicuticular multilayer; **103**: *Oreina* (*Protorina*) sp. (non-metallic red); **104**: *Chrysolina grossa* (non-metallic red); **105**: *Chrysolina rossia* (black).

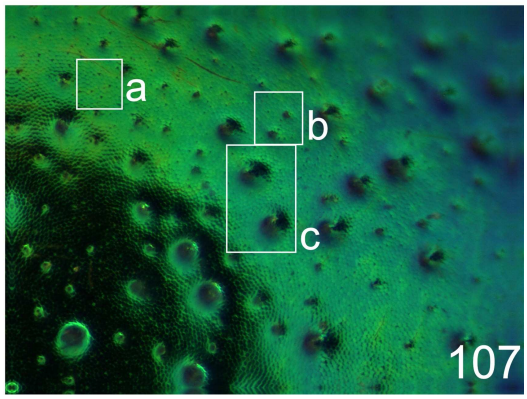
106. Coincidence of the elytral pattern structure in *Chrysolina cerealis* (a) and *Eulasia vittata* (Glaphyridae) (b).

107. Classes of punctuation on the pronotum of *Chrysolina graminis*. a: micropunctuation; b: punctures of the first order; c: punctures of the second order.


200 nm
100
101
102


700 nm
103
104
105


Rs
M
Sc
Cu
a
Rs
M
Cu
Sc
b
106


a
b
c
107

APPENDICES

Appendix 1. Species used in the phylogenetic reconstruction

Species	Code used in phylogeny
Leptinotarsa decemlineata (OUTGROUP)	Leptinotarsa
Chrysolina ? kinabaluensis	Ch.kinabaluensis
Chrysolina (*Allohypericia*) *aeruginosa*	Ch.Allohyp.aeruginosa
Chrysolina (*Allohipericia*) *fuliginosa*	Ch.All.fuliginosa
Chrysolina (*Anopachys*) *asclepiadis*	Ch.Anop.asclepiadis
Chrysolina (*Apterosoma*) *angusticollis*	Ch.Apt.angusticollis
Chrysolina (*Atechna*) *vigintiquatorsignata*	Ch.Ate.vigintiquatorsignata
Chrysolina (*Bechynea*) *platypoda*	Ch.Bechy.platypoda
Chrysolina (*Bechynia*) *nikolskii*	Ch.Bech.nikolskii
Chrysolina (*Camerounia*) *ornata*	Ch.Cam.ornata
Chrysolina (*Centoptera*) *bicolor*	Ch.Cent.bicolor
Chrysolina (*Chalcoidea*) *marginata*	Ch.Chal.marginata
Chrysolina (*Chrysocrosita*) *spectabilis*	Ch.Chrysocr.spectabilis
Chrysolina (*Chrysolina*) *staphylaea*	Ch.Chrys.staphylaea
Chrysolina (*Chrysolinopsis*) *gemina*	Ch.Chrysolin.gemina
Chrysolina (*Chrysomorpha*) *cerealis*	Ch.Chrysom.cerealis
Chrysolina (*Colaphodes*) *haemoptera*	Ch.Col.haemoptera
Chrysolina (*Colaphosoma*) *hemisphaerica*	Ch.Colaph.hemisphaerica
Chrysolina (*Colaphosoma*) *sturmi*	Ch.Colaphos.sturmi
Chrysolina (*Craspeda*) *limbata*	Ch.Cras.limbata
Chrysolina (*Crositops*) *pedestris*	Ch.Cros.pedestris
Chrysolina (*Diachalcoidea*) *sacarum*	Ch.Diach.sacarum
Chrysolina (*Erythrochrysa*) *polita*	Ch.Eryth.polita
Chrysolina (*Euchhrysolina*) *graminis*	Ch.Euch.graminis
Chrysolina (*Fastuolina*) *fastuosa*	Ch.Fast.fastuosa
Chrysolina (*Ghesquiereita*) *katangana*	Ch.Ghes.katangana
Chrysolina (*Heliostola*) *lichenis*	Ch.Hel.lichenis
Chrysolina (*Hypericia*) *geminata*	Ch.Hyp.geminata
Chrysolina (*Hypericia*) *hyperici*	Ch.Hyp.hyperici
Chrysolina (*Lithopteroides*) *hexanthematica*	Ch.Lith.hexanthematica
Chrysolina (*Maenadochrysa*) *femoralis*	Ch.Maen.femoralis
Chrysolina (*Melasomoptera*) *grossa*	Ch.Melas.grossa
Chrysolina (*Naluhia*) *confluens*	Ch.Nal.confluens
Chrysolina (*Ovosoma*) *sahlbergi*	Ch.Ovos.sahlbergi
Chrysolina (*Ovosoma*) *vernalis*	Ch.Ovos.vernalis
Chrysolina (*Ovostoma*) *olivieri*	Ch.Ovost.olivieri
Chrysolina (*Palaeosticta*) *diluta*	Ch.Pal.diluta
Chrysolina (*Paradiacalhoidea*) *vignai*	Ch.Parad.vignai
Chrysolina (*Pierryvettia*) *stictica*	Ch.Pierr.stictica
Chrysolina (*Pseudotaeniochrysea*) *superba*	Ch.Pseudot.superba
Chrysolina (*Rhyssoloma*) *fragariae*	Ch.Rhys.fragariae
Chrysolina (*Sphaeromela*) *varians*	Ch.Sph.varians
Chrysolina (*Stichoptera*) *rossia*	Ch.Stich.rossia
Chrysolina (*Sulcicollis*) *oricalcia*	Ch.Sul.oricalcia
Chrysolina (*Synerga*) *coerulans*	Ch.Syn.coerulans
Chrysolina (*Synerga*) *viridana*	Ch.Syn.viridana
Chrysolina (*Taeniochrysea*) *americana*	Ch.Taen.americana
Chrysolina (*Taeniosticha*) *bakuensis*	Ch.Taen.bakuensis
Chrysolina (*Threnosoma*) *helopioides*	Ch.Thr.helopioides
Chrysolina (*Timarcholina*) *haemochlora*	Ch.Timar.haemochlora
Chrysolina (*Timarchoptera*) *templetoni*	Ch.Tim.templetoni
Chrysolina (*Vittatochrysa*) *vittata*	Ch.Vitt.vittata
Crosita altaica	Cr.altaica
Oreina (*Chrysochloa*) *elegans*	Or.Chrys.elegans
Oreina (*Chrysochloa*) *elongata*	Or.Chrys.elongata
Oreina (*Frigidorina*) *frigida*	Or.Frig.frigida
Oreina (*Oreina*) *speciosa*	Or.Ore.speciosa
Oreina (*Oreina*) *viridis*	Or.Ore.viridis
Oreina (*Protorina*) *plagiata*	Or.Prot.plagiata
Semenovia mirabilis	Sem.mirabilis

Appendix 2. Characters coding

A list of the characters coded for the phylogenetic study is given below. A new terminology was applied to specify different kinds of punctures occurring together on the integument. The elytral integument of all the species, and the pronotal integument of most of them, shows three different classes of punctures, mostly well distinct from each other. The smallest punctures (micropunctuation), barely perceivable only at higher magnification (60-100x), likely corresponding to sensilla emerging between the integument cell, were not taken into account. Intermediate size punctures, always present both on pronotum and elytra are called here "punctuation of the first order". Larger punctures, always present on the elytra but not always present on the pronotum, are called "punctuation of the second order". See fig. 107.

