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Morphological analysis of two Paussinae  
species: *Paussus favieri* and *P. cridaei* (Coleoptera:  
Carabidae). A geometric morphometric approach.

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*I have called this principle,  
by which each slight variation,  
if useful, is preserved,  
by the term of Natural Selection.*

(Charles Darwin)



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## **Supplementary materials**

*Appendix A:* Materials and Methods of the thesis, with references.

*Appendix B:* Additional Materials to Chapter 2, section 2:

1. Table 2.2a - Pairwise comparisons by the canonical variate analysis performed between the studied populations in *P. cridaae*. Mahalanobis distances and *p*-values, after 10000 permutation runs. Significant values are reported in bold red. (Angola – AN; Democratic Republic of Congo-former Zaire- CN; Kenya – KN; Rwanda – RW; South Africa – SA; Tanzania – TZ; Uganda – UG; Zambia – ZA; Zimbabwe – ZB).
2. Table 2.2b - Pairwise comparisons by the canonical variate analysis performed between the studied populations in *P. cridaae*. Procrustes distances and *p*-values, after 10000 permutation runs. Significant values are reported in bold red. (Angola – AN; Democratic Republic of Congo-former Zaire- CN; Kenya – KN; Rwanda – RW; South Africa – SA; Tanzania – TZ; Uganda – UG; Zambia – ZA; Zimbabwe – ZB).

*Appendix C:* other papers published and submitted during the PhD:

1. Scalici M., Bravi R. 2012. Solving alpha-diversity by morphological markers contributes to arranging the systematic status of a crayfish species complex (Crustacea, Decapoda). *J Zool Syst Evol Res.* 50: 89-98.
2. Bravi R., Ruffini M., Scalici M. Morphological variation in riverine cyprinids: a geometric morphometric contribution. *Italian Journal of Zoology.* Accepted.

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2. Bravi R., Ruffini M., Scalici M. Morphological variation in riverine cyprinids: a geometric morphometric contribution. *Italian Journal of Zoology.* Accepted.
3. Benitez H., Bravi R., Vallebuona F. Left-Right Asymmetry and Shape Analysis in Multiple Austral Populations of *Ceroglossus chilensis* (Coleoptera: Carabidae). *Journal of Zoological Systematics and Evolutionary Research.* In rev.
4. Benítez H., Pizarro-Araya J., Bravi R., Sanzana M.J. Alfaro FM. Variation and island effect on isolated populations of *Praocis* (*Praocis*) *spinolai* Gay and Solier, 1840 (Coleoptera: Tenebrionidae) with a geometric morphometric approach. *Journal of Arid Environment.* In rev.
5. Bravi R., Scalici M., Klingenberg C.P., Di Giulio A. Directional asymmetry in *Paussus favieri* (Coleoptera: Carabidae: Paussini). *Biological Journal of Linnean Society.* (In preparation).
6. Bravi R., Scalici M., Klingenberg C.P., Di Giulio A. Morphological variation in ant nest beetle *Paussus favieri* (Coleoptera: Carabidae, Paussinae). *Biological Journal of Linnean Society.* (In preparation).
7. Bravi R., Scalici M., Klingenberg C.P., Di Giulio A. Morphological variation in *Paussus favieri* Fairmaire, 1851. In prep.
8. Bravi R., Scalici M., Klingenberg C.P., Di Giulio A. Morphological variation in *Paussus cridae/spinicoxis* Westwood 1850. In prep.
9. Bravi R., Scalici M., Klingenberg C.P., Di Giulio A. Directional asymmetry in two Paussinae species: *Paussus cridae* and *P. spinicoxis* (Coleoptera: Carabidae: Paussini). In prep.

## **Preface**

The thesis is structured in:

- ✓ Brief introduction on the issue of the Ph.D. project, discussing the importance of morphology and how it can be used to infer characters involved in intraspecific relationships, upon the light of coevolutionary interactions. In particular the study focuses on myrmecophilous beetles, which have revealed as an interesting model of study for they strictly morphological adaptations. *Paussus favieri* and *Paussus spinicoxis/cridae* were used as model species for this purpose. Moreover, morphological characters are used to deepen the taxonomic status of the group *Paussus spinicoxis/cridae*, which some authors consider as two separate species, some others as one.

Chapters are structured as manuscripts, with brief introductions, results and discussions, including tables and figures.

- ✓ Chapter 1 is focused on the investigation of morphological variability in two myrmecophilous beetles, and in particular on how the coevolutionary interactions with ants could infer on the morphology of the two species. This chapter is divided in to three sections:
  1. A brief introduction on the study of morphological variability and its importance in this parasite beetles, upon the light of coevolution;
  2. Is focused on the investigation of morphological variability on several parts of the entire body, in different populations of the myrmecophilous beetle *P. favieri*;
  3. Is centred on morphological analyses on the taxonomically questioned species of myrmecophilous beetles *Paussus cridae* and *P. spinicoxis*.
- ✓ Chapter 2 is centred on the analysis of evolutionary patterns that shaped the coevolutionary interactions between myrmecophiles beetles and their ant hosts. This chapter is subdivided in to three sections
  1. Briefly introduction of coevolutionary theories and their importance in morphological adaptations.

2. Analysis of evolutionary patterns in *P.favieri*.
  3. Analysis of evolutionary patterns in *P.spinicoxis* and *P. cridae*.
- ✓ Conclusions section briefly synthetize and link the main results of the thesis, also suggesting future directions for researchers

Additionally, abstract and materials and methods are separated from the main text and included in the supplementary material section. References include all the citations in the thesis. Finally, chapters correspond to manuscripts, submitted or in preparations.





## **INTRODUCTION**

### **Morphology**

The importance of morphology is stated from ancient time, in fact from Linnaeus to present days, differences in forms were used to infer differences between organisms, and finally to describe and classify species.

Over the past 20 years systematic biology has enlarged the view of morphology to include in the study many different kinds of patterns to phylogenetic studies. Among these, macroscopic structural characters are traditionally used to infer patterns of phylogenetic relationships through the time (e.g. ancestors and descents) (Lauder 1990). Systematists have largely used morphology as the foundation for biogeographic, ontogenetic, and morphometric analyses, but more recently they have included DNA sequences, electrophoretic banding patterns and ontogenetic sequences to disentangle genealogical relationships (Lauder 1990). Since this genetic approach to phylogeny increased in its diffusion and applications, the use of morphology to describe forms and infer their phylogeny has decreased.

More recently a rediscover of the importance of morphological characters happened. Morphological evidence in fact, helps to solve the hypotheses of phylogenetic relationships and characters evolution, and excluding it from the analyses could result in less exploratory and explanatory outcomes (Assis 2009). Furthermore molecular hypotheses could fail in providing explanations and reconstructions regarding the morphological evolution of traits, omitting the importance of coherently linking morphological evidence to phylogenetic relationships.

Under these considerations, it emerges the importance of morphological data in testing phylogenetic relationships among taxa. Consequently, morphology must have its own role in phylogenetic reconstructions, and this could not be omitted (Assis 2009; Hermesen and Hendricks 2008).

### **Geometric morphometrics**

The study of the shape is fundamental for many biological studies such as taxonomic classification and understanding the variety of many biological forms, both based on the descriptions of these biological forms (Zelditch et al. 2012). The so-called traditional morphology took place in the early twentieth century, when the biological studies passed from merely describe,

to scientifically quantify the different morphologies of the forms (Adams et al. 2004). This “quantitative approach” was possible because the development of multivariate statistical methodologies, and made possible the beginning of the morphometric studies (Adams et al. 2004). Enclosed in this context, morphometrics is the study of shape variation and its covariation with other variables. Traditionally it takes applications in analyzing quantitative variables as length, width, and height. Unfortunately with this traditional method one could obtain the same set of variables from two different shapes, and this because the two shapes could have for instance the same length or the same width even if the two shapes are completely different.

Nowadays modern morphometrics studies include and emphasize the geometry of morphology in the shape analyses, and this new technique is known as “geometric morphometrics” (GMM). Including the geometric information in the analyses avoid obtaining the same set of variables. This new method of analysis started what Rohlf and Marcus called the “morphometric revolution”(Rohlf and Marcus 1993).

One of the methods used for catching the information regarding the geometry of shapes is the landmarks based method. This begins with the collection of coordinates, which characterize the shape of the object. Then the superimposition (Procrustes fit) mathematically eliminates all the non-shape information (rotation, orientation and location). After that it is possible to compare the shape of the objects through the coordinate data and obtain the variation in shape between objects (described in terms of differences in deformation grids or shapes) (Adams et al. 2004; Klingenberg 2011).

Starting from 90’, advances in morphometric methods allow integrating the geometric morphometric with other methods, coming from different fields. The study of symmetry and asymmetry for instance, had fascinated researchers for years. Detecting random departures from perfect symmetry (so called fluctuating asymmetry-FA) had taken to identify the stability or instability of developmental processes (Møller and Swaddle 1997; Palmer and Strobeck 1986; Palmer 1996, 2004), and thus to assess the environmental processes that could influence these departures from symmetry, and their implications in evolutionary processes (Palmer 2004). A great innovation on this field took place when Klingenberg and McIntyre (1998) developed a technique for assessing the departure of symmetry with the use of geometric morphometrics, and used the shape variation to depict

levels of integrations, thus benefit from the powerful of analyses and visualization of this method.

More recently the interests of researchers in quantify and depict variation in symmetry in apparently symmetrical forms has increased (Klingenberg et al. 1998, 2002, 2010a,b; Savriama et al. 2012) and promise to begin another “revolutionary era” in studies of symmetries.

### **Myrmecophily and Parasitism on ants**

Ants inhabit well-structured and stable nests, composed by a variety of microhabitats (*e.g.* foraging trunks, nest chambers, guard nests, storage chambers, brood chambers divided into pupae, larvae and eggs chambers, queen chamber, etc.) that are suitable and attractive for a variety of organisms, including symbionts and social parasites (Hölldobler and Wilson 1990). Nests are well defended, and only members of the colony are allowed to enter the nest, the alien individuals being severely rejected. Specialized castes are variously able to block and hurt the enemies when defending both the nest and the colony members (Hölldobler and Wilson 1990). Where this strategies are not enough, ants are also equipped with poison glands, releasing toxic chemicals, the most known of which is the formic acid (Hölldobler and Wilson 1990). In a so strongly defended environment, organisms that want to access and use the abundant resources stored inside the nest, have to infiltrate into the defensive system.

Nevertheless many organisms (especially arthropods) have succeeded into penetrate the ant nests, that is, they have “broken the code”, interrupting the honest signal transmitted among the ants’ colonies, and becoming in various grade ants’ parasites.

In general, the parasite-host interaction involves an intimate association with host and an unfavorable impact on it (Foitzik 2009). Parasites have evolved several times, in several taxa from free-living ancestors and experimented a continuous implementation of their specialization during their evolution (Poulin 2006).

Parasites can also act as mediators in biological interactions between two or more populations of the same or different species. Price et al. (1986) inferred that the parasite mediation is one of the most important kind of interactions in ecological systems, comparable with the importance that competition, parasitism or mutualism could have. This kind of interaction

includes several types of mediation by parasites: i) in a species-species competition parasites could act as agent of interference; ii) parasites could be an instrument of defense for a species against its enemies; iii) consumers could benefit from parasites during the eating processes (Price et al. 1986).

A particular type of parasitic social interaction may refer to social parasites. In this type of parasitism, parasite takes advantage from the interactions between members of social species. Avian brood parasitism is an example: cuckoos and cowbirds lay their eggs in nests of different species, and in this manner they avoid the costs of parental cares (Foitzik et al. 2003). Another suitable example is the parasitism on social insects such as ants or termites. In ants in particular, the social parasitism is widely found with a variety of manifestations: xenobiosis, temporary parasitism, permanent parasitism, the latter with or without slavery (Wilson 1971).

Originally the definition of social parasite was referred to ants that parasite other ants (Hölldobler and Wilson 1990), and today is still used in this sense (Foitzik 2001, 2003, 2009); however, from its formulation since now, the term “social parasite” is increasingly used referring also to other organisms (especially arthropods) which live in ant nests, causing various degree of damages to the colony (Hölldobler and Wilson 1990; Barbero et al. 2009).

It's thought that about 10,000 insects species have evolved as social parasites, and “learned” how to speak the ants’ behavioral, chemical and acoustical languages. Consequently, ants do not attack them; instead in many cases they are actively fed and cared by the ants (Hölldobler and Wilson 1990; Barbero et al. 2009; Thomas et al. 2010). This kind of relationship involved “durable interactions” between parasite and its host, starting a process of co-evolutionary changes between them (Combes 2001).

Thompson (2001) referred to this process as coevolution: “Coevolution is the process of reciprocal evolutionary change between interacting species, driven by natural selection. It is one of the major processes organizing the Earth’s biodiversity into interrelated communities of species”.

Coevolution between parasites and their hosts has been widely studied in many biological systems (May and Anderson 1990; Toft and Karter 1990; Bolen et al. 2000; Gandon 2002). During time, particularly appropriate to explain this peculiar type of coevolution both in animals and plants has revealed the arms races theory (Dawkins and Krebs 1979; Davies et al. 1989; Holub 2001; Foitzik 2003; Maor and Shirasu 2005). In particular Foitzik et al. (2001) showed the evidence of the arms race model in social parasites (ants) and also related it to the local co-adaptation of the populations examined. According to this model, hosts are expected to evolve

resistance against parasites when the parasite pressure is stronger. As a part of the counter adaptation, parasites constantly have to break the host defenses in order to guarantee their survival. It is a never-ending battle between two opponents, which climaxes in adaptation, shaping behaviors, communications and characters of the opponents.

## **Paussinae**

The subfamily Paussinae (Coleoptera: Carabidae) is considered a monophyletic group within Carabidae, divided into five tribes: Metriini, Mystromomini, Ozaenini, Protopaussini and Paussini (Figure 1) (Di Giulio et al. 2003). They are small (2.8-22 mm) and generally brownish, characterized by nocturnal predatory habits. They have chemical defensive glands in adults based on quinones releasing, which give them the appellation of bombardier beetles. Moreover they have a unique type of larva, highly derived and extremely modified, with a U-shaped curved abdomen, and the three last abdominal segments and the urogomphi transformed into a terminal disk (Di Giulio and Vigna Taglianti, 2000; Di Giulio et al. 2003, 2011; Di Giulio and Moore, 2004; Moore and Di Giulio, 2006; Di Giulio 2007; Moore et al. 2011).