Characters 1-15 describe (or depend on) continuous characters, whose values were recorded, with precision to the second decimal number. These characters were considered additive by default from the software; in addition, characters marked with an * were also set as additive, since they were deemed to be expression of a morphocline.

1. Body length
(mm)

2. Body ratio
(length/width). Length measured from the anterior margin of the pronotum to the apex of the elytra. Width measured at its maximum (usually, across the anterior half of the elytra).

3. Pronotal ratio
(length/width). Length measured along the medial line. Width measured at its maximum.

4. Pronotal maximum width/basal width

5. Body length/ body thickness ratio
Length measured from the anterior margin of the pronotum to the apex of the elytra. Thickness measured at its maximum (usually, across anterior half of the elytra).

6. Mentum index
(length/width)

7. Labrum index
(length/width)

8. Hind femur index, length/maximum width
(length/width). Width measured at its maximum (subapical width)

9. Hind leg tarsus, 1st article ratio
(length/width). Measures taken on the hairy sole

10. Hind leg tarsus, 2nd article ratio
(length/width). Measures taken on the hairy sole

11. Hind leg tarsus, 3rd article ratio
(length/width). Measures taken on the hairy sole

12. Hind leg tarsus, length 3rd article / length 2nd article
Measures taken on the hairy sole

13. Hind leg tarsus, width 3rd article/width 2nd article
Measures taken on the hairy sole

14. Ratio length of the metathorax /length of the first abdominal sternite.
Measures taken along the midline. According to taxonomic literature, the ratio between these two measures would be a diagnostic character to part the genus *Oreina* from the genus *Chrysolina*.

15. Metathorax, medial length/minimal length

MOUTHPARTS

16. Mouthparts: last article of the maxillar palps, relative width
0 - about half of the width of the penultimate article
1 - as large as the penultimate or slightly narrower

17. Mouthparts: maxillary palps, whether sexually dimorphic
0 - no
1 - yes

***18. Mouthparts: maxillary palps of the male, shape**
0 - pointed or rounded
1 - truncate, with subparallel sides
2 - truncate, with divergent sides (securiform)

19. Mouthparts: labial palps of the male, shape
0 - pointed to rounded
1 - truncate to securiform

HEAD

20. Head, dorsal side: punctuation of the clypeus stronger than the punctuation of the head
0 - yes
1 - no

21. Head, ventral side: subocular sulcus, degree of impression
0 - not perceivable
1 - shallow, with sloped margins
2 -well incised, with sharp margins

22. Head, ventral side: subocular sulcus, direction
0 - towards the eye, not tangent to the eye
1 - towards the eye, not tangent to the eye
2 - sinuated, directed away from the eye

THORAX, DORSAL SIDE

23. Thorax, pronotum: shape of sides
0 - unevenly rounded
1 - evenly rounded
2 - sides subparallel
3 - sides straight narrowing from the base

***24. Thorax, pronotum: reticulation of sides**
0 - distinct

1 - faint
2 - absent

***25. Thorax, pronotum: reticulation of the medial (discal) area**
0 - distinct
1 - faint
2 - absent

***26. Thorax, pronotum: basal pit/sulcus of the callum**
0 - absent or virtually so
1 - irregular, not sharp, defined by denser punctures
2 - in form of fovea or punctuated sulcus
3 - in form of sharp sulcus

27. Thorax, pronotum: degree of development of the lateral callum in its anterior half
0 - callum absent or indistinct, the pronotum is regularly rounded
1 - surface flattened or at most gently sloped
2 - strong impression
3 - narrow sulcus prolonging the basal pit/sulcus

***28. Thorax, pronotum: punctuation of the pre-callum impression**
0 - stronger than that of the discal area, foveolated
1 - stronger than that of the discal area, but not foveolated
2 - as that of the discal area

29. Thorax, pronotum: distribution of the large punctures of the pre-callum impression
0 - present also on the callum
1 - limited to the pre-callum impression

30. Thorax, pronotum: second order of punctuation present
0 - yes, widely distributed on the discal area
1 - yes, only near the anterior margin
2 - no

31. Thorax, pronotum: punctuation of the discal area evenly distributed
0 - yes
1 - no

***32. Thorax, pronotum: punctuation of first order on the callum compared to punctuation of first order of the pronotal disc**
0 - equal
1 - thinner
2 - stronger

***33. Thorax, pronotum: relative size of the first order of punctuation**
0 - small
1 - medium
2 - large

***34. Thorax, pronotum: condition of the marginal furrow along the base**
0 - absent
1 - present, only near posterior angles
2 - present, complete

35. Thorax, pronotum: setigerous pores at the angles
0 - present at the anterior and at the posterior angles
1 - present only at the posterior angles
2 - absent

36. Thorax, pronotum: setigerous pores, whether contained within the marginal furrow or not
0 - yes
1 - no

37. Thorax, pronotum: setigerous pores, whether contained within a deep fovea
0 - no
1 - yes

THORAX, VENTRAL SIDE

***38. Thorax, ventral side, prosternum: reticulation**
0 - distinct
1 - faint
2 - absent

39. Thorax, ventral side, prosternum: shape of prosternal sulci
0 - present only at the external end of prosternum
1 - disappearing in punctures towards the medial process
2 - complete, large, shallow
3 - complete, strong, narrow
4 - obliterated or missing

40. Thorax, ventral side, prosternum: prosternal sulci distance in the middle
0 - well parted from each other
1 - connecting or almost so

***41. Thorax, ventral side, proepimera: reticulation of ventral side**
0 - absent
1 - faint
2 - distinct

42. Thorax, ventral side, proepimera: shape of the posterior fovea
0 - absent or indistinct
1 - distinct, not incised on the posterior side
2 - distinct, incised on the posterior side