Among the five tribes, Metriini (3 species distributed in North America and 1 in China) and Mystromomini (2 Australian endemic species) are considered as basal tribes with free-living predatory habits, Ozaenini (180 species distributed predominantly in Neotropical Region) have mostly free-living lifestyle but some species are associated with ants, whereas Protopaussini (monogeneric tribe with 8 described species, distributed in the Oriental Region) and Paussini (572 species distributed in the Afrotropical Region) are more derived tribes and obligate myrmecophiles (Di Giulio et al. 2003; Geiselhardt et al. 2007; Moore 2008; Moore et al. 2011). Myrmecophily (obligate association with ants) seems to have evolved in the most derived groups of the Paussinae (Protopaussini and Paussini), and in *Physeia* and a few other genera of the Ozaenini (see Moore et al. 2011).

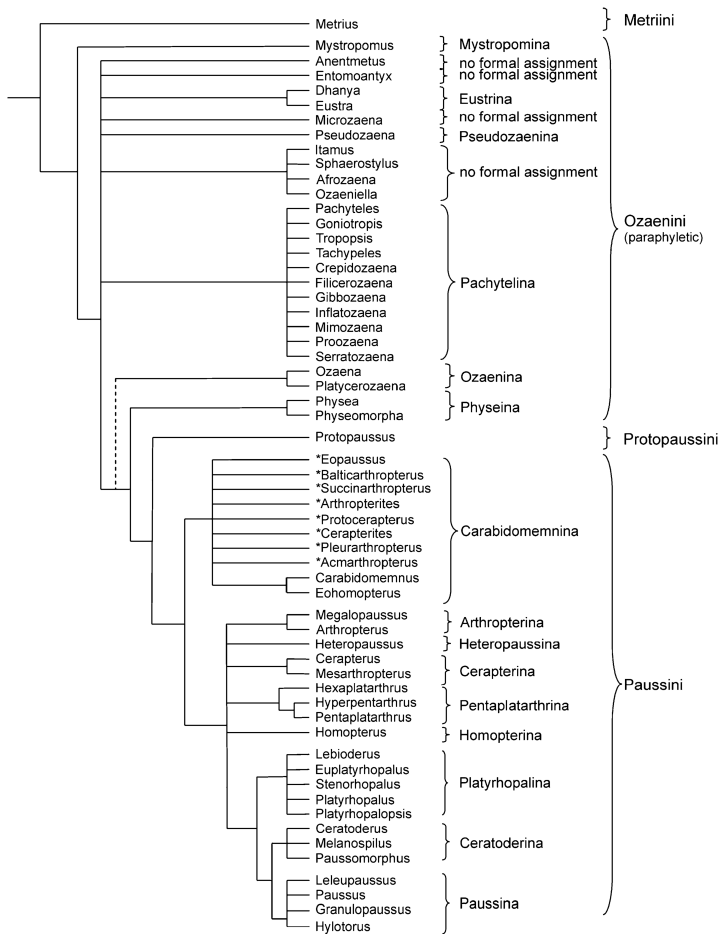


Figure 1. Phylogenetic relationships of Paussinae beetles, based on structural characters. Maked with asterisk are fossil genera (from Geiselhardt et al. 2007).

## **Paussini**

The tribe Paussini is composed by typically tropical and subtropical ant's parasites: they are rare, and spend most part of their life cycle in hidden ant nests, making them difficult to observe and understand their interaction with ants in the field. Within this tribe only two species show a European distribution: *Paussus favieri*, Fairmaire 1851 and *P. turcicus* Frivaldszky von Frivald, 1835. Paussini are obligatory associated with ants' subfamilies Myrmicinae and Formicinae and considered as social parasites, since they prey on ant broods (Geiselhardt et al. 2007). It seems that evolution of myrmecophilous lifestyle in Paussini have been complex, as suggested by the evolution and regression of trichome systems and other structures (Darlington 1950; Geiselhardt et al. 2007).

Due to their morphological and functional adaptations to myrmecophilous lifestyle, Paussini can be subdivided into the "protective type" and the "symphile type" on the base of their defensive strategies. The former is a type characterized by a compact hard body with smooth surface and retractable appendages; by this way the beetle is protected against the ants' aggressions. This body shape is also present in other myrmecophilous coleopterans (Rasa 1996; Geiselhardt et al. 2007). Slim body, with long thin appendages and the presence of many trichomes, characterizes the "symphile type" that is common to most derived taxa, and denotes beetles completely integrated in the ant nests (Geiselhardt et al. 2007).

The tribe shows further fascinating morphological myrmecophilous adaptations: antennae very modified in a variety of shapes (flattened, enlarged, lenticular, globular, concave, elongate, etc.) (Figure 2) and with antennal joints often reduced, compact and slim bodies, and elongated legs. Moreover on the antennae, abdomen, and prothorax some "myrmecophilous organs" are present, composed of trichomes (specialized tufts of hairs) and connected to exocrine glands releasing some chemicals (of still unknown composition) that result attractive and appeasing for ants (Darlington 1950; Geiselhardt et al. 2007; Di Giulio et al. 2011).



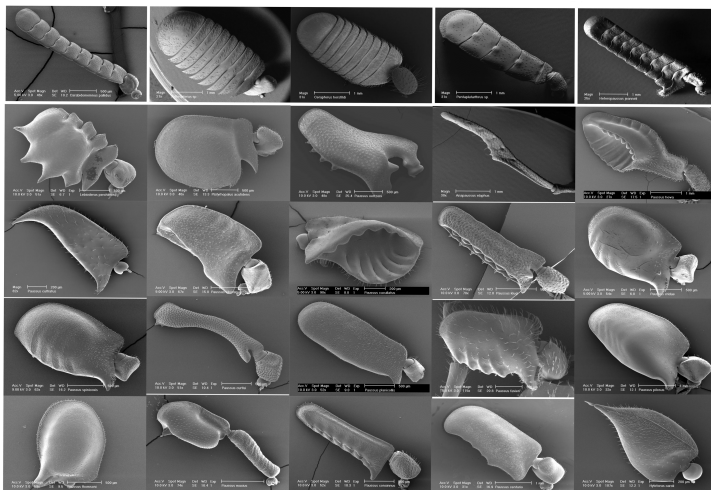


Figure 2. Morphological variability in Paussini antennal clubs (SEM micrographs by A. Di Giulio).

### ***Paussus favieri*.**

*Paussus favieri* Fairmaire, 1851 lives in nests of the polygynic ant *Pheidole pallidula* (Nylander, 1849) (Figure 3), and is one of the two Paussini species present also in Europe. It is an Atlanto-Mediterranean species and its distribution range comprises Algeria, France, Morocco, Spain, Portugal and Tunisia (Casale et al. 1982; Di Giulio et al. 2011). Some specimens from Sardinia and Sicily (Casale et al. 1982) and one from Corsica (Zerche, 1990) are present in museum collections, however no specimen was found there during the last 100 years (Di Giulio et al. 2011).

Due to its rarity and bizarre structural and morphological adaptations to myrmecophilous life style, this species has attracted the interest of collectors and researchers since the past 150 years.

Escherich (1899) made the first ethological observations of *P. favieri* in captivity describing some interactions between the beetles and their hosts (e.g. grooming, dragging, and grabbing), and on the acceptance of the beetle in the nest. Le Masne (1961a,b,c) later, focused his observations on the predatory strategy (the beetle feeding on the ants brood), and on the mechanisms of acceptance of the beetle. According to both Echerich (1899) and Le Masne (1961 a,b,c) *P. favieri* is promptly accepted and fully

integrated in the nest, without ostility. These observations were recently confirmed and implemented by the sexual and cleaning behavior by Maurizi et al. (2012).

The morphology of *P. favieri* can be described as follows: antennal clubs with subtriangular shape, composed by three joints: subrectangular scape, small rounded pedicel and flagellum represented by a unique “antennal club”; analysis of the antennae at scanning electron microscope (SEM) revealed the presence of different types of modified sensilla and glandular pores of different sizes that represent the openings of cuticular ducts; head is shiny and covered with a long spiny trichome in the middle; prothorax is constricted in the middle and covered with bristles; elytrae are trimmed along the edges and covered with branched bristles (Westwood 1850; Di Giulio et al. 2009, 2012).

Apart from the number of past and recent studies, many gaps on the life history, biology and morphological variations of *P. favieri* needed to be filled.



Figure 3. Two specimens of *Paussus favieri* in a nest of *Pheidole pallidula* with eggs, larvae and workers of the host ant (photo by R. Bravi).

## ***Paussus cridae***

*Paussus cridae* Gestro 1915 is reported from the west part of Central Africa (Angola, Cameroun, Congo Republic, Democratic Republic of Congo - former Zaire). Moreover it is a host of ants of unidentified *Pheidole* species (Luna de Carvalho, 1989).

From a preliminary analysis, it seems to be very similar in morphology (A. Di Giulio *in verbis*) to its closest relative *Paussus spinicoxis* Westwood 1850. According to Luna de Carvalho (1989), the only character that separates the two species is the antennal club: slightly shorter and less convex in *P. spinicoxis*. However we observed that there is a morphological cline when long series of well geographically distributed specimens are considered.

*P. spinicoxis* is reported to occupy the eastern part of Central Africa (Botswana, Democratic Republic of Congo, Kenya, Mozambique, Ruanda, South Africa, Zambia, Zimbabwe); finally, it is a host of the ant species *Pheidole megacephala atrox* (Luna de Carvalho, 1989).

The two species potentially occupy ant nests of the same host, they have a very close distribution range, and finally they are reported as sympatric in Democratic Republic of Congo (Luna de Carvalho, 1989).

Nagel (1983) assigned both species to the “*Paussus spinicoxis*” group, and Luna de Carvalho (1989) considered the two species belonging to the same “super-species”. For this reason when investigating the taxonomical validity of the *P. cridae* species it is not possible to exclude the species *P. spinicoxis* from the analyses.

Very few studies have been conducted on these species, and due to their morphological similarities and their vicinity in the distribution area, the debate on their species status could be considered still open.

Moreover *P. cridae* and *P. spinicoxis* are widely represented in the entomological collections of the European museums, ongoing to be misidentified, and their natural status requires additional studies.

## **Aim and objectives**

Aim of the present Ph.D. thesis is to contribute to knowledge about the still poorly known tribe Paussini, focusing on morphology of the model species: *Passus favieri*, *P. cridae*, and *P. spinicoxis*.

In particular the main objectives of the present study are:

1. To analyze and describe the morphological variability of the beetle *Paussus favieri*, in order to depict and describe its geographical variability in its distribution range. We perform this approach analyzing several parts of the whole body of the two species. Several W-Mediterranean populations are studied, representative of its main distribution range;
2. To investigate through a morphological approach the differences between the described species group *Paussus cridae* and *P. spinicoxis*, in order to contribute to the debate about their taxonomic status. We perform this approach analyzing several parts of the whole body of the two species. Several populations, representative of their main African distribution range are studied;
3. To describe the morphological variability of the species *Paussus cridae* and *P. spinicoxis*, in their distribution range, in order to depict and assess their geographical variation.
4. To investigate how the selective pressure operate on the beetles by the ants, could influence the morphological variability, especially on the structures most involved in intraspecific interactions (principally antennal clubs and head), upon the light of coevolutionary myrmecophilous interactions.



## **CHAPTER 1**

### **1) Introduction**

Coevolution is reported as the evolution among two or more interacting species, and is a dynamic process that reshapes the interactions among species over the time and over the geographic landscapes (Thompson 2009). This kind of evolutionary force is particularly strong in the parasite-host interactions. In this way parasites lead to strong evolutionary interactions with their hosts, often reducing the fitness of the hosts. This strong selective pressure causes a counter-adaptation in the host, leading to an evolutionary arms race between parasite and its host (Dawkins and Krebs 1979). This is particularly true in ant nest parasites (Foitzik et al. 2001, 2003, 2009).

Ant nests represent a great supply in terms of resources (food, ants, brood, waste materials etc.), and they can be viewed as stable environments. For these reasons, ant nests are very attractive for a large number of organisms, many of which establishing various types of relationships: from commensalism to selective predation, mutualism, and parasitism (Maurizi et al. 2012). Ant parasites represent about 10% of known myrmecophilous insects (considered to be ~ 10,000 species) and most of them belong to the families Coleoptera, Lepidoptera, Orthoptera, and Diptera (Thomas and Settle 2004).

Members of the tribe Paussini (Coleoptera, Carabidae, Paussini) are ground myrmecophilous beetles considered to be ant nest parasites, generally associated with the ants' subfamilies Myrmicinae (that comprise the genus *Pheidole*) and Formicinae (Geiselhardt et al. 2007). Due to their unique morphological adaptations to myrmecophily (e.g. myrmecophilous organs, very modified antennal clubs and bodies) the study of Paussini is very challenging, mostly for the higher morphological variability inside the tribe.

Within Paussini the subtribe Paussina comprises on the whole 12 genera and 29 subgenera, and all are obligate myrmecophiles (Di Giulio et al. 2003; Lorenz 2005; Geiselhardt et al. 2007; Moore 2008; Moore et al. 2011).

Paussini have been extensively studied from a taxonomic point of view, and recently many are the researches concerning their life cycle and their interactions with their hosts (Darlington 1950; Nagel 1987, 2003; Luna de Cravalho 1986; Di Giulio et al. 2003, 2009, 2001; Maurizi et al. 2011, 2012), but the majority of the studies concern the species *Paussus favieri*, a relatively abundant species with an Atlanto-Mediterranean distribution.

## 2) Morphological variation in *Paussus favieri*.<sup>1</sup>

### I. Introduction

Despite the rarity of Paussinae species, *Paussus favieri* Fairmaire, 1851 is quite common in northern Africa, and one of the two species present also in Europe (the other one is *Paussus turcicus* Frivaldszky von Frivald, 1835) (Figure 1.1). It is an Atlanto-Mediterranean species and its distribution range comprises Algeria, France, Morocco, Portugal, Spain and Tunisia (Casale Portugal 1982; Di Giulio et al. 2011). Among this species some authors found an intraspecific variability (A. Di Giulio personal observation), probably due to the partial geographic isolation the ant nest offer.

Although many studies investigate on *P. favieri*, they are for the majority about the behaviour and the interactions with its host *Pheidole pallidula*, or regard the fine structural morphology of specific structures as antennal clubs or head (Di Giulio et al. 2009, 2012; Maurizi et al. 2012); no study regarded instead the analysis and description of the morphological variability of *P. favieri*.

The aim of the present study is to analyze and describe the morphological variability within and among populations (Algeria, France, Morocco, Spain) of *Paussus favieri*, located among the distribution range of the species, investigated with a geometric morphometric approach.

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<sup>1</sup> This work corresponds to the paper: Bravi R., Scalici M., Klingenberg C.P., Di Giulio A. Morphological variation in *Paussus favieri*. In prep.

<sup>2</sup> This work corresponds to the paper: Bravi R., Scalici M., Klingenberg C.P., Di Giulio A.



Figure 1.1. *Paussus favieri* (photo by A. Di Giulio).

## II. Results

### Antennal clubs

Canonical Variate Analysis (CVA) showed that significative differences exist between right and left antennal clubs (Mahalanobis distance = 4.63,  $P < 0.0001$ ; Procrustes distance = 0.032,  $P < 0.0001$ ) and they are not influenced by sexual dimorphism (Mahalanobis distance = 0.61,  $P = 0.64$ ; Procrustes distance = 0.007,  $P = 0.58$ ). ANOVA test confirmed the existence of left-right differences for both size and shape in the antennal clubs (for size Sums of Squares = 1.83, Means of Squares = 1.83,  $P < 0.0001$ ; for shape Sums of Squares = 0.041, Means of Squares = 0.0023,  $P < 0.0001$ ). Concerning the shape variation, Principal Component Analysis (PCA) analysis showed that the majority of the variation is due to the firsts four PC components (76.93% for the right antennal club, 78.27% for the left antennal club), with each principal component (PC) that account for no more than 37% of the total shape variation (for right antennal club PC1 + PC2 + PC3 +



PC4 = 36.93% + 19.35% + 10.80% + 9.85%; for left antennal club PC1 + PC2 + PC3 + PC4 = 37.02% + 17.55% + 13.47% + 10.24%) (Figure 1.2). Finally CVA analysis showed that significant differences between the four populations analyzed emerged, and were highest for the Moroccan population (Table 1.1).

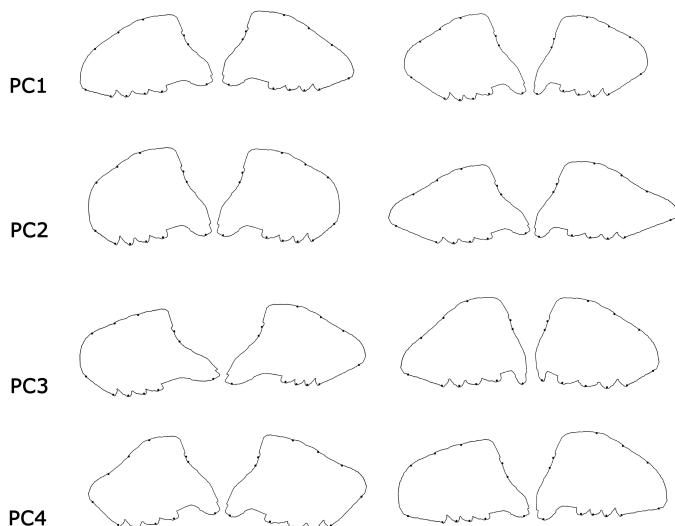


Figure 1.2. Decomposition of shape variation in left and right antennal clubs. The firsts four principal components (PCs) of shape variation are shown. For each PC the diagrams show the shapes that correspond to scores -1.0 (on the left, for both left and right antennal clubs) or +1.0 (on the right, for both left and right antennal clubs).

## Head

CVA analysis didn't show the existence of sexual dimorphism (Mahalanobis distance = 0.51,  $P = 0.70$ ; Procrustes distance = 0.20,  $P = 0.32$ ). Shape and size variation were assessed using ANOVA and MANOVA tests for both symmetric and asymmetry component of shape variation. ANOVA test showed a high morphological variation in shape in the whole sample (Sums of Squares = 0.22, Means of Squares = 0.00087,  $P = 0.001$ ); in particular variation between populations for both shape and size were evident (for size Sums of Squares = 0.010, Means of Squares = 0.0033,  $P = 0.018$ ; for shape Sums of Squares = 0.026, Means of Squares = 0.0017,  $P = 0.018$ ). MANOVA test confirmed the differences between populations for symmetric component of the shape variation, but not for asymmetry (for symmetric component Pillai trace = 0.57,  $P = 0.0072$ ; for asymmetry component Pillai trace = 0.25,  $P = 0.61$ ). Then we used PCA analysis to quantify the shape variation. The firsts three PCs were sufficient to explain the majority of the variation for both symmetric and asymmetry components (for symmetry component  $PC1 + PC2 + PC3 = 89.78\%$ ; for asymmetric component  $PC1 + PC2 + PC3 = 95.28\%$ ) (Figure 1.3). Finally CVA analysis were used to assess and quantify the differences emerged between population, enlighten that majority of the differences were found in the Moroccan population, but differences also emerged from the Algerian population (Table 1.1).

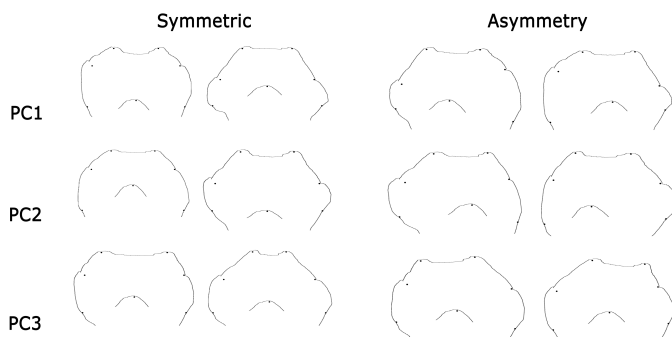


Figure 1.3. Decomposition of shape variation in head. The firsts three PCs for each category of symmetry or asymmetry are shown. For each PC the diagrams show the shapes that correspond to scores -1.0 (on the left) or +1.0 (on the right) for both symmetric and asymmetry components of shape variation.

### Pronotum

CVA analysis showed that morphological variation of pronotum was not influenced by sexual dimorphism (Mahalanobis distance = 0.46,  $P = 0.58$ ; Procrustes distance = 0.025,  $P = 0.21$ ). ANOVA test showed the presence of morphological variation in the whole sample (Sums of Squares = 0.51, Means of Squares = 0.0013,  $P < 0.0001$ ), and differences high for shape variation (Sums of Squares = 0.11, Means of Squares = 0.008,  $P < 0.0001$ ) and less evident for size (Sums of Squares = 0.011, Means of Squares = 0.0036,  $P = 0.06$ ). MANOVA test confirmed the presence of morphological variation in both symmetric and asymmetry components of shape variation (for symmetric component Pillai trace = 0.90,  $P < 0.0001$ ; for asymmetry component Pillai trace = 0.59,  $P < 0.0001$ ). PCA analysis then, was used for describe the shape variation. The firsts three PCs accounted for the majority of variation for both symmetric and asymmetry components (for symmetric component  $PC1 + PC2 + PC3 = 88.58\%$ ; for asymmetry component  $PC1 + PC2 + PC3 = 93.89\%$ ) (Figure 1.4). Finally CVA was used to assess morphological differences between populations, and the Moroccan one emerged as the population more different from the others (Table 1.1).

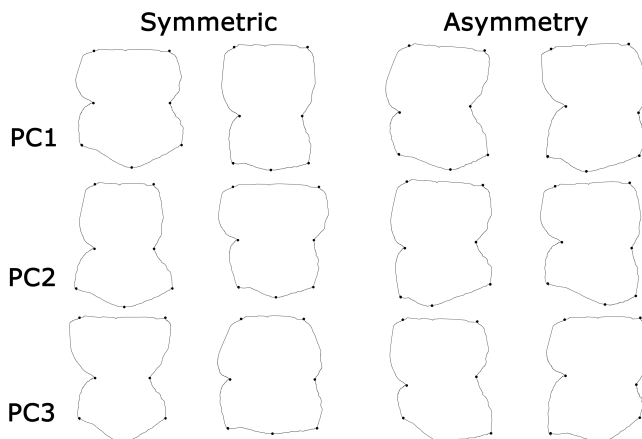


Figure 1.4. Decomposition of shape variation in pronotum. The firsts three PCs for each category of symmetry or asymmetry are shown. For each PC the diagrams show the shapes that correspond to scores -1.0 (on the left) or +1.0 (on the right) for both symmetric and asymmetry components of shape variation.

### Elytrae

CVA test for sexual dimorphism revealed there were no differences between sexes (Mahalanobis distance = 0.76,  $P = 0.80$ ; Procrustes distance = 0.0081,  $P = 0.61$ ). We examined then the morphological variation in the entire sample, which resulted high and significative (Sums of Squares = 0.52, Means of Squares = 0.0011,  $P < 0.0001$ ). Differences emerged between populations for shape (Sums of Squares = 0.0051, Means of Squares = 0.00017,  $P = 0.035$ ) but not for size (Sums of Squares = 0.65, Means of Squares = 0.022,  $P = 0.56$ ), and were not confirmed by MANOVA test (for symmetric component Pillai trace = 0.79,  $P = 0.087$ ; for asymmetry component Pillai trace = 0.53,  $P = 0.67$ ). Shape variation for symmetric and asymmetry components was described by PCA analysis. In both cases the firsts four PCs were sufficient to explain the majority of shape variation (for symmetry component PC1 + PC2 + PC3 + PC4 = 94.19%; for asymmetric component PC1 + PC2 + PC3 + PC4 = 89.30%) (Figure 1.5). Finally differences between populations were assessed with CVA, and Moroccan population emerged also in this case as the most morphologically different from other populations (Table 1.1.1 and Figure 1.1.5).

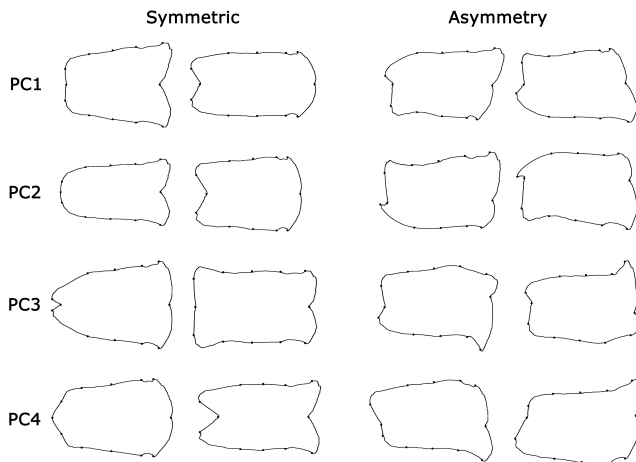


Figure 1.5. Decomposition of shape variation in elytrae. The firsts three PCs for each category of symmetry or asymmetry are shown. For each PC the diagrams show the shapes that correspond to scores -1.0 (on the left) or +1.0 (on the right) for both symmetric and asymmetry components of shape variation.

Table 1.1. Pairwise comparisons by the canonical variate analysis performed between the studied four populations for all the investigated body parts. Results are reported as Mahalanobis distance (and *p*-values), after 10000 permutation runs. AG = Algeria, FR = France, MK = Morocco, SP = Spain.

|          |    | AG             | FR             | MK             |
|----------|----|----------------|----------------|----------------|
| Antennae | FR | 1.63 (0.28)    |                |                |
|          | MK | 1.83 (<0.0001) | 1.70 (0.0015)  |                |
|          | SP | 1.53 (0.019)   | 1.68 (0.015)   | 1.67 (<0.0001) |
| Head     | FR | 4.86 (0.0008)  |                |                |
|          | MK | 4.53 (<0.0001) | 1.49 (0.025)   |                |
|          | SP | 5.43 (<0.0001) | 1.18 (0.33)    | 1.86 (<0.0001) |
| Pronotum | FR | 2.65 (0.02)    |                |                |
|          | MK | 2.84 (<0.0001) | 1.84 (<0.0001) |                |
|          | SP | 2.84 (<0.0001) | 0.92 (0.47)    | 1.89 (<0.0001) |
| Elytrae  | FR | 1.45 (0.68)    |                |                |
|          | MK | 1.59 (0.039)   | 2.64 (0.0002)  |                |
|          | SP | 1.46 (0.25)    | 1.86 (0.3)     | 1.95 (0.0001)  |

### III. Discussion

The present study aimed to describe the morphological variability of *P. favieri*. In particular we took for the analyses four different populations, quite representative of the whole distribution range of this species.

All results revealed the presence of a high morphological variability among populations, especially due to the antennal clubs, even if all other body parts presented a certain morphological variability as well.

Regarding the antennal clubs morphological differences emerged in both size and shape. In particular the antennal clubs presented a variation mostly associated with the length of the antennal club that passed from elongated shape to a more rounded shape. This is well evident in the Moroccan population, emerged as the most different from our analyses: the apical part of both left and right antennal clubs is more elongate, even best distinguishable in the left antennal club than in the right one (Figure 1.6).

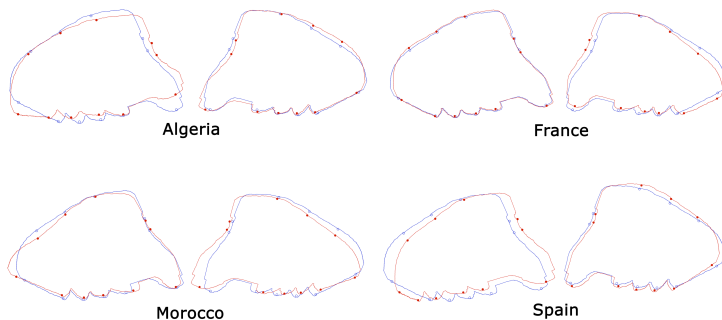


Figure 1.6. Average shape variation in left and right antennal clubs in different population analyzed. Shape deformation is shown in red, whereas starting shapes are shown in blue.