43. Thorax, ventral side, proepimera: impression of the posterior angle
0 - impressed
1 - not impressed

44. Thorax, ventral side, proepimera: radial sulci around the border
0 - absent to faint
1 - distinct to strong

***45. Thorax, ventral side, proepimera: degree of projection at the middle of the lateral ridge**
0 - null
1 - indistinctly projecting
2 - evidently but poorly projecting
3 - evidently and strongly projecting

***46. Thorax, ventral side, proepimera: degree on apical enlargement of the lateral ridge**
0 - null
1 - moderate (double thickness of middle)
2 - strong (more than double thickness of middle)

47. Thorax, ventral side, proepimera: sharp sulcus along the external edge
0 - absent
1 - present

48. Thorax, ventral side, mesosternun: shape of the central process

0 - flat, close to the metasternum
1 - thickened, without central crest and lateral fovea
2 - thickened, with central crest and lateral fovea

49. Thorax, ventral side, metasternum: punctuation along the anterior furrow
0 - smooth, glabrous
1 - smooth, hair on small punctures
2 - smooth, hair on large punctures
3 - indented by large punctures

50. Thorax, ventral side, metasternum: shape of the lateral furrow
0 - sharp, clearly reaching the posterior angle, parted from the basal sulcus
1 - blunt and/or not clearly reaching the posterior angle
2 - sharp and linked to the basal sulcus

51. Thorax, ventral side, metasternum: shape of the posterior sulcus
0 - distinct, large and poorly incised
1 - distinct, narrow and well incised
2 - obliterated, almost absent

52. Thorax, ventral side, metasternum: setation of the posterior sulcus
0 - absent to faint
1 - distinct to strong

53. Thorax, metasternum: anterior margin with complete ridge
0 - no
1 - yes

***54. Thorax, ventral side, metaepimera: marginal furrow**
0 - present on the external side and also in form of apical impression
1 - present on the external side only
2 - absent on the external side (present only on the anterior side)

ELYTRA

55. Elytra, shape: whether strongly convex at sides, with maximum width not corresponding to the epipleura
0 - no
1 - yes

***56. Elytra: reticulation of the surface**
0 - distinct
1 - faint
2 - absent

***57. Elytra: arrangement of punctuation of the second order**
0 - not ordered
1 - partially ordered in rows
2 - well ordered in not geminated rows
3 - well ordered in geminated rows

58. Elytra: leaks around punctures
0 - absent
1 - poor
2 - strong

59. Elytra, apical sulcus near the suture: degree of impression
0 - strongly impressed
1 - faint to absent

***60. Elytra, apical sulcus near the suture: elongation**
0 - short
1 - reaching half of the elytra

61. Elytra, whether having protruding ridges
0 - no
1 - yes

62. Elytra, epipleura: strongly turning apically
0 - no
1 - turning inwards
2 - turning outwards

63. Elytra, epipleura: epipleura very thin near the apex (almost inexistent)
0 - yes
1 - no

LEGS

64. Legs, tarsi: tarsi of males larger than tarsi of females
0 - no
1 - yes

65. Legs, tarsi: in males, sole of the first article of protarsi longitudinally divided
0 - no
1 - yes

66. Legs, tarsi: in males, sole of the first article of mesotarsi longitudinally divided
0 - no
1 - yes

67. Legs, tarsi: in males, sole of the metatarsi longitudinally divided
0 - no
1 - yes, up to the first article
2 - yes, up to the third article

68. Legs, tarsi: in females, sole of the protarsi longitudinally divided
0 - no
1 - yes, up to the first article
2 - yes, up to the second article
3 - yes, up to the third article

69. Legs, tarsi: longitudinal division of tarsi large or narrow
0 - large
1 - narrow

70. Legs, protibia: presence of tomentose stripe along the internal side
0 - no
1 - yes

ABDOMEN

71. Abdomen, first sternite: margin of the medial process with complete ridge along the edge
0 - yes
1 - no

72. Abdomen, last sternite: reticulation of the medial area
0 - present
1 - poor
2 - absent

73. Abdomen, last sternite: whether slightly inflated in the male
0 - no
1 - yes

***74. Abdomen, last sternite: whether protruding in an "ovopositor"**
0 - no
1 - mildly protruding
2 - yes

75. Abdomen, last sternite: presence of an apical pit in the male
0 - no
1 - yes
76. Abdomen, last sternite: presence of a longitudinal sulcus in the male
0 - yes
1 - no
77. Abdomen, last sternite: presence of a transversal impression in the male
0 - no
1 - yes
78. Abdomen, last sternite: presence of an apical pit in the female
0 - no
1 - yes
79. Abdomen, last sternite: posterior edge thickened in the middle in the male
0 - no
1 - yes
80. Abdomen, last sternite: posterior border twisted downwards in the middle and exposing fine and dense punctuation
0 - no
1 - yes
81. Abdomen, last sternite: shape of the marginal furrow
0 - narrow, with smooth bottom
1 - large, flat, sculptured/punctuated bottom
82. Abdomen, last sternite: whether the marginal furrow reaches the base
0 - yes, complete
1 - yes, but interrupted at sides
2 - no
83. Abdomen, last sternite: shape of the edge in the male
0 - truncate, straight
1 - truncate, slightly concave
2 - bisinuated
3 - normally rounded
84. Abdomen, last sternite: shape of the edge in the female
0 - normally rounded
1 - truncated, straight
2 - truncated, concave

PYGIDIUM

***85. Pygidium: length of the longitudinal groove**
0 - only present at the base
1 - reaching the distal half
2 - reaching the very apex
86. Pygidium: edge thickened in a ridge
0 - no
1 - yes

AEDEAGUS

87. Aedeagus, shape: whether tubular or flat and spatular
0 - tubular
1 - intermediate shape
2 - spatular
88. Aedeagus, shape: whether sinuated
0 - no
1 - yes
89. Aedeagus, apex: presence of denticles
0 - no
1 - yes, reflexed
2 - not reflexed, anchor-like
90. Inner aedeagic apodeme of the last sternite: shape
0 - distinctly grooved and elevated
1 - poorly grooved and flat

BIOLOGY AND CITOLOGY

91. Host plants: family
0 - Solanaceae
1 - Lamiaceae
2 - Asteraceae
3 - Scrophulariaceae
4 - Apiaceae
5 - Plantaginaceae
6 - Clusiaceae
92. Diploid chromosome number
0 - 23/24
1 - 40
2 - 42
3 - 38
4 - 34
5 - 32
6 - 36
7 - 48
8 - 46
93. Defensive chemicals: presence of cardenolides
0 - no
1 - yes
94. Defensive chemicals: presence of pyrrolizidine alkaloids
0 - no
1 - yes
95. Defensive chemicals: presence of polyoxygenated steroids
0 - no
1 - yes

Appendix 3. Data matrix.