Regarding the head, morphological variations resulted associated to the structure on the whole, and the higher variation are related with the eyes and the upper part of the head, the latter having the higher morphological variability. The Moroccan population emerged from the analyses as the one with majority of variation, even if the shape modifications were not so evident as in the last cases. Moreover some differences emerged for the head also in the Algerian population, even less subtle than the Moroccan ones (Figure 1.7).

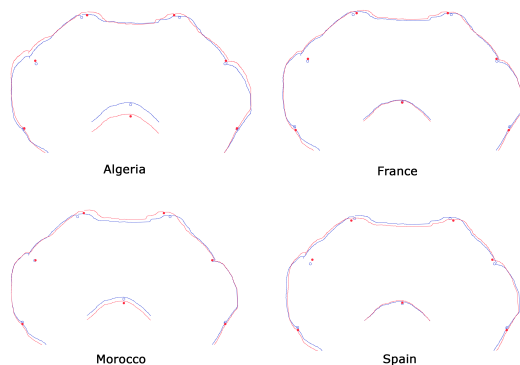


Figure 1.7. Average shape variation in head, in different population analyzed. Shape deformation is shown in red, whereas starting shape is shown in blue.

Pronotum resulted in quite high morphological variability, the variations regard the structure on the whole, and goes from a larger to quite tiny shape and the modifications involved the upper or the lower part, but not both in the same time. Regarding differences between populations, even in this case the Moroccan population resulted the one with the highest variability and the pronotum assumes a short and large form (Figure 1.8).

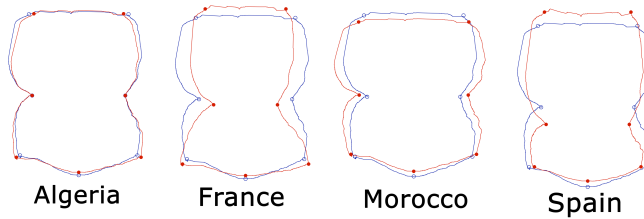


Figure 1.8. Average shape variation in pronotum in different population analyzed. Shape deformation is shown in red, whereas starting shapes are shown in blue.

Finally the elytrae resulted as the structures less under variation, with a certain morphological variability mostly associated to the upper and lower extremities of the abdomen. Regarding the Moroccan population emerged as the mostly different from the others even in this case, with the majority of shape variation concentrated at the extremities of the structure (Figure 1.9).

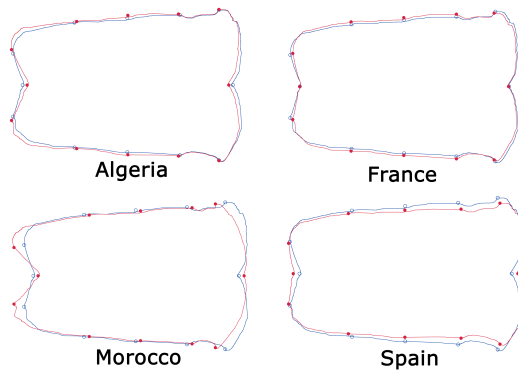


Figure 1.9. Average shape variation in elytrae in different population analyzed. Shape deformation is shown in red, whereas starting shapes are shown in blue.

On the whole from our analyses a certain morphological variability emerged. It was bigger in the antennal clubs, the organs mostly involved in the ant-beetle interactions, and less pronounced in the other body parts. Regarding the intra-populations variability, the latter appeared as present and mostly due to the Moroccan population. The specimens from the Moroccan population were from the Atlas Mountains; the ant nests were at ~2000 m (a.s.l.), whereas the other populations' specimens were caught from 600 m (a.s.l.) up to 1000 m (a.s.l.). This geographically differences could be revealed in the morphological differences emerged from our analyses. The high morphological variability assumed among the population we examined was confirmed with the approach of geometric morphometric, and although solving some questions, our analysis raise up other questions about the evolutionary interpretation of this so high variability. Further studies are necessary to solve the question.



### 3) Morphological variation in *Paussus cridae*.<sup>2</sup>

#### I. Introduction

*Paussus cridae* (Figure 1.10) is an African species, very close in morphology to its relative *P. spinicoxis*, with an apparently morphological cline that makes the species not easy to distinguish one another (A. Di Giulio personal observation). The only taxonomical character for distinguish the two species is the morphology of the antennal club (Luna de Carvalho 1989).

The African species have attracted many researchers (Nagel 1983, 1987; Luna de Carvalho 1989; Geiselhardt et al. 2007), but due to their rarity, and to particular political condition of many countries in the distribution range, the African species are still lacking of studies.



Figure 1.10. *Paussus spinicoxis* (photo by R. Bravi).

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<sup>2</sup> This work corresponds to the paper: Bravi R., Scalici M., Klingenberg C.P., Di Giulio A. Morphological variation in *Paussus cridae*. In prep.

Very few studies have been conducted on *P. crida* and *P. spinicoxis*, and due to their morphological similarities, their vicinity in the distribution area, the debate on their species status is to be considered still open.

The aim of present study is to contribute to the debate on the taxonomical status of the two species from a morphological point of view. In particular we were interested in study the whole body, subdivided in five different anatomical parts (left and right antennal clubs, head, pronotum and elytrae), and analyzed them with a geometric morphometric approach.

## Results

### Antennal clubs

Canonical Variate Analysis (CVA) analysis showed the existence of subtle differences between species (Mahalanobis distance = 1.88,  $P < 0.0001$ ; Procrustes distance = 0.029,  $P < 0.0001$ ), confirmed by the ANOVA (for size Sums of Squares = 0.67, Means of Squares = 0.67,  $P < 0.0001$ ; for shape Sums of Squares = 0.013, Means of Squares = 0.00075,  $P < 0.0001$ ) but not by MANOVA (Pillai trace = 0.64,  $P = 0.12$ ). Given these results the two species were analyzed separately.

#### *Paussus cridaae*

CVA showed that certain differences exist between right and left antennal clubs (Mahalanobis distance = 28.83  $P < 0.0001$ ; Procrustes distance = 0.021,  $P = 0.25$ ) and they are shortly influenced by sexual dimorphism (Mahalanobis distance = 4.89,  $P < 0.0001$ ; Procrustes distance = 0.020,  $P = 0.38$ ). ANOVA test confirmed the existence of left-right differences for size but not for shape (for size Sums of Squares = 1.46, Means of Squares = 1.46,  $P < 0.0001$ ; for shape Sums of Squares = 0.0022, Means of Squares = 0.00012,  $P = 0.12$ ). These differences were not influenced by sexual dimorphism (Sums of Squares = 0.014, Means of Squares = 0.014,  $P = 0.14$ ) or by populations' differences (Sums of Squares = 0.007, Means of Squares = 0.007,  $P = 0.28$ ).

Concerning the shape variation, Principal Component Analysis (PCA) showed that the majority of the variation is due to the firsts three PC components (75.65% for the right antennal club, 80.78% for the left antennal club), with each other principal component (PC) that account for no more than 14% of the total shape variation (for right antennal club  $PC1 + PC2 + PC3 = 43.06\% + 20.07\% + 12.52\%$ ; for left antennal club  $PC1 + PC2 + PC3 = 47.80\% + 18.09\% + 14.89\%$ ) (Figure 1.11).

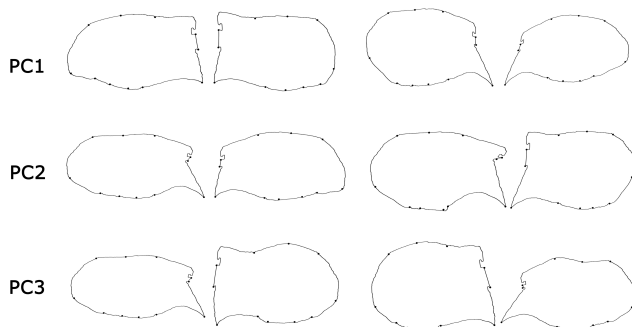


Figure 1.11. Decomposition of shape variation in left and right antennal clubs of *P. cridae*. The firsts three principal components (PCs) of shape variation are shown. For each PC the diagrams show the shapes that correspond to scores -1.0 (on the left, for both left and right antennal clubs) or +1.0 (on the right, for both left and right antennal clubs).

### *Paussus spinicoxis*

CVA showed that certain differences exist between right and left antennal clubs (Mahalanobis distance = 2.61  $P < 0.0001$ ; Procrustes distance = 0.012,  $P = 0.23$ ) and they are influenced by sexual dimorphism (Mahalanobis distance = 2.89,  $P < 0.0001$ ; Procrustes distance = 0.035,  $P < 0.0001$ ). ANOVA test confirmed the existence of left-right differences for both size and shape (for size Sums of Squares = 2.90, Means of Squares = 2.90,  $P < 0.0001$ ; for shape Sums of Squares = 0.0025, Means of Squares = 0.00014,  $P = 0.12$ ). These differences were influenced by sexual dimorphism (for size Sums of Squares = 1.31, Means of Squares = 0.65,  $P < 0.0001$ ; for shape Sums of Squares = 0.025, Means of Squares = 0.0007,  $P < 0.0001$ ) and partially by populations' differences (for size Sums of Squares = 0.69, Means of Squares = 0.12,  $P = 0.0024$ ; for shape Sums of Squares = 0.019, Means of Squares = 0.00017,  $P = 0.0018$ ).

Concerning the shape variation, Principal Component Analysis (PCA) analysis showed that the majority of the variation is due to the firsts three PC components (75.65% for the right antennal club, 73.32% for left antennal club), with each other principal component (PC) that account for no more than 14% of the total shape variation (for right antennal club PC1 + PC2 + PC3 = 43.06% + 20.07% + 12.52%; for left antennal club PC1 + PC2 + PC3 = 40.77% + 20.74% + 11.79% + (Figure 1.12).

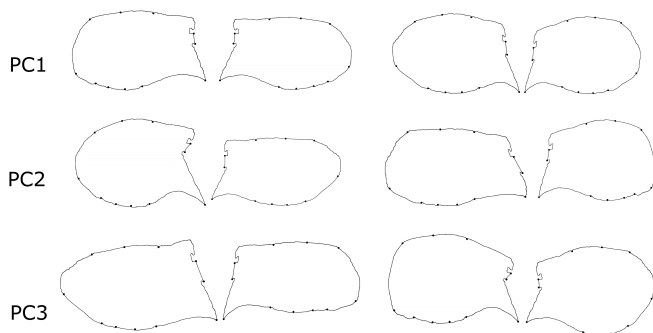


Figure 1.12. Decomposition of shape variation in left and right antennal clubs of *P. spinicoxis*. The firsts three principal components (PCs) of shape variation are shown. For each PC the diagrams show the shapes that correspond to scores -1.0 (on the left, for both left and right antennal clubs) or +1.0 (on the right, for both left and right antennal clubs).

### Head

CVA analysis showed the existence of subtle differences between species (Mahalanobis distance = 1.46,  $P = 0.052$ ; Procrustes distance = 0.0038,  $P = 0.023$ ), partially confirmed by the ANOVA (for size Sums of Squares = 0.018, Means of Squares = 0.018,  $P = 0.01$ ; for shape Sums of Squares = 0.011, Means of Squares = 0.022,  $P = 0.013$ ) and MANOVA (for symmetric component Pillai trace = 0.49,  $P = 0.0042$ ; for asymmetry component Pillai trace = 0.16,  $P = 0.49$ ). Given these results the two species were analyzed separately.

### *Paussus cridaae*

CVA showed a subtle indication about the presence of sexual dimorphism (Mahalanobis distance = 3.85,  $P = 0.061$ ; Procrustes distance = 0.029,  $P = 0.56$ ). Shape and size variation were assessed using ANOVA and MANOVA tests for both symmetric and asymmetry component of shape variation. ANOVA test showed a certain morphological variation in shape in the whole sample (Sums of Squares = 0.024, Means of Squares = 0.00069,  $P < 0.0001$ ), due nor to variation between populations (for shape Sums of Squares = 0.044, Means of Squares = 0.00088,  $P = 0.29$ ; for size Sums of Squares = 0.00, Means of Squares = 0.00,  $P = 0.98$ ), neither to a strong signal of sexual dimorphism (for size Sums of Squares = 0.0031, Means of Squares = 0.0031,  $P = 0.18$ ; for shape Sums of Squares = 0.031, Means of

Squares = 0.00062,  $P = 0.49$ ). MANOVA test confirmed the absence of differences between populations (for symmetric component Pillai trace = 0.81,  $P = 0.24$ ; for asymmetry component Pillai trace = 0.67,  $P = 0.46$ ) and sexes (for symmetric component Pillai trace = 0.90,  $P = 0.10$ ; for asymmetry component Pillai trace = 0.57,  $P = 0.61$ ).

Then we used PCA analysis to quantify the shape variation. The firsts two PCs were sufficient to explain the majority of the variation for both symmetric and asymmetry components (for symmetry component PC1 + PC2 = 96.53% = 70.93% + 25.60%; for asymmetric component PC1 + PC2 = 83.24% = 55.20% + 28.05%) (Figure 1.13).

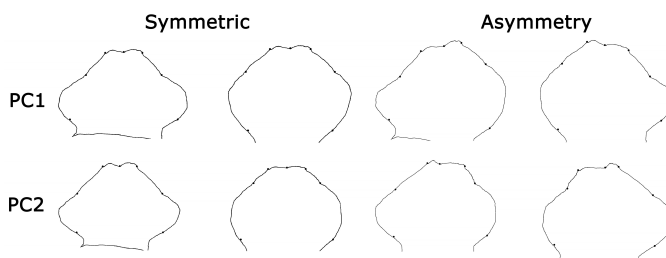


Figure 1.13. Decomposition of shape variation in head in *P. cridae*. The firsts three PCs for each category of symmetry or asymmetry are shown. For each PC the diagrams show the shapes that correspond to scores -1.0 (on the left) or +1.0 (on the right) for both symmetric and asymmetry components of shape variation.