Character

Taxon	01	02	03	04	05	06	07	08	09	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95
Leptinotarsa	1.000	1.400	0.500	1.000	1.900	3.000	1.000	4.700	1.100	0.400	1.400	2.200	1.400	1.400	0.900	0	?	1	0	0	1	0	3	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	2	0	1	0	0	?	0	0	2	0	0	1	0	0	0	0	3	2	0	0	0	0	0	0	0	0	0	0	?	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	4	?	?	?
Ch.Allohyp.aeruginosa	0.600	1.500	0.500	1.000	2.100	3.300	2.100	5.500	1.400	0.500	1.600	2.200	1.600	1.600	0.900	1	0	2	0	1	2	0	1	1	0	1	1	0	0	0	0	1	1	2	1	0	0	0	3	0	2	1	1	1	2	2	0	2	2	0	1	0	1	0	0	0	3	1	0	0	0	0	1	1	1	1	1	2	?	1	1	2	0	0	0	1	0	0	0	0	0	0	3	0	1	0	1	?	?	?	2	?	?	?	?
Ch.Taen.americana	0.900	1.500	0.500	1.000	2.100	2.600	1.900	6.200	1.600	0.400	1.500	2.500	1.500	1.500	1.000	1	0	1	0	0	2	1	1	0	0	0	1	0	0	2	0	0	0	2	0	1	0	1	3	0	2	1	1	0	1	0	0	2	2	0	1	0	1	1	0	2	3	1	0	0	0	0	1	?	0	0	0	0	?	1	1	2	?	0	0	1	0	?	1	0	0	?	0	0	0	0	1	0	1	?	1	0	1	0	0
Ch.Apt.angusticollis	0.900	1.600	0.600	1.150	2.100	3.000	1.900	6.200	1.500	0.600	1.400	1.500	1.400	1.700	0.700	1	0	1	1	1	2	0	0	1	1	0	1	0	0	0	1	?	0	2	0	0	0	1	3	0	0	0	1	0	0	?	0	2	2	0	1	1	0	0	0	1	0	1	0	0	0	0	1	1	0	0	0	0	?	1	1	1	0	2	0	1	0	0	1	0	0	1	2	?	0	0	1	1	0	?	2	2	?	?	?
Ch.Anop.asclepiadis	0.800	1.600	0.500	1.040	2.100	4.100	2.500	7.200	1.600	0.800	1.500	1.200	1.500	1.600	0.800	1	0	2	1	0	2	0	0	0	0	1	1	0	0	0	0	1	0	2	0	1	0	1	3	0	0	1	1	0	0	0	0	2	1	1	1	1	1	2	0	2	1	0	0	0	0	0	0	0	0	0	0	0	?	1	1	1	0	1	0	1	0	0	1	0	0	1	2	0	0	0	0	0	2	?	2	?	0	0	0
Ch.Cent.bicolor	1.200	1.600	0.500	1.000	2.000	2.700	1.900	6.900	1.400	0.400	1.000	1.600	1.000	1.400	1.000	1	1	1	0	0	2	1	1	0	0	1	1	0	?	2	0	0	0	2	0	1	0	2	3	0	0	2	0	1	0	1	0	1	2	0	1	1	1	1	0	0	3	1	0	0	0	0	1	0	1	1	1	3	0	1	1	2	1	0	0	1	0	0	0	0	?	0	0	0	1	0	2	0	1	0	12	0	?	?	?
Ch.Chrysom.cerealis	0.900	1.400	0.500	1.000	1.900	4.000	1.700	5.600	1.400	0.400	1.400	1.900	1.400	1.500	1.000	1	0	1	0	0	2	2	1	0	0	2	0	1	0	0	0	?	1	2	0	1	0	0	1	0	2	1	0	0	2	1	0	2	2	0	1	0	1	1	0	2	0	2	0	0	0	0	0	0	0	0	0	0	?	1	1	2	1	0	0	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	1	0	?	?	?
Ch.Syn.coerulans	0.700	1.500	0.600	1.000	2.300	2.600	1.900	6.200	1.900	0.600	1.600	1.600	1.600	1.400	1.100	1	0	1	0	0	2	1	1	1	0	0	0	0	0	0	0	0	0	2	0	?	0	2	3	0	0	1	0	1	2	1	0	2	2	0	0	0	1	2	0	2	0	2	0	0	0	0	0	0	0	0	0	0	?	1	1	2	?	0	0	0	0	1	?	0	0	?	2	0	0	0	?	0	0	?	1	?	1	0	0
Ch.Nal.confluens	0.800	1.300	0.500	1.000	2.100	2.200	2.000	5.500	1.400	0.500	1.300	1.600	1.300	1.600	1.000	1	?	2	0	0	2	1	1	1	0	0	1	0	1	0	0	1	0	2	0	1	0	1	4	0	2	0	0	0	3	0	1	1	2	0	1	1	1	0	0	0	2	0	0	0	0	0	1	0	0	0	0	0	?	1	1	2	0	0	0	0	0	0	?	0	1	0	2	0	0	0	1	0	0	?	?	?	?	?	?
Ch.Pal.diluta	0.600	1.300	0.500	1.000	1.900	3.300	2.200	4.700	0.800	0.400	0.600	2.100	1.400	1.700	0.900	1	0	2	0	1	0	0	1	0	0	1	0	0	0	2	0	0	0	1	2	?	?	0	3	0	1	1	1	0	0	1	0	1	2	0	0	1	1	1	0	0	1	1	0	?	0	0	1	1	0	0	0	0	?	1	1	0	?	0	0	1	0	0	0	0	1	0	0	0	2	0	2	0	0	?	25	6	?	?	?
Ch.Fast.fastuosa	0.600	1.500	0.600	1.050	2.100	2.600	2.100	6.600	1.700	0.600	1.700	1.700	1.700	1.500	0.900	1	0	1	0	1	2	1	1	0	0	0	0	1	0	0	1	0	0	2	0	?	0	0	1	0	2	1	0	0	1	1	0	2	2	1	1	0	1	1	0	2	0	2	0	0	0	2	1	0	0	0	0	0	?	1	1	0	1	0	0	1	0	0	1	0	0	0	0	0	0	0	1	1	2	0	1	0	1	0	0
Ch.Maen.femoralis	0.800	1.300	0.500	1.000	1.800	3.100	1.900	6.900	1.400	0.300	1.300	2.500	1.300	1.400	0.900	1	?	1	0	1	2	0	3	0	0	1	1	1	?	2	0	1	1	1	2	?	?	0	3	0	2	0	1	0	1	2	0	2	3	2	1	0	1	1	?	0	0	1	0	0	0	?	1	0	1	1	1	1	1	1	1	1	0	0	0	1	0	0	0	0	1	0	0	1	2	0	2	0	0	1	1	?	?	?	?