### *Paussus spinicoxis*

CVA showed a subtle indication about the presence of sexual dimorphism (Mahalanobis distance = 1.65,  $P = 0.039$ ; Procrustes distance = 0.029,  $P = 0.16$ ). Shape and size variation were assessed using ANOVA and MANOVA tests for both symmetric and asymmetry component of shape variation. ANOVA test showed a certain morphological variation in shape in the whole sample (Sums of Squares = 0.043, Means of Squares = 0.00042,  $P < 0.0001$ ), due to certain variation between populations (for shape Sums of Squares = 0.046, Means of Squares = 0.0013,  $P < 0.0001$ ; for size Sums of Squares = 0.035, Means of Squares = 0.0049,  $P = 0.11$ ), but not to sexual dimorphism (for size Sums of Squares = 0.0031, Means of Squares = 0.00069,  $P = 0.77$ ; for shape Sums of Squares = 0.0049, Means of Squares = 0.00049,  $P = 0.32$ ). MANOVA test confirmed the absence of differences

between populations (for symmetric component Pillai trace = 1.65,  $P = 0.067$ ; for asymmetry component Pillai trace = 1.28,  $P = 0.43$ ) and sexes (for symmetric component Pillai trace = 0.51,  $P = 0.30$ ; for asymmetry component Pillai trace = 0.31,  $P = 0.76$ ).

Then we used PCA analysis to quantify the shape variation. The firsts two PCs were sufficient to explain the majority of the variation for both symmetric and asymmetry components (for symmetry component  $PC1 + PC2 = 75.73\% = 41.86\% + 33.86\%$ ; for asymmetric component  $PC1 + PC2 = 76.59\% = 53.08\% + 23.52\%$ ) (Figure 1.14).

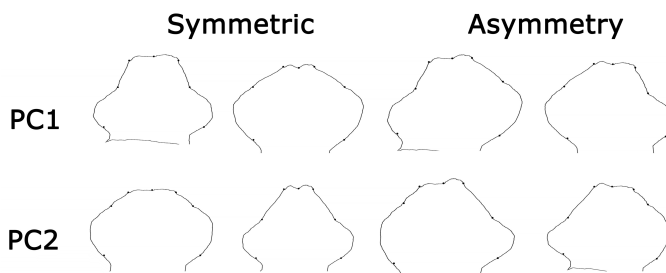


Figure 1.14. Decomposition of shape variation in head in *P. spinicoxis*. The firsts three PCs for each category of symmetry or asymmetry are shown. For each PC the diagrams show the shapes that correspond to scores -1.0 (on the left) or +1.0 (on the right) for both symmetric and asymmetry components of shape variation.

### *Pronotum*

CVA analysis showed the existence of certain differences between species (Mahalanobis distance = 1.89,  $P < 0.0001$ ; Procrustes distance = 0.019,  $P = 0.0016$ ), partially confirmed by the ANOVA (for size Sums of Squares = 0.058, Means of Squares = 0.058,  $P = 0.06$ ; for shape Sums of Squares = 0.062, Means of Squares = 0.00089,  $P = 0.0009$ ) and MANOVA (for symmetric component Pillai trace = 0.54,  $P = 0.0002$ ; for asymmetry component Pillai trace = 0.16,  $P = 0.49$ ). Given these results the two species were analyzed separately.

### *Paussus cridae*

Sample size comprised only one population, so we didn't perform the analyses on populations differences.

CVA showed a subtle indication about the presence of sexual dimorphism (Mahalanobis distance = 6.18,  $P < 0.0001$ ; Procrustes distance = 0.020,  $P = 0.041$ ). Shape and size variation were assessed using ANOVA and MANOVA tests for both symmetric and asymmetry component of shape variation. ANOVA test showed a certain morphological variation for both shape and size in the whole sample (for shape Sums of Squares = 0.0093, Means of Squares = 0.00015,  $P < 0.0001$ ; for size Sums of Squares = 0.049, Means of Squares = 0.0055,  $P = 0.0054$ ), and a very subtle signal of sexual dimorphism (for size Sums of Squares = 0.030, Means of Squares = 0.030,  $P = 0.046$ ; for shape Sums of Squares = 0.0019, Means of Squares = 0.00027,  $P = 0.10$ ). MANOVA test confirmed the presence of subtle sexual dimorphism (for symmetric component Pillai trace = 0.96,  $P = 0.035$ ; for asymmetry component Pillai trace = 0.60,  $P = 0.71$ ).

Due to the fact we found a certain morphological variability we performed PCA analysis to quantify the shape variation. The firsts two PCs were sufficient to explain the majority of the variation for both symmetric and asymmetry components (for symmetry component  $PC1 + PC2 = 80.04\% = 50.80\% + 29.24\%$ ; for asymmetric component  $PC1 + PC2 = 73.51\% = 37.70\% + 35.81\%$ ) (Figure 1.15).

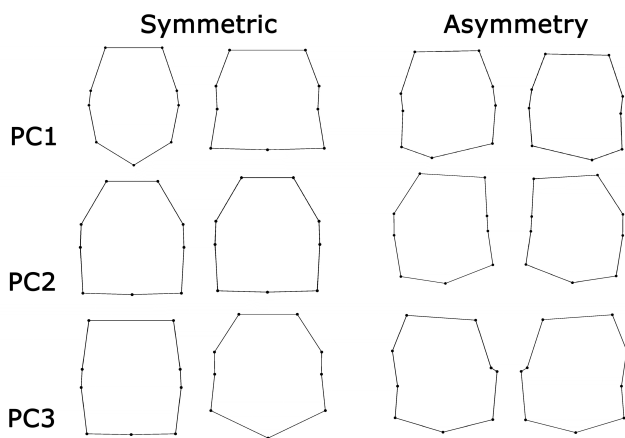


Figure 1.15. Decomposition of shape variation in pronotum in *P. spinicoxis*. The firsts three PCs for each category of symmetry or asymmetry are shown. For each PC the diagrams show the shapes that correspond to scores -1.0 (on the left) or +1.0 (on the right) for both symmetric and asymmetry components of shape variation.



*Paussus spinicoxis*

CVA showed the presence of sexual dimorphism (Mahalanobis distance = 1.77,  $P < 0.0001$ ; Procrustes distance = 0.027,  $P < 0.0001$ ). Shape and size variation were assessed using ANOVA and MANOVA tests for both symmetric and asymmetry component of shape variation. ANOVA test showed a certain morphological variation in size and shape in the whole sample (for size Sums of Squares = 0.57, Means of Squares = 0.018,  $P < 0.0001$ ; for shape Sums of Squares = 0.057, Means of Squares = 0.00026,  $P < 0.0001$ ), a subtle variation between populations (for shape Sums of Squares = 0.019, Means of Squares = 0.00040,  $P = 0.024$ ; for size Sums of Squares = 0.18, Means of Squares = 0.025,  $P = 0.23$ ), and present but little sexual dimorphism on shape (for size Sums of Squares = 0.025, Means of Squares = 0.013,  $P = 0.51$ ; for shape Sums of Squares = 0.018, Means of Squares = 0.0013,  $P < 0.0001$ ). MANOVA test partially confirmed the absence of differences between populations (for symmetric component Pillai trace = 1.92,  $P = 0.0071$ ; for asymmetry component Pillai trace = 1.03,  $P = 0.87$ ) and sexes (for symmetric component Pillai trace = 0.89,  $P = 0.0020$ ; for asymmetry component Pillai trace = 0.42,  $P = 0.47$ ).

Then we used PCA analysis to quantify the shape variation. The firsts three PCs were sufficient to explain the majority of the variation for both symmetric and asymmetry components (for symmetry component  $PC1 + PC2 + PC3 = 80.39\% = 49.44\% + 18.77\% + 12.18\%$ ; for asymmetric component  $PC1 + PC2 + PC3 = 82.38\% = 47.03\% + 22.76\% + 12.59\%$ ) (Figure 1.16).

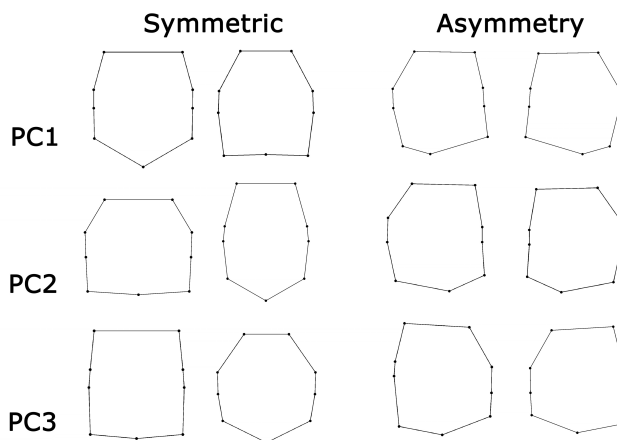


Figure 1.16. Decomposition of shape variation in pronotum in *P. cridae*. The firsts three PCs for each category of symmetry or asymmetry are shown. For each PC the diagrams show the shapes that correspond to scores -1.0 (on the left) or +1.0 (on the right) for both symmetric and asymmetry components of shape variation.

### *Elytrae*

CVA analysis didn't show the differences between species (Mahalanobis distance = 1.13,  $P = 0.35$ ; Procrustes distance = 0.0075,  $P = 0.76$ ), partially confirmed by the ANOVA (for size Sums of Squares = 2.20, Means of Squares = 2.20,  $P < 0.0001$ ; for shape Sums of Squares = 0.062, Means of Squares = 0.00089,  $P = 0.0009$ ) and totally by MANOVA (for symmetric component Pillai trace = 0.22,  $P = 0.72$ ; for asymmetry component Pillai trace = 0.20,  $P = 0.79$ ). Given these results the two species were analyzed together.

CVA showed a very subtle presence of sexual dimorphism (Mahalanobis distance = 1.35,  $P = 0.043$ ; Procrustes distance = 0.017,  $P = 0.21$ ). Shape and size variation were assessed using ANOVA and MANOVA tests for both symmetric and asymmetry component of shape variation. ANOVA test showed a certain morphological variation for shape in the whole sample (Sums of Squares = 0.060, Means of Squares = 0.00018,  $P < 0.0001$ ), and no signal of sexual dimorphism (for size Sums of Squares = 0.11, Means of Squares = 0.057,  $P = 0.56$ ; for shape Sums of Squares = 0.00078, Means of Squares = 0.000039,  $P = 0.99$ ). MANOVA test completely confirmed the

absence of sexual dimorphism (for symmetric component Pillai trace = 0.34,  $P = 0.95$ ; for asymmetry component Pillai trace = 0.31,  $P = 0.97$ ).

Then PCA analysis was used to quantify the shape variation. The firsts two PCs were sufficient to explain the majority of the variation for both symmetric and asymmetry components (for symmetry component  $PC1 + PC2 = 94.47\% = 82.70\% + 11.77\%$ ; for asymmetric component  $PC1 + PC2 = 81.88\% = 56.41\% + 25.48\%$ ) (Figure 1.17).

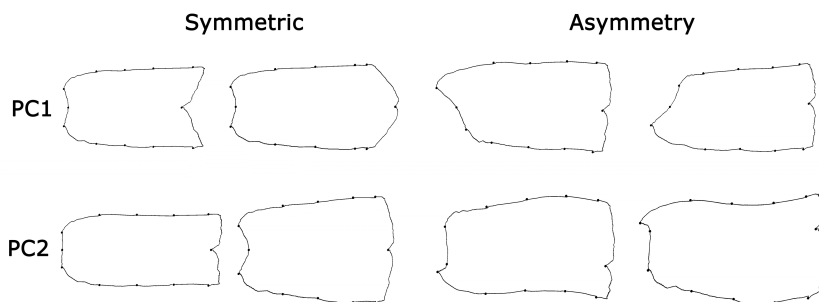


Figure 1.17. Decomposition of shape variation in elytrae. The firsts two PCs for each category of symmetry or asymmetry are shown. For each PC the diagrams show the shapes that correspond to scores -1.0 (on the left) or +1.0 (on the right) for both symmetric and asymmetry components of shape variation.

## II. Discussion

The present study aimed to describe the morphological variability of the two species *P. cridaae* and *P. spinicoxis*, helping to clarify their taxonomic status.

These two species emerged as controversial from literature: some authors define them belonging to a subgenus, others to a species group (Luna de Carvalho 1989; Nagel 1983, 1987, 2003), making not easy to identify their taxonomic status. Additionally, the two species occupy near distribution areas, and are sympatric in Democratic Republic of Congo (Luna de Carvalho 1989).

Moreover, the specimens we analyzed were all identified as *P. spinicoxis*, even they came from areas identified as distribution areas of both species, evidence this of the difficulties in taxonomically distinguish the two species.

Hence, our analyses were made on different populations, quite representative of the whole distribution range of the two species, and

additionally a population in their geographical contact point in Democratic Republic of Congo.

From the analyses emerged a high morphological variability within the sample, not coherent with the taxonomic species identification.

Regarding the antennal clubs, differences between the two species emerged. The shape passes from rounded and bigger in *P. crida*, to little more elongate in *P. spinicoxis* (Figure 1.18).

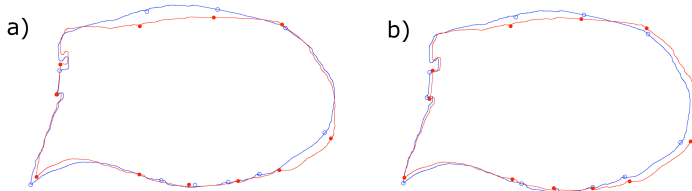


Figure 1.18. Average shape variation in left and right antennal clubs. Shape deformation is shown in red, whereas starting shapes are shown in blue. All the shape changes are exaggerated 5-fold for better visibility. a) *P. crida*; b) *P. spinicoxis*.

Regarding the head, very subtle differences emerged between species, and were more statistical than morphological: the shapes were not so different in the two species, even if the majority of variation is associated with *P. crida* (Figure 1.19).