Ch.Rhys.fragariae	1.100	1.700	0.500	1.000	2.500	3.500	1.600	5.400	1.400	0.500	1.500	1.900	1.500	1.600	0.600	1	0	2	1	1	1	1	1	2	2	0	2	1	0	2	0	?	0	2	0	1	0	0	3	0	2	0	1	0	0	?	0	1	2	2	1	1	0	1	0	2	3	1	0	0	1	1	0	0	0	0	0	0	?	1	1	2	1	0	0	1	0	0	1	0	0	2	0	0	0	0	1	1	0	0	1	?	?	?	?
Ch.All.fuliginosa	0.800	1.400	0.500	1.000	1.900	2.400	2.100	4.800	1.400	0.300	1.400	2.600	1.400	0.900	1.400	1	0	1	1	1	2	0	3	0	0	1	0	1	1	2	0	2	0	1	0	1	0	0	1	0	2	0	?	1	2	0	0	1	2	0	1	1	1	1	1	0	0	2	0	0	0	0	0	1	0	0	0	0	?	1	1	0	0	0	0	1	0	?	0	0	1	0	0	0	1	0	2	0	0	1	2	2	0	0	0
Ch.Chrysolin.gemina	0.800	1.500	0.500	1.000	2.200	2.500	1.700	6.800	1.800	0.500	1.500	2.200	1.500	1.500	1.000	1	1	3	1	1	1	1	1	0	0	0	0	1	?	2	0	0	0	2	0	0	0	0	3	0	1	1	1	0	0	?	0	1	2	0	1	0	0	1	0	2	3	1	0	0	0	1	1	1	0	0	0	0	?	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	0	?	?
Ch.Hyp.geminata	0.600	1.600	0.500	1.000	2.200	2.900	?	?	?	?	?	?	?	1.400	1.000	?	?	?	1	1	2	1	1	0	0	2	0	1	1	2	0	0	?	2	0	1	0	0	2	1	2	1	0	0	2	1	0	2	2	0	1	0	?	1	?	0	3	2	0	0	0	0	0	?	?	?	?	?	?	1	1	0	1	?	0	1	1	?	1	?	1	0	0	?	1	0	0	0	0	?	6	3	0	0	1
Ch.Euch.graminis	1.000	1.600	0.600	1.000	2.300	3.100	1.800	6.900	1.800	0.500	1.200	1.600	1.200	1.700	1.000	1	0	1	0	0	2	1	1	0	0	0	0	0	0	0	1	1	0	2	0	0	0	2	1	0	0	1	0	1	2	1	0	2	2	0	1	0	1	0	0	2	0	2	0	1	0	2	0	?	0	0	0	0	?	1	1	2	1	0	0	0	0	0	0	0	0	?	2	0	0	0	1	0	0	?	1	0	1	0	0
Ch.Melas.grossa	1.000	1.500	0.500	1.000	2.500	2.700	2.200	6.200	1.800	0.700	1.300	1.300	1.300	1.700	1.100	1	?	2	0	1	2	1	1	1	1	2	2	0	1	1	0	0	0	2	0	1	0	0	3	0	2	1	0	0	2	1	0	2	2	0	1	0	1	0	0	2	0	0	0	0	0	0	1	1	0	0	0	0	?	1	1	2	0	0	0	1	0	0	1	0	1	0	3	0	0	0	1	0	0	?	1	0	1	0	0
Ch.Timar.haemochlora	0.800	1.400	0.600	1.080	2.100	2.500	2.000	5.200	1.200	0.400	1.300	1.900	1.300	2.100	0.800	1	1	2	0	0	2	0	0	0	0	0	1	2	0	?	0	1	1	2	2	?	?	0	0	0	2	1	1	0	0	0	0	0	1	0	2	0	1	2	0	0	0	2	0	0	0	0	1	1	0	0	0	0	?	1	1	2	1	0	0	1	0	0	1	0	0	0	0	0	2	0	2	0	2	?	?	?	?	?	?
Ch.Col.haemoptera	0.700	1.300	0.400	1.000	1.800	3.000	1.800	5.300	1.300	0.500	1.400	1.800	1.400	1.500	0.800	1	1	2	0	0	2	2	3	0	0	0	0	1	0	0	0	1	?	2	2	?	?	0	3	0	2	2	0	0	1	1	0	2	2	1	0	0	1	1	1	1	0	0	0	0	0	2	1	1	0	0	0	0	?	1	1	1	?	0	0	0	0	0	0	1	0	0	?	2	2	0	2	0	0	?	5	1	1	0	0
Ch.Thr.helopioides	1.100	1.300	0.500	1.000	1.900	2.000	2.200	7.300	1.500	0.400	1.400	2.300	1.400	1.700	0.900	1	0	1	0	0	2	2	1	0	0	2	0	0	0	2	0	0	0	2	0	1	0	0	3	0	2	1	0	0	0	?	0	1	1	0	1	0	1	1	0	0	0	1	0	0	0	0	1	1	0	0	0	0	?	1	?	0	1	0	0	1	0	0	0	1	1	0	1	2	2	0	2	0	2	?	24	7	?	?	?
Ch.Colaph.hemisphaerica	0.800	1.400	0.500	1.000	2.000	3.000	2.000	5.400	1.700	0.500	1.300	2.000	1.300	1.700	0.800	1	0	2	1	1	0	?	3	2	1	3	2	2	?	2	0	1	0	1	2	?	?	0	3	0	0	1	0	0	0	1	0	2	2	1	1	0	1	2	1	2	1	0	1	0	0	2	1	1	0	0	0	0	?	1	1	2	0	0	0	1	0	0	0	?	0	0	1	0	1	0	1	0	1	?	1	0	?	?	?
Ch.Lith.hexanthematica	0.800	1.600	0.500	1.000	2.300	3.100	2.400	6.900	2.200	0.600	1.400	1.400	1.400	1.500	0.900	1	1	1	0	1	2	2	1	1	1	1	2	0	1	2	0	0	2	2	1	1	0	1	?	0	1	1	1	1	2	2	0	2	3	0	0	1	1	1	0	0	0	0	0	0	0	0	1	0	1	1	1	1	1	1	1	1	0	0	0	1	0	0	0	0	0	0	3	0	2	0	2	0	1	?	1	?	?	?	?
Ch.Hyp.hyperici	0.600	1.600	0.500	1.000	2.200	2.900	1.800	4.700	1.200	0.600	1.500	1.800	1.500	1.400	1.000	1	0	2	1	0	1	1	1	0	0	2	0	1	?	2	0	2	0	2	0	1	0	1	2	1	1	0	1	1	2	1	0	2	2	0	1	0	1	1	0	0	3	1	0	0	0	0	1	1	0	0	0	0	?	1	1	0	1	0	0	1	1	0	0	0	?	0	0	1	2	0	1	0	1	?	6	3	0	0	1
Ch.Ghes.katangana	0.800	1.400	0.500	1.000	1.900	3.400	1.800	5.500	1.600	0.400	1.300	2.600	1.300	1.900	0.800	1	0	2	0	1	2	0	1	2	0	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	0	0	0	1	3	1	0	2	3	1	0	1	1	1	0	0	3	1	0	0	0	0	?	0	0	0	0	0	?	1	1	2	0	0	0	1	0	0	0	0	0	0	3	0	2	0	2	0	1	0	?	?	?	?	?
Ch.kinabaluensis	1.000	1.400	0.500	1.000	2.100	3.300	2.000	6.700	1.600	0.800	1.400	1.300	1.400	1.300	1.000	1	0	1	0	0	2	0	1	0	0	0	1	0	0	0	1	1	0	0	0	1	0	1	0	0	2	0	1	0	0	0	0	1	0	0	0	0	1	1	0	2	1	0	1	0	0	0	1	0	0	0	0	0	?	1	1	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0	?	1	1	?	?	?	?	?	?
Ch.Hel.lichenis	0.700	1.500	0.500	1.080	2.000	4.000	2.100	4.700	1.300	0.400	1.400	1.800	1.400	1.700	0.700	1	0	1	0	0	1	2	0	0	0	1	2	2	?	2	0	1	1	2	2	?	?	0	0	0	1	0	1	1	0	?	0	2	0	1	0	0	1	2	0	0	0	1	0	0	0	0	0	1	0	0	0	0	?	1	0	1	1	0	0	1	0	0	0	0	0	0	3	0	0	0	1	0	0	?	?	?	?	?	?
Ch.Cras.limbata	0.700	1.600	0.500	1.040	2.200	2.900	1.600	5.200	1.300	0.500	1.400	1.900	1.400	1.700	0.800	1	1	3	0	1	2	0	0	0	0	2	1	0	0	2	0	0	0	2	2	?	?	0	3	0	2	1	0	0	2	2	0	2	0	1	0	1	1	1	0	0	1	2	0	0	0	0	1	1	0	0	0	0	?	1	0	1	1	0	0	1	0	0	0	1	1	0	0	0	2	0	1	0	0	?	5	2	?	?	?
Ch.Chal.marginata	0.600	1.700	0.500	1.000	2.400	3.500	2.400	5.900	1.300	0.300	1.400	2.800	1.400	1.500	1.200	1	0	1	0	0	2	0	1	0	0	1	0	0	0	2	0	0	0	2	0	1	0	1	2	1	0	1	0	0	2	1	0	2	0	0	1	1	1	1	0	0	3	1	0	0	0	0	1	1	0	0	0	0	?	1	0	0	0	0	0	1	0	0	0	0	0	0	3	0	1	0	2	1	0	0	25	1	?	?	?
Ch.Bech.nikolskii	0.900	1.400	0.600	1.100	2.000	4.000	1.800	6.400	1.400	0.600	1.200	1.600	1.200	2.100	0.800	1	0	2	0	0	2	0	0	0	0	0	1	2	?	2	0	0	0	2	1	1	0	0	2	0	2	1	1	0	0	0	0	2	1	0	0	0	1	1	0	0	0	1	1	0	0	0	0	1	0	0	0	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2	0	1	?	2	?	?	?	?
Ch.Ovost.olivieri	1.000	1.200	0.500	1.020	1.700	4.500	2.200	6.800	1.600	0.500	1.500	2.000	1.400	1.500	0.800	1	1	3	1	0	2	2	0	1	0	1	0	1	?	2	0	0	1	2	2	0	0	0	3	0	2	1	0	1	3	2	0	2	0	0	0	0	1	2	0	0	0	2	1	0	0	0	1	1	0	0	0	1	1	1	1	1	?	0	0	1	0	0	0	0	0	0	0	0	2	1	2	0	1	?	1	?	?	?	?
Ch.Sul.oricalcia	0.800	1.500	0.500	1.000	2.300	2.800	1.900	5.900	1.400	0.400	1.400	2.200	1.500	1.600	0.900	1	0	1	0	0	2	2	3	0	1	3	3	2	?	2	0	0	0	2	0	1	0	0	2	0	1	1	0	0	1	1	0	2	2	0	1	0	1	1	1	0	2	2	0	0	0	?	1	1	0	0	0	0	?	1	1	1	1	0	0	1	0	0	1	0	1	0	0	1	2	0	2	0	1	?	4	8	0	0	0
Ch.Cros.pedestris	1.000	1.600	0.500	1.070	2.000	2.600	2.100	5.300	1.300	?	?	?	?	1.600	0.700	1	0	2	0	1	2	2	0	1	0	1	0	0	1	2	0	1	2	2	1	1	?	0	3	0	1	1	0	1	1	2	0	0	2	1	0	1	1	2	0	0	0	1	0	0	0	0	1	1	0	0	0	1	1	1	0	1	1	0	0	1	0	0	?	1	1	0	1	0	1	0	2	0	0	?	?	?	?	?	?
Ch.Bechy.platypoda	1.000	1.600	0.500	1.000	2.100	2.700	2.300	4.700	1.200	0.400	1.300	2.100	1.300	1.800	0.700	1	0	1	1	1	2	0	1	0	0	2	1	1	0	1	0	0	0	2	0	1	0	0	2	0	2	0	1	0	1	1	0	1	2	1	1	0	1	1	1	0	0	1	0	0	0	2	?	1	0	0	0	0	?	1	1	0	0	0	0	0	0	0	0	1	0	0	0	2	0	2	0	0	0	?	?	?	?	?	?
Ch.Eryth.polita	0.700	1.500	0.600	1.000	2.200	3.100	2.200	7.600	1.600	0.600	1.500	1.800	1.500	1.600	1.000	1	1	2	0	1	2	1	1	0	0	1	2	0	0	1	0	1	0	2	0	1	0	0	3	0	2	1	0	0	0	?	0	2	2	0	1	0	1	1	0	2	0	0	0	0	0	0	1	1	0	0	0	0	?	1	1	2	0	0	0	1	0	0	1	0	0	0	0	0	1	0	1	0	0	?	1	?	1	0	0
Ch.Stich.rossia	0.800	1.300	0.400	1.000	1.900	2.900	2.000	7.200	1.800	0.500	1.200	2.200	1.200	1.700	0.900	1	1	3	1	1	2	2	3	0	0	2	1	1	0	2	0	0	0	1	2	?	?	0	3	0	2	1	0	0	1	0	0	1	2	1	1	1	1	1	0	0	0	2	0	0	0	2	1	1	0	0	0	0	?	1	1	0	0	0	0	1	0	0	0	1	1	0	0	0	2	0	2	0	1	?	1	4	?	?	?