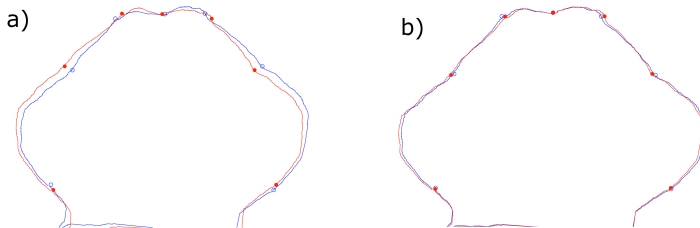


Figure 1.19. Average shape variation in head. Shape deformation is shown in red, whereas starting shapes are shown in blue. All the shape changes are exaggerated 5-fold for better visibility. a) *P. crida*; b) *P. spinicoxis*.

Pronotum showed to be morphologically different in the two species and also in this case the difference were most statistical than morphological. (Figure 1.20).

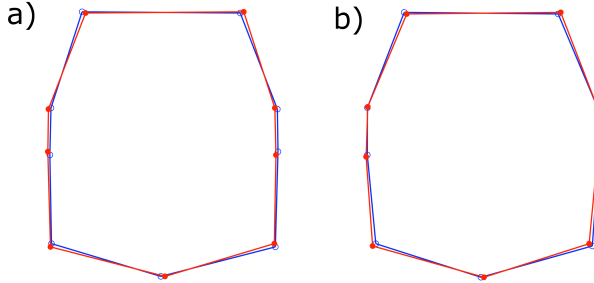


Figure 1.20. Average shape variation in pronotum. Shape deformation is shown in red, whereas starting shapes are shown in blue. All the shape changes are exaggerated 5-fold for better visibility. a) *P. cridae*; b) *P. spinicoxis*.

The elytrae resulted from the analyses the part less differentiated, and didn't show differences between the two species analyzed, even if a subtle variation in shape in the terminal part of the elytrae could be detected (Figure 1.21).

Finally all differences emerged from the analyses are indicative of a high similarity between the two species, more than of a separation between them. In fact the morphological variation emerged was not present in all structures analyzed, and where present, not all structures showed the same grade of differentiation between the two species.

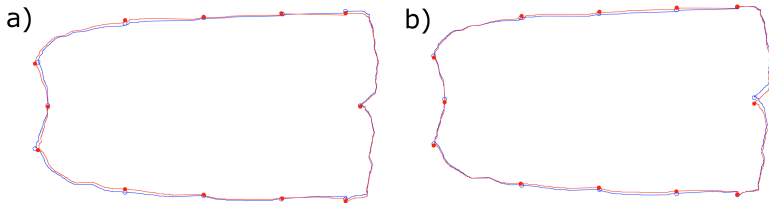


Figure 1.1. Average shape variation in elytrae. Shape deformation is shown in red, whereas starting shapes are shown in blue. All the shape changes are exaggerated 5-fold for better visibility. a) *P. cridae*; b) *P. spinicoxis*.

Results showed a certain morphological variability between the two described species, with the exception of the elytrae, in which no differences had been found. Regarding the populations' differences, a certain variability emerged, but not referred to a population in particular. Interestingly the Angolan population, the one denoted as belonging to *P. cridaae* species, seems to be not separated to the other populations, reinforcing the findings of a certain morphological similarities between the two species. At the same time the differences emerged showed high morphological variability within *P. spinicoxis*, mostly due to the antennal clubs. This is a part intensively used in ant-beetle interactions, and resulted very modified within all members of the subfamily Paussinae (Geiselhardt et al. 2007; Di Giulio et al. 2009; Maurizi et al. 2012). Moreover previous analyses under the scanning electron microscope (S.E.M.) revealed the presence of sensilla and pores similar to those already described in *P. favieri* (Di Giulio personal observation; Di Giulio et al. 2009, 2011). This observation let us to conclude that in the species *P. cridaae* and *P. spinicoxis* antennae are the part most involved in interspecific interactions and consequently they are the part highly under coevolutionary pressures.

On the whole, variation among populations was higher than the one expected from the two species, indicating a very high morphological variability, but not sufficient to distinguish and separate two different species. The results we obtained brought us to the conclusion that we could be in presence of only one species, with a very high intra-specific morphological variability.

However, even if the geometric morphometric approach has demonstrated its validity in enlightening still subtle morphological variation, further studies are necessary to better understand and explain the variability we observed.



## **CHAPTER 2**

### **1) Introduction**

Results of analyses discussed in chapter 1 indicated the presence of high morphological variability in all the species we examined. Due to the novelty of our results, we were interested in deepened the significance of them, upon the light of coevolutionary interactions.

Evolutionary interactions between species and their environment, and among different species, have attracted an increasing number of evolutionists. Coevolutionary interactions between species constitute a challenging topic in evolutionary theories.

In 1979 Dawkins and Krebs formulated the arms races theory with the aims to explain how two interacting species do coevolve. “An adaptation in one lineage (e.g. predators) may change the selection pressure on another lineage (e.g. prey), giving rise to a counter-adaptation. If this occurs reciprocally, an unstable runaway escalation or ‘arms race’ may result” (Dawkins and Krebs 1979). The arms races could be inter-specific or intra-specific, and helpfully explains the prey-predator and host-parasites interactions, as well as the other types of interactions between lineages. The host-parasite interaction is what Dawkins and Krebs (1979) identified as an asymmetric interspecific arms race. From its formulation this theory has been demonstrated to be suitable for various host-parasite interactions (Anderson and May 1982; Foitzik et al. 2001, 2003, 2009).

This theory implies also that lineages posses the capacity to change plastically as the environment change. This capacity is called evolutionary plasticity, and allows a lineage to respond to the changes of its environment, or otherwise it may be going extinct.

Most animals have a bilaterally symmetric body plan, at least externally, although other types of symmetry exist as well. Morphological symmetry outcomes from the repetition of an organ or part of an organ in different positions or orientations, making the spatial arrangement strongly patterned. Because of its interest, bilateral symmetry is largely studied in various contexts (e.g., Finnerty et al. 2004, Manuel 2009) and several studies have approached it by means the geometric morphometrics (Debat et al. 2000, Klingenberg et al. 2002, Drake and Klingenberg 2010, Klingenberg et al. 2010 a,b).



Bilateral symmetry can manifest itself in two ways: as object or matching (Mardia et al. 2000, Klingenberg et al. 2002). The first case happens whenever a structure is symmetric in itself, and the plane of symmetry can divide the structure into two halves that are mirror images of each other. Otherwise matching symmetry happens every time a structure is present in one left and one right copies that can be matched to each other, as they are mirror images of each other (Klingenberg et al. 2002, Savriama and Klingenberg 2012).

It is expected that the left and the right side of a bilateral organism, or a part of it which shows bilateral symmetry, are separate copies of the same structure. They share the same genome, and in a homogeneous environment these two parts develop as identical as an effect of environmental pressure (Klingenberg 2003). In this deterministic system the left and right sides of an organism should develop the same morphology, as expected. But real developing systems are not deterministic systems, and small random perturbations could produce non perfect symmetric organisms (McAdams and Arkin 1999, Klingenberg 2003). Developmental systems are no linear systems (Klingenberg 2003), and the genetic interactions between loci may have different effects that could be difficult to disentangle upon the light of developmental interactions; for this purpose some authors have investigated developmental effects with the method of quantitative genetics with the simplified model of quantitative trait loci (Leamy et al. 2002, Leamy and Klingenberg 2005, Leamy et al. 2008), for trying to understand the interaction between organisms and their environment. In this context left-right asymmetries emerged as expression of developmental noise, and they accumulate through development (Palmer 1996, 2004, Klingenberg 2003).

Asymmetrical forms in animals can generally fall into two main categories: within a single species most individuals are asymmetrical towards the same side, that is directional asymmetry (DA), or asymmetries can be equally frequent to both the both body sides, so dextral and sinistral forms are equally common within a taxon, that is antisymmetry (AS). These two types of symmetry can be used to infer the developmental interactions that could be considered as the base of evolutionary processes. A third type of asymmetry has to be considered: fluctuating asymmetry (FA) is defined as the non-directional deviation from bilateral symmetry (right-left differences,  $r-l$ ), and has usually been considered as a measure of developmental instability (Palmer 1996, 2004). As a consequence of non-linear developmental perturbations, the distribution of traits on left and right sides deviates from original distribution (Klingenberg 2003).

Asymmetrical forms in animals can generally fall into two main categories: within a single species most individuals are asymmetrical towards the same side, that is directional asymmetry (DA), or asymmetries can be equally frequent to both the left and right body sides, so dextral and sinistral forms are equally common within a taxon, that is antisymmetry (AS). These two types of symmetry can be used to infer the developmental interactions that could be considered as the base of evolutionary processes. A third type of asymmetry has to be considered: fluctuating asymmetry (FA) is defined as the non-directional deviation from bilateral symmetry (right-left differences,  $r-l$ ), and has usually been considered as a measure of developmental instability (Palmer 1996, 2004). As a consequence of non-linear developmental perturbations, the distribution of traits on left and right sides deviates from original distribution (Klingenberg 2003).

The expression of DA is mediated by a left-right axis that drives the development of the structures on either body sides (Klingenberg et al. 1998). The presence of DA is well known since past decades now (Van Valen 1962, Palmer and Strobeck 1986, Møller and Swaddle 1997), but assessing and quantifying it in organisms is still matter of debate (Graham et al. 1993, Bell et al. 2007). Although it was first thought that DA is relatively rare, subtle DA has been found in almost every study that examined asymmetries with geometric morphometric techniques (Klingenberg et al. 1998, 2002, Debat et al. 2000, Klingenberg and Zaklan 2000, Willmore et al. 2005), however the developmental processes that produce asymmetry are still poorly known (Gangestad and Thornhill 1999, Houle 2000, Gangestad and Thornhill 2003).

Because DA is used to infer developmental interactions, its analysis with a morphometric approach represents an opportunity to investigate patterns and processes that are the base for evolution. Depending on the type of symmetry considered, morphometric method allows separating symmetry into its components of fluctuating and directional asymmetry, and investigating them in structures with both object and matching symmetry. Organisms have different parts that are integrated to each other to various degrees and complexity, and do not evolve in isolation, but are integrated in some way (Klingenberg 2008).

Until now all the geometric morphometric studies have looked at only one structure or at the comparison between modules of the same structure (Debat et al. 2000, Klingenberg and Zaklan 2000, Drake and Klingenberg 2010, Figueirido et al. 2012). In the present two studies we aim to analyse the evolutionary pattern of different body parts, and moreover the

evolutionary integration between them, for assessing how different parts evolve together and act as a whole.

Ant nest beetles *P. favieri*, *P. cridae*, and *P. spinicoxis*, emerged from two previous studies to constitute a good model species for exploring coevolutionary integration through morphological variation, because of their high morphological variability. Hence, the three species were analyzed a second time with geometric morphometric approach, with the aim to explore, assess and quantify their evolutionary plasticity.

## 2) Directional asymmetry in *Paussus favieri* (Coleoptera: Carabidae: Paussini).<sup>3</sup>

### I. Introduction

The present study was conducted analyzing symmetry components of body shape variation in five different body parts (left and right antennal clubs, head, pronotum and elytrae) showing matching and object symmetry, of the charismatic ground beetle *Paussus favieri*. Although the subfamily Paussinae comprises typically rare insects with myrmecophilous lifestyle that live in tropical and subtropical regions, *P. favieri* is a relatively common species with an Atlanto-Mediterranean distribution (Di Giulio et al. 2011). Moreover this species is considered as parasite of ant nests, feeding on brood and adults of the species *Pheidole pallidula*. Due of its myrmecophilous lifestyle *P. favieri* has particular morphological adaptations (Di Giulio et al. 2011, Maurizi et al. 2012) making it an interesting model in evolutionary and morphological studies.

Our purpose is to evaluate the amount of DA as signal of evolutionary patterns in this model species, at the level of some sclerotized external body structures (antennal clubs, head, pronotum and abdomen), and how evolutionary integrated they are.

The case study also aims to demonstrate the utility of analyzing separately different body parts, for quantifying DA for studies on evolutionary processes.

### II. Results

#### Error evaluation

Procrustes ANOVA for assessing error showed that the mean square for individual variation and fluctuating asymmetry exceeded the measurement error, which was consequently negligible for all parts analyzed ( $P < 0.0001$  for each part, except the head that had  $P = 0.043$ ).

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<sup>3</sup> This work corresponds to the paper: Bravi R., Scalici M., Klingenberg C.P., Di Giulio A. Directional asymmetry in *Paussus favieri* (Coleoptera: Carabidae: Paussini). In prep.

### *Antennal clubs*

We found a high and significant pattern of DA in the antennal clubs that are morphologically different in both shape and size. Procrustes ANOVA showed a clear high pattern of DA in antennal clubs (Figure 2.1a). The latters showed a significant pattern of DA for both size ( $SS = 1.83$ ,  $MS = 1.82$ ,  $P < 0.0001$ ) and shape ( $SS = 0.040$ ,  $MS = 0.0023$ ,  $P < 0.0001$ ), confirmed by MANOVA (Pillai tr. = 0.92,  $P < 0.0001$ ). For the right antennal club these differences emerged in a shorter and rounder club, with an elongated terminal part. Otherwise for the left antennal club shape is stretched and elongated, with a shorted and curved terminal part. Finally the left antennal club emerged also as thicker than the right one. Principal component analysis was used to assess the pattern of DA (Figure 2.2a). The analysis of symmetric component of PCA showed that PC1 alone took up 35.55% of the total variation with the following PC that accounted 18.28%. ( $PC1 + PC2 = 53.82\%$ ); the others PCs explain each no more than 11% of the variation. Given this DA in the antennal clubs, we examined DA between populations. CVA analysis showed that differences are significative between all populations, with the Moroccan one showing the highest differences than the others (see Table 1.1).

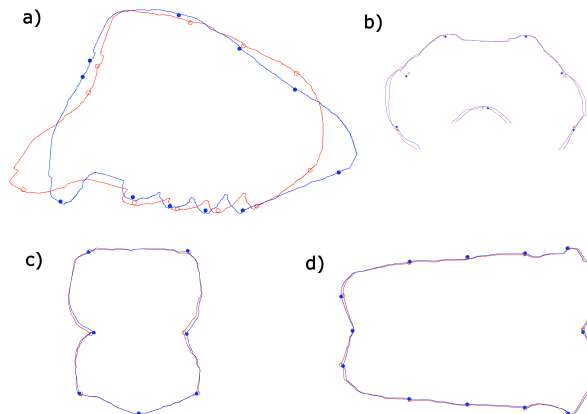


Figure 2.1. Shape changes associated with DA. The shape changes are shown as the difference between the shape averages of right-left sides (blue and red outline respectively) for the antennal club, and as the mean differences from the target shape in all other parts. All the shape changes are exaggerated 5-fold for better visibility. a) left and right antennal clubs; b) head; c) pronotum; d) elytrae.

## Head

DA was statistically significant in both symmetric and asymmetry component. A high pattern of DA for shape and size emerged as well. Even if those differences resulted as more subtle as the ones emerged from antennal clubs, they were however statistically significant and denote the existence of systematically left-right differences even in the head. Procrustes ANOVA showed the existence of a clear pattern of DA in both shape ( $SS = 0.027$ ,  $MS = 0.0054$ ,  $P < 0.0001$ ) and size ( $SS = 0.012$ ,  $MS = 0.0025$ ,  $P < 0.0001$ ), and confirmed by MANOVA test (Pillai tr. = 2.66,  $P = 0.018$ ), which was used to examine the amount of non isotropic variation among landmarks and thus taking into account the structure of shape variation (Figure 2.1b). Principal component analysis was used to assess the pattern of DA (Fig 2.2b). The analysis of symmetric component of PCA showed that PC1 alone took up 52.40% of the total variation, representing the most of the whole shape variation, and the PC2 that accounted 27.54% ( $PC1 + PC2 = 79.93\%$ ); remnants PCs explain each no more than 9%. Moreover CVA test showed significant differences between all populations, but highest distances emerged in the Moroccan one (see Table 1.1).

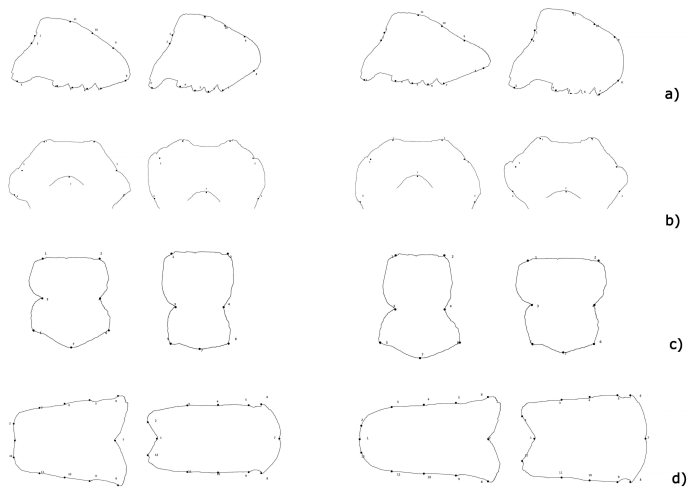


Figure 2.2. Shape variation in body parts under study. The first two PCs component of shape variation associated with DA are shown. For each PC, the diagram shows the shape corresponding with the score of -0.1 (left diagram) or +0.1 (right diagram). The percentage is the variation that each PC accounts for. a) antennal clubs; b) head; c) pronotum; d) elytrae.

### Pronotum

DA in the pronotum was mostly associated with shape and with both symmetric and asymmetry components of shape variation. Procrustes ANOVA showed pattern of DA mostly associated with shape ( $SS = 0.024$ ,  $MS = 0.0048$ ,  $P < 0.0001$ ) (Figure 2.1c), and confirmed by MANOVA test (Pillai tr. = 0.30,  $P < 0.0001$ ), used also in this case to examine the amount of non isotropic variation. We then used the symmetric component of PCA analysis to assess the pattern of DA (Figure 2.2c). PC1 alone took up 61.37% of the total variation with PC2 that accounted 18.73% ( $PC1 + PC2 = 80.10\%$ ); the other PCs accounted each for no more than 8% of the variation. CVA test showed significative differences between all populations also in this case, with highest distances emerged in the Moroccan population (see Table 1.1).

### Elytrae

A subtle, but not statistically significant DA, for both shape and size emerged in the elytrae. Procrustes ANOVA was significative neither for size ( $SS = 0.46$ ,  $MS = 0.051$ ,  $P = 0.22$ ) nor for shape ( $SS = 0.00023$ ,  $MS = 0.000023$ ,  $P = 0.056$ ) (Figure 2.1d). However a subtle DA emerged with the MANOVA test (Pillai tr. = 5.34,  $P = 0.037$ ), and PCA analysis for the symmetric component of variation was used to explore this pattern of DA (Figure 2.2d). PC1 alone took up 47.46% of the total variation with the PC2 that accounted 29.45% ( $PC1 + PC2 = 76.91\%$ ); the others PCs accounted each for no more than 11% of the whole variation, indicating that the majority of variation is associated with the firsts two component of PCA. Moreover CVA test showed significative differences only for the Moroccan population, but no differences emerged between the others (see Table 1.1).

### Evolutionary integration

PLS analysis was used to assess the pattern of evolutionary covariation trough integration, between all parts analyzed and revealed a high pattern of covariation in all connected anatomical parts (Table 2.1).

Table 2.1. Partial Least Square (PLS) analysis between different body parts. This analysis give the measure of the correlation among parts. ANT = antennae; CP = head; PN = pronotum; EL = elytrae.

|        | <i>RV</i> coefficient | <i>p</i> -value   |
|--------|-----------------------|-------------------|
| ANT/CP | 0.19                  | <b>0.017</b>      |
| ANT/PN | 0.29                  | <b>&lt;0.0001</b> |
| ANT/EL | 0.18                  | 0.18              |
| CP/PN  | 0.14                  | <b>0.019</b>      |
| CP/EL  | 0.18                  | 0.22              |
| PN/EL  | 0.19                  | <b>0.013</b>      |

### III. Discussion

In the present study we have applied the tool of geometric morphometrics to the study of several different body parts, with the aims: a) to characterize the presence of DA; b) to infer about its implications in evolutionary processes; c) to assess the evolutionary integration among different structures. We used the approach developed by Klingenberg et al. (2002) for symmetrical structures, and amplified it to different structures with both matching and object symmetry, belonging to the same specimen.

All differences that came out from the analyses are associated with both shape and size in the whole sample, and revealed the presence of a high and significant DA, and a subtle but clear variability between populations.

All results revealed the presence of a high evolutionary pattern (DA) on the antennal clubs, indicating that differences exist between right and left parts. The analyses on the head confirmed the presence of high and significant DA in both size and shape. Regarding the pronotum, coherently with results on antennal clubs and head, DA emerged as high and mostly associated with shape, reinforcing our findings about the existence of an evolutionary pattern. In the analysis of the elytrae subtle pattern of DA emerged, but no differences between right and left sides were found. These results display the presence of morphological differences between parts, and antennal club emerged as the structure most under the DA, with a high shape and size variability. Additionally the analyses showed the presence of differences among populations, with the Moroccan one that emerged as the most differentiated population in every structure analyzed.

Differences emerged between the Moroccan population and the other populations, could be explained with the isolation of the Atlas Mountains chain. This is a very antique chain, and considered one of the main



biogeographical areas within the Mediterranean (Krijgsman et al. 1999, Brown et al. 2002). The Miocene Atlas uplift and climate change generally provide a good explanation for speciation and differentiation of many species with Northwest African distribution (Brown et al. 2002, Harris et al. 2010). Our results suggest that isolation happened between the Moroccan and the others populations, and are coherent with the Atlas uplift during Miocene and analogous to results obtained from other authors' analyses with different species (Brown et al. 2002, Harris et al. 2010). Although the isolation of the Moroccan population came into high differences between this one and the others populations, there was no signal strong enough to assess some kind of evolutionary divergence of the Moroccan from the other populations analyzed.

While subtle DA was found in almost every study analyzing symmetric structures with geometric morphometrics in different organisms, such as plants (Savriama et al. 2012), animals (Klingenberg et al. 1998, 2002) and humans (Klingenberg et al. 2010b), in *P. favieri* DA emerged as high and significant, and present in almost all body parts analyzed.

The high and statistically significant DA we found could be indicative of an evolutionary process that is in progress, and could be referred to the presence of a high plasticity, the latter due to the parasitic life habit of *P. favieri*. This species in facts shows high morphological adaptations to a myrmecophilous life style, especially at the level of antennae, transformed into glandular organs (antennal clubs) covered of trichomes, and connected to exocrine glands that release chemical secretions attractive for ants (Di Giulio et al. 2009, 2012; Maurizi et al. 2012).

All organisms have to respond to environmental pressure to survive and evolve, and they respond in many different ways. Phenotypic plasticity is the capacity of a single genotype to produce adaptive forms in response to environmental conditions, and subjected to natural selection and evolutionary changes (West-Eberhard 2009). This is particularly true in parasites, which are involved in what Dawkins and Krebs (1979) called the arms races; parasitism is a strong driving force in evolution, and upon the light of the arms race theory, in the parasite-host interaction parasites need to have a high evolutionary plasticity to respond to their high hosts' selective pressure (Dawkins and Krebs 1979; Foitzik et al. 2001, 2003, 2009). This theory could be the key for understanding the high phenotypic plasticity, and the high DA emerged in our case study.

DA represents the mean asymmetry in a sample, its presence is not a consequence of the morphological outcome of random perturbations due to developmental noise, but it is a special kind of answer to environmental

pressure that affects two body sides unequally, and come out as systematic left-right differences (Klingenberg 2003).

Finally, different parts analyzed showed a high pattern of integration, evidence that those different parts are linked and evolve together, and even if those different parts were analyzed separately, nevertheless they have not to be considered as separate anatomical parts, but constituting the body as a whole. Moreover the different pattern of DA shows that body parts have a different evolutionary pattern, as result of non-equal selective pressure that act on different structures.

More studies could be necessary for better understanding those evolutionary processes that constitute the base that guide the morphological modifications under selective pressure, on parasites as *P. favi*eri, and on other free living species as well.

### 3) Directional asymmetry in two Paussinae species: *Paussus cridae* and *P. spinicoxis* (Coleoptera: Carabidae: Paussini).<sup>4</sup>

#### I. Introduction

The present study was conducted analyzing symmetry components of body shape variation in five different body parts (left and right antennal clubs, head, pronotum and elytrae) showing matching and object symmetry, of two species of ground beetles: *Paussus cridae* and *P. spinicoxis* (Coleoptera, Carabidae, Paussinae).

Our purpose is to evaluate the amount of DA in *P. cridae* and *P. spinicoxis* species, at the level of some sclerotized external body structures, and to discuss our findings under evolutionary and morphological points of view, quantifying DA for studies on evolutionary processes. We also aim to analyse and assess the pattern of evolutionary integration between several body parts considered as separate one another.

#### II. Results

From previous analyses (Chapt. 1, Par. 2), the two species of *P. cridae* and *P. spinicoxis* resulted in having a high morphological variability, however not sufficient to indicate a clear separation in two well-differentiated species. Hence, the analyses were performed in some cases considering the two species as one, and investigating the pattern of DA within the entire sample. This method doesn't prevent us to detect any pattern of species differentiation.

#### Error evaluation

Procrustes ANOVA for assessing error showed that the mean square for individual variation and fluctuating asymmetry exceeded the measurement error, which was consequently negligible for all parts analyzed ( $P < 0.0001$  for each part).

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<sup>4</sup> This work corresponds to the paper: Bravi R., Scalici M., Klingenberg C.P., Di Giulio A. Directional asymmetry in two Paussinae species: *Paussus cridae* and *P. spinicoxis* (Coleoptera: Carabidae: Paussini). In prep.

### *Antennal clubs*

ANOVA revealed a high and significant pattern of DA in the antennal clubs that are morphologically different in both shape and size (for size  $SS = 4.27$ ,  $MS = 4.27$ ,  $P < 0.0001$ ; for shape  $SS = 0.0040$ ,  $MS = 0.00022$ ,  $P < 0.0001$ ) confirmed by MANOVA (Pillai tr. = 0.84,  $P < 0.0001$ ). For the right antennal club these differences emerged in a shorter and rounder club, with a subtle elongated terminal part (Figure 2.3a). Otherwise for the left antennal club shape is quite stretched and elongated, with a shorted and curved terminal part. Principal component analysis (PCA) was used to assess the pattern of DA (Figure 2.4a). The analysis of symmetric component of PCA showed that PC1 alone took up 38.57% of the total variation with the following two PCs that accounted 16.55% and 13.85% respectively. ( $PC1 + PC2 + PC3 = 68.97\%$ ); the others PCs explain each no more than 7% of the variation. Given this DA in the antennal clubs, we examined DA between populations. CVA analysis showed that there are some significative differences between all populations, but no one merged as most different from the others (Table 2.2).

### *Head*

Pattern of DA for shape emerged in the head. Even if differences resulted as more subtle as the one emerged from antennal clubs, they were however statistically significative and denote the existence of some left-right differences even in the head. Procrustes ANOVA showed the existence of a pattern of DA in shape ( $SS = 0.0016$ ,  $MS = 0.00031$ ,  $P = 0.0009$ ), confirmed by MANOVA test (Pillai tr. = 0.28,  $P = 0.03$ ), the latter used to examine the amount of non isotropic variation among landmarks and thus taking into account the structure of shape variation (Fig. 2.3b). Moreover principal component analysis was used to assess the pattern of DA (Figure 2.4b). The analysis of symmetric component of PCA showed that PC1 alone took up 46.30% of the total variation, representing the most of the whole shape variation, and the other two PCs accounted for 23.97% and 19.29% respectively ( $PC1 + PC2 + PC3 = 89.56\%$ ); remnants PCs explain each no more than 9%. Moreover CVA test showed significative differences between all populations, but any population emerged as highly different from the others (Table 2.2).