Ch.Diach.sacarum	0.600	1.400	0.500	1.000	2.100	2.900	2.300	5.800	1.400	0.300	1.300	2.300	1.300	1.400	0.900	1	0	2	0	0	2	0	1	0	0	1	0	0	0	2	0	0	0	0	2	?	?	2	2	0	1	2	1	1	1	2	0	2	2	1	1	1	1	1	1	0	3	1	0	0	0	0	1	0	0	0	0	3	0	1	0	2	0	0	0	1	0	0	0	0	0	0	3	1	2	0	2	0	0	?	?	?	?	?	?
Ch.Ovos.sahlbergi	0.900	1.400	0.400	1.000	1.900	2.800	1.800	6.200	0.900	0.300	1.300	2.600	1.300	1.500	0.900	1	0	2	1	0	1	2	3	0	0	3	0	1	1	2	0	0	0	1	2	?	?	0	3	0	2	2	0	0	0	0	0	2	2	0	1	1	1	1	1	0	2	1	0	0	0	2	1	1	0	0	0	0	?	1	1	0	0	0	0	1	1	?	0	1	1	0	0	1	1	0	2	1	0	?	1	?	?	?	?
Ch.Chrysocr.spectabilis	0.900	1.500	0.500	1.080	2.200	3.900	1.800	6.100	1.400	0.400	1.300	2.300	1.300	1.700	0.700	1	0	2	1	?	2	0	0	0	0	1	1	1	0	2	0	1	1	1	2	?	?	0	1	0	1	1	0	1	1	1	0	2	2	1	0	0	1	1	0	0	0	1	0	0	0	0	1	1	0	0	0	2	0	1	1	0	0	0	0	1	0	0	0	0	0	0	0	0	2	1	2	0	0	?	?	?	?	?	?
Ch.Chrys.staphylaea	0.800	1.500	0.400	1.000	2.100	3.300	1.900	6.200	1.700	0.600	1.200	1.600	1.200	1.600	0.800	1	1	3	1	1	2	1	1	0	0	2	2	0	1	1	0	1	0	0	2	?	?	0	3	0	2	1	1	0	2	2	0	1	0	0	1	0	1	2	0	1	0	1	0	0	0	0	1	0	0	0	0	0	?	1	1	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2	0	0	?	1	0	1	0	0
Ch.Pierr.stictica	0.800	1.400	0.500	1.050	2.100	3.300	1.600	5.600	1.900	0.700	1.400	1.500	1.400	1.500	0.900	0	1	1	0	1	2	0	0	1	0	0	0	0	0	0	1	0	0	2	2	?	?	0	3	0	2	1	1	0	2	0	0	2	2	0	1	1	1	1	0	2	1	1	0	0	0	0	1	0	0	0	0	0	?	1	1	2	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	1	?	?	?	?	?	?
Ch.Colaphos.sturmi	0.700	1.300	0.500	1.000	1.800	2.700	2.300	6.100	1.300	0.500	1.200	1.500	1.200	0.700	1.600	1	1	2	1	1	0	?	1	1	0	0	0	1	1	2	0	1	0	2	2	?	?	1	3	0	2	1	0	0	3	0	0	1	2	1	1	0	1	2	1	0	0	1	1	0	0	0	1	1	0	0	0	0	1	1	1	0	1	0	0	1	0	0	?	0	0	0	0	0	2	1	2	0	1	1	1	?	?	?	?
Ch.Pseudot.superba	1.000	1.400	0.500	1.000	2.200	2.800	2.000	6.300	1.600	0.700	1.500	1.900	1.500	0.900	1.300	1	0	2	0	0	2	0	1	0	0	0	1	0	0	1	0	0	0	2	0	1	0	1	0	0	0	1	1	1	1	0	0	2	2	0	1	0	0	1	0	2	3	2	0	0	0	0	1	0	0	0	0	0	?	1	1	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	1	?	?	?	?	?	?
Ch.Tim.templetoni	0.700	1.300	0.500	1.090	1.900	3.600	2.000	7.200	1.700	0.600	1.700	1.700	1.700	1.800	0.900	1	0	2	1	1	2	0	0	1	1	1	1	0	0	1	1	?	0	2	2	?	?	1	0	0	2	1	0	0	0	?	0	1	2	0	0	1	1	1	0	1	3	0	0	0	0	1	?	?	0	0	0	0	?	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2	0	1	0	?	?	?	?	?
Ch.Sph.varians	0.500	1.400	0.500	1.000	2.100	3.100	1.800	6.000	1.300	0.600	1.300	1.500	1.300	1.500	0.900	1	0	1	0	1	1	1	1	0	0	0	0	1	0	2	0	1	1	2	0	1	0	2	2	1	0	1	1	0	2	1	0	2	2	0	1	0	1	0	0	1	0	1	0	0	0	2	1	1	0	0	0	0	?	1	1	0	0	0	1	1	0	0	?	0	0	2	0	0	1	0	2	0	0	?	6	5	0	0	1
Ch.Ovos.vernalis	1.000	1.300	0.500	1.000	1.700	2.300	1.700	6.000	1.200	0.500	1.300	2.000	1.300	1.500	0.700	1	0	2	1	0	2	2	3	0	0	3	3	2	?	2	0	0	0	1	2	?	?	0	3	0	2	1	0	0	0	2	0	2	2	0	0	0	1	1	1	0	1	2	0	0	0	2	1	1	0	0	0	0	?	1	1	0	0	0	0	1	1	?	0	1	1	0	0	1	2	0	?	1	0	1	5	1	?	?	?
Ch.Parad.vignai	0.600	1.600	0.600	1.000	2.300	3.200	2.000	5.200	0.800	0.400	1.000	1.700	1.000	1.700	0.600	1	?	1	0	1	2	0	1	1	1	1	0	1	0	2	0	0	0	2	2	?	?	1	3	0	2	0	0	0	0	?	0	2	2	2	1	0	1	1	1	0	2	0	0	0	0	2	1	1	0	0	0	?	?	1	1	1	1	?	0	1	0	?	0	1	?	0	0	?	1	0	2	0	0	1	?	?	?	?	?
Ch.Syn.viridana	0.800	1.600	0.600	1.000	2.200	2.600	1.800	6.800	1.600	0.700	1.600	1.400	1.600	1.500	1.200	1	0	1	0	1	2	1	1	0	0	0	0	0	0	0	1	0	0	2	0	1	0	2	3	0	0	1	0	1	2	?	0	2	2	0	1	0	1	1	0	2	0	2	0	0	0	0	0	0	0	0	0	0	?	1	1	2	?	0	0	0	0	1	?	0	0	0	2	0	0	0	1	0	0	0	1	?	1	0	0
Ch.Vitt.vittata	0.600	1.400	0.500	1.000	1.900	3.400	2.100	4.700	1.600	0.400	1.300	2.200	1.300	1.600	0.700	1	?	1	0	1	0	1	1	0	0	0	1	1	0	0	0	1	0	2	2	?	?	0	3	0	2	0	1	0	0	?	0	1	1	0	2	0	1	?	0	1	0	2	0	0	0	1	0	0	0	0	0	0	?	1	1	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2	0	0	?	?	?	?	?	?
Ch.Taen.bakuensis	0.700	1.500	0.500	1.000	2.100	3.400	1.900	5.000	1.100	0.400	1.300	1.800	1.300	1.500	0.800	1	0	2	0	0	2	0	1	0	0	2	0	1	0	2	0	0	0	1	2	?	?	0	3	0	1	2	1	0	0	1	0	2	2	1	?	0	1	2	?	0	2	1	0	0	0	2	0	0	0	0	0	1	1	1	0	1	1	0	0	1	0	0	0	0	1	0	0	0	1	0	1	0	0	?	5	?	?	?	?
Ch.Cam.ornata	0.900	1.300	0.400	1.140	2.100	3.300	2.300	5.600	1.500	0.500	1.400	1.900	1.400	1.500	1.100	1	1	2	1	0	0	?	0	0	0	0	0	2	0	0	0	0	0	1	0	1	0	0	4	0	2	0	1	0	3	2	1	0	1	0	2	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	?	1	1	1	0	0	0	1	0	0	0	0	1	0	1	0	1	0	0	0	0	1	?	?	?	?	?
Ch.Ate.vigintiquatorsignata	0.700	1.400	0.500	1.000	2.000	4.200	1.200	5.900	1.200	0.400	1.200	1.600	2.000	1.700	1.000	1	0	1	0	0	2	1	1	1	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1	1	0	1	1	0	1	2	0	2	0	1	1	1	0	2	2	0	0	0	0	1	0	0	0	0	0	?	1	1	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	0	1	?	?	?	?	?
Cr.altaica	0.900	1.600	0.600	1.160	2.200	5.500	2.000	7.000	1.700	0.700	0.900	1.200	0.900	1.500	0.700	1	1	2	1	?	2	1	0	1	0	2	2	0	1	2	0	1	2	2	0	?	0	2	0	0	0	1	1	0	0	0	0	1	2	1	?	1	1	1	0	0	1	2	1	?	?	0	1	1	0	0	2	3	0	1	1	2	0	0	0	0	0	0	?	0	0	0	0	1	2	0	2	0	?	?	2	?	?	?	?
Or.Chrys.elegans	0.700	1.700	0.600	1.000	2.500	3.000	1.500	7.200	2.200	0.600	1.600	1.300	1.600	1.500	0.900	1	?	1	1	1	2	1	1	0	0	1	2	0	1	0	1	0	?	2	0	0	1	0	1	0	0	1	1	1	0	0	0	2	2	0	1	0	1	1	0	2	0	2	0	0	0	0	0	0	0	0	0	0	?	1	0	0	?	0	0	1	0	0	1	0	0	0	0	0	0	0	2	0	0	?	2	?	?	?	?
Or.Chrys.elongata	0.800	1.700	0.600	1.000	2.600	3.200	1.700	5.800	1.700	0.400	1.400	2.000	1.400	1.400	0.900	1	0	1	0	1	2	1	1	1	2	1	2	0	0	0	1	?	0	2	0	0	0	2	3	0	0	1	0	1	0	0	0	2	2	0	1	0	1	0	0	1	0	2	0	1	0	0	1	?	0	0	0	0	?	1	0	0	1	0	0	0	0	0	1	0	0	2	0	0	0	0	0	1	0	0	2	?	1	1	0
Or.Frig.frigida	0.600	1.700	0.500	1.000	2.400	3.400	1.700	4.600	1.390	0.400	0.500	1.700	1.200	1.000	1.400	1	0	1	1	1	2	1	1	0	0	0	1	0	1	0	0	0	?	2	0	0	1	0	1	0	1	1	1	1	0	0	0	2	1	0	1	0	1	0	0	2	0	2	0	0	0	0	0	0	0	0	0	0	?	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	2	0	1	?	2	?	1	1	0
Or.Prot.plagiata	1.000	1.900	0.500	1.110	2.600	3.300	1.700	7.400	2.000	0.500	1.400	1.800	1.400	0.900	1.400	1	0	1	1	1	2	1	1	0	0	1	2	0	0	0	1	0	?	2	0	0	?	0	1	0	0	1	0	1	1	0	0	2	1	0	1	0	1	1	0	2	0	2	0	0	0	0	0	?	0	0	0	0	?	1	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	?	0	0	0
Sem.mirabilis	0.900	1.500	0.600	1.030	2.000	3.300	1.900	7.800	1.300	0.500	1.300	1.500	1.300	1.400	0.700	1	0	2	1	1	2	2	0	2	2	0	2	0	1	2	0	0	?	2	2	?	?	0	3	0	0	0	0	0	?	2	0	2	0	1	0	0	1	2	1	2	1	1	0	0	0	2	1	1	0	0	0	0	?	1	1	2	1	0	0	1	0	0	0	0	?	0	1	0	0	0	2	0	?	?	?	?	?	?	?
Or.Ore.speciosa	1.000	1.600	0.600	1.000	2.300	3.100	1.700	5.900	1.300	0.400	0.600	2.400	1.400	1.200	1.500	1	1	2	1	1	2	1	0	0	0	1	1	0	0	1	1	0	0	2	0	0	0	0	3	0	2	1	0	1	1	0	0	2	1	0	1	0	0	0	0	0	0	2	0	0	0	0	0	1	0	0	0	0	?	1	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	?	4	?	1	0	0
Or.Ore.viridis	1.000	1.600	0.500	1.000	2.100	2.900	1.800	5.700	0.000	0.000	0.000	0.000	1.400	1.000	1.300	1	1	3	1	1	2	1	0	0	0	1	0	0	0	1	1	0	0	2	0	0	?	0	3	0	0	1	0	1	1	0	0	2	1	0	1	0	1	0	0	0	0	2	0	0	0	0	0	1	0	0	0	0	?	1	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	?	4	?	?	?	?

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