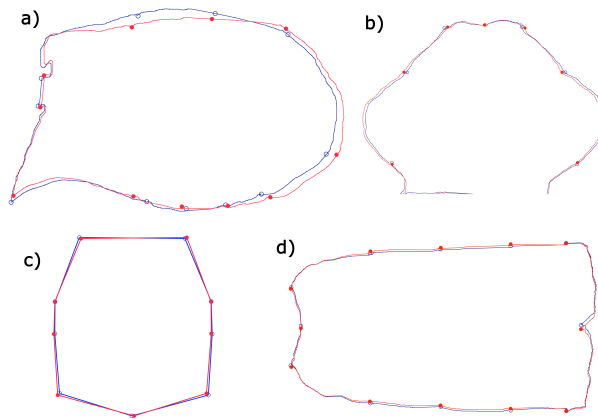


Figure 2.3. Shape changes associated with DA. The shape changes are shown as the difference between the shape averages of right-left sides (blue and red outline respectively) for the antennal club, and as the mean differences from the target shape in all other parts. All the shape changes are exaggerated 5-fold for better visibility. a) left and right antennal clubs; b) head; c) pronotum; d) elytrae.

### Pronotum

DA in the pronotum was mostly associated with shape and with asymmetry components of shape variation. Procrustes ANOVA showed subtle but significant pattern of DA, mostly associated with shape ( $SS = 0.0014$ ,  $MS = 0.00020$ ,  $P = 0.020$ ) (Fig. 2.3c), and confirmed by MANOVA test (Pillai tr. = 0.43,  $P = 0.0004$ ), used also in this case to examine the amount of non isotropic variation. We then used the symmetric component of PCA analysis to assess the pattern of DA (Figure 2.4c). PC1 alone took up 45.15% of the total variation with the others two PCs that accounted for 24.24% and 11.88% respectively ( $PC1 + PC2 + PC3 = 81.17\%$ ); the other PCs accounted each for no more than 9% of the variation. CVA test showed significative differences between all populations also in this case, and as previous cases no population emerged as the most differentiated (Table 2.2).

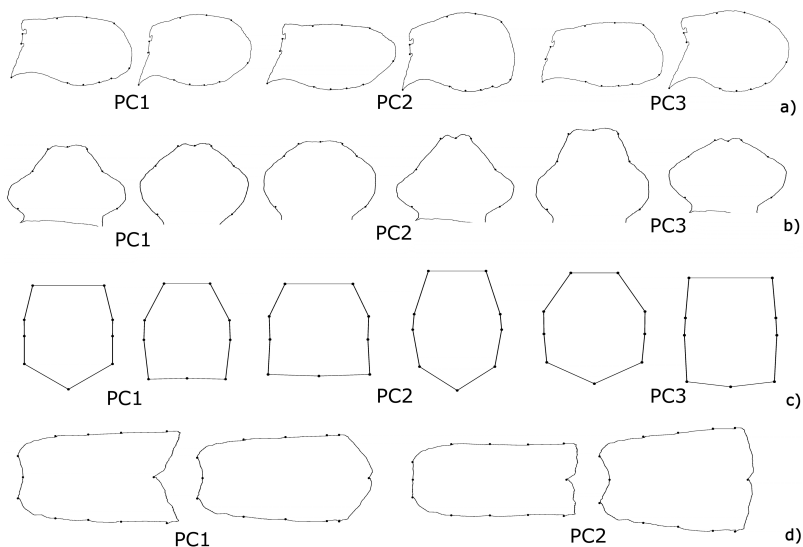


Figure 2.4. Shape variation in body parts under study. The first PC components of shape variation associated with DA are shown. For each PC, the diagram shows the shape corresponding with the score of -0.1 (left diagram) or +0.1 (right diagram). a) antennal clubs (PC1 + PC2 + PC3); b) head (PC1 + PC2 + PC3); c) pronotum (PC1 + PC2 + PC3); d) elytrae (PC1 + PC2).

### Elytrae

Very subtle but statistically significant DA emerged in the elytrae (Fig. 2.3d). Procrustes ANOVA showed a pattern of DA mostly associated with shape (SS = 0.00041, MS = 0.000041, P = 0.0047), and confirmed by MANOVA test (Pillai tr. = 0.45, P = 0.013). PCA analysis for the symmetric component of variation was used to explore this pattern of DA (Figure 2.4d). PC1 alone took up 82.70% of the total variation with the PC2 that accounted 11.77% (PC1 + PC2 = 94.47%); the others PCs accounted each for no more than 2% of the whole variation, indicating that the majority of variation is associated with the first two component of PCA. Moreover CVA test showed some differences between populations, but more subtle than in other parts (Table 2.2).

Table 2.2. Pairwise comparisons by the canonical variate analysis performed between the studied populations, for all the investigated body parts. Results are reported as Mahalanobis distances'  $p$ -values, after 10000 permutation runs. Significant values are reported in bold red. Angola – AN; Democratic Republic of Congo-former Zaire- CN; Kenya – KN; Rwanda – RW; South Africa – SA; Tanzania – TZ; Uganda – UG; Zambia – ZA; Zimbabwe – ZB.

| ANTENNAL CLUBS | AN     | CN     | KN     | RW     | SA     | TZ     | UG     | ZA     | ZB   |
|----------------|--------|--------|--------|--------|--------|--------|--------|--------|------|
| CN             | 0.00   |        |        |        |        |        |        |        |      |
| KN             | <.0001 | 0.01   |        |        |        |        |        |        |      |
| RW             | 0.00   | 0.33   | 0.00   |        |        |        |        |        |      |
| SA             | 0.00   | 0.25   | 0.89   | 0.10   |        |        |        |        |      |
| TZ             | <.0001 | 0.02   | 0.00   | 0.04   | 0.15   |        |        |        |      |
| UG             | 0.03   | 0.09   | 0.00   | 0.01   | 0.13   | 0.00   |        |        |      |
| ZA             | <.0001 | 0.02   | <.0001 | 0.03   | 0.04   | 0.01   | <.0001 |        |      |
| ZB             | <.0001 | 0.01   | <.0001 | 0.01   | 0.04   | <.0001 | 0.00   | <.0001 |      |
| ZW             | <.0001 | 0.34   | 0.02   | <.0001 | 0.43   | 0.02   | 0.01   | 0.00   | 0.17 |
| HEAD           | AN     | CN     | KN     | RW     | SA     | TZ     | UG     | ZA     | ZB   |
| CN             | 0.59   |        |        |        |        |        |        |        |      |
| KN             | 0.00   | 0.85   |        |        |        |        |        |        |      |
| RW             | 0.40   | 1.00   | 0.12   |        |        |        |        |        |      |
| SA             | 0.40   | 1.00   | 0.09   | 1.00   |        |        |        |        |      |
| TZ             | 1.00   | <.0001 | 0.00   | 0.08   | 0.25   |        |        |        |      |
| UG             | 0.86   | 0.67   | 0.06   | 0.33   | <.0001 | 0.80   |        |        |      |
| ZA             | 0.05   | 0.50   | <.0001 | 0.31   | 0.77   | 0.25   | 0.77   |        |      |
| ZB             | 0.01   | 0.63   | 0.73   | 0.05   | 0.08   | 0.03   | 0.07   | 0.00   |      |
| ZW             | 0.23   | 0.33   | 0.01   | <.0001 | 0.34   | 0.20   | 0.33   | 0.47   | 0.02 |
| PRONOTUM       | AN     | KN     | RW     | SA     | TZ     | UG     | ZA     | ZB     |      |
| KN             | <.0001 |        |        |        |        |        |        |        |      |
| RW             | 0.42   | 0.03   |        |        |        |        |        |        |      |
| SA             | <.0001 | <.0001 | 0.29   |        |        |        |        |        |      |
| TZ             | 0.00   | <.0001 | 0.02   | 0.15   |        |        |        |        |      |
| UG             | 0.04   | 0.00   | 0.01   | 0.01   | 0.04   |        |        |        |      |
| ZA             | 0.00   | <.0001 | 0.01   | <.0001 | <.0001 | 0.00   |        |        |      |
| ZB             | 0.00   | <.0001 | 0.03   | 0.00   | 0.00   | 0.08   | 0.05   |        |      |
| ZW             | 1.00   | 0.03   | 0.66   | 0.10   | 0.18   | 0.15   | 0.34   | 0.53   |      |
| ELYTRAE        | AN     | CN     | KN     | TZ     | UG     | ZA     | ZB     |        |      |
| CN             | 0.67   |        |        |        |        |        |        |        |      |
| KN             | 0.00   | 0.64   |        |        |        |        |        |        |      |
| TZ             | 0.72   | 0.95   | 0.00   |        |        |        |        |        |      |
| UG             | 0.67   | 0.66   | 0.89   | 0.94   |        |        |        |        |      |
| ZA             | 0.24   | 0.97   | 0.02   | 0.28   | 0.93   |        |        |        |      |
| ZB             | 0.91   | 0.91   | <.0001 | 0.52   | 0.43   | 0.01   |        |        |      |
| ZW             | 0.34   | <.0001 | 0.00   | 0.03   | <.0001 | 0.01   | 0.05   |        |      |

### Evolutionary integration

PLS analysis was used to assess the pattern of evolutionary covariation through integration, between all parts analyzed and revealed no pattern of covariation in different parts analyzed, with the exception of antennal clubs and pronotum, that resulted integrated (Table 2.3).

Table 2.3. Partial Least Square (PLS) analysis between different body parts. This analysis give the measure of the correlation among parts. ANT = antennae; CP = head; PN = pronotum; EL = elytrae.

|        | <i>RV</i> coefficient | <i>p</i> -value |
|--------|-----------------------|-----------------|
| ANT/CP | 0.12                  | 0.23            |
| ANT/PN | 0.17                  | <b>0.017</b>    |
| ANT/EL | 0.10                  | 0.14            |
| CP/PN  | 0.12                  | 0.11            |
| CP/EL  | 0.08                  | 0.17            |
| PN/EL  | 0.089                 | 0.16            |

### III. Discussion

In this study the tool of geometric morphometrics was applied to the study of several different body parts, with the aim to characterize the presence of DA and its implications in evolutionary processes, and to assess the evolutionary integration among different body parts as well. We used the approach developed by Klingenberg et al. (2002) for symmetrical structures, and applied it to different structures with both matching and object symmetry, belonging to the same specimen.

Differences came out from the analyses were associated principally with shape, and revealed the presence of significant pattern of DA, and a subtle but clear variability between all populations.

All results revealed the presence of an evolutionary pattern (DA) on the antennal clubs in both size and shape, indicating that differences between right and left parts exist. Regarding the head the analyses confirmed the presence of DA for shape as well. Regarding the pronotum, coherently with results on head, DA emerged as mostly associated with shape, confirming the presence of an evolutionary pattern. In the analysis of the elytrae subtle pattern of DA emerged and was mostly associated with shape even in this case. These results display the presence of morphological differences



between parts, and antennal club emerged as the structure most under the DA, with a high shape and size variability. Moreover the pattern of DA emerged was not high enough to revealed differences between the two species.

Additionally, the analyses showed the presence of differences between populations, with no population emerged as high differentiated from the others (differently on what previously obtained in *P. favieri*), indicating a high grade of morphological differences, but no differentiation between populations. Moreover the differences emerged between the two species were not high enough to bring us to the conclusion they are two well-separated species.

Even in the present study DA emerged as significant, and present in all body parts analyzed.

The high and statistically significant DA we found could be indicative of an evolutionary process that is in progress, and could be referred to the presence of a high plasticity, the latter due to the parasitic life habit of the species. *P. cridae* and *P. spinicoxis* in facts, shows high morphological adaptations to a myrmecophilous life style (as in all Paussini happened), especially at the level of antennae, transformed into glandular organs (antennal clubs) and covered of trichomes (Di Giulio personal observation), probably connected to exocrine glands releasing chemical secretions, as already reported for *P. favieri* (Di Giulio et al. 2009, 2012; Maurizi et al. 2012).

As discussed in the previous study, the presence of DA in a sample could be a special kind of answer to environmental pressure that affects two body sides unequally, and outcome as systematic left-right differences (Klingenberg 2003).

Finally, different parts analyzed didn't show a high pattern of integration, with the exception of antennal clubs and pronotum that resulted highly integrated. In our sample almost all body parts seem to act as separate anatomical parts, evidence of different selective pressure acting on them.

The different patterns of DA obtained, confirmed the different strength of evolutionary processes, result of non-equal selective pressure that act on different structures. In this way ants could act as a driving selective pressure factor on the morphology of the beetle, and the force of this selective pressure depends upon the interactions on the structures strictly involved in the interspecific communications between beetle and ants. Our results enlightened the strength of interactions between the beetle and its host, mostly focused on antennal clubs and pronotum.

From our analyses emerged that different body parts have not to be treated together for not misleading any evolutionary difference in the way selective pressure acts. Moreover, an analysis of integration is necessary in any cases, for assessing how linked the different body parts are.

Even in this study the morphometric approach emerged as a valid tool for study evolutionary processes from a morphological point of view, making possible to detect even subtle evolutionary signals in the sample.

More studies could be necessary for better understanding those evolutionary processes that constitute the base that guide the morphological modifications under selective pressure, both on parasites such as *P. crida* and *P. spinicoxis*, and on other free living species as well.



## CONCLUSIONS

The present work represents a pioneer study on the morphology of three *Paussus* species, investigated with a geometric morphometric approach. Beetles represent a good model for morphometrics studies: they have sclerotized bodies, in which points are easy to identified and fixed; different parts are well distinguishable and they demonstrated to be suitable for several morphometric approaches (Alibert et al. 2001; Pretorius and Scholtz 2001; Pizzo et al. 2008).

Morphometric study on three species of the genus *Paussus* was very challenging, because Paussini are rare, and in some cases their biology is still poorly known. Moreover they live in ants' nests as parasite, and show strict morphological adaptations that make them a good model for studying morphological modification to an evolutionary point of view. *Paussus favieri* was chose as a species with an Atlanto-Mediterranean distribution, quite abundant in its distribution range; the other two species, *P. cridae* and *P. spinicoxis* were chose because from literature their taxonomic status resulted still not clear (Nagel 1983, 1987, 2003; Luna de Carvalho 1989), they are quite abundant in their distribution range, and they are sympatric in Democratic Republic of Congo (Luna de Carvalho 1989).

Anatomical parts were analyzed independently from each other; this was done for avoiding overestimating or even underestimating the amount of the variation, case that could happen if we performed the analyses on the body as a whole. This approach allowed us to detect even subtle morphological modifications. And in fact results confirmed the presence of great morphological variability in all species, with the group *P. cridae* - *P. spinicoxis* that showed the higher amount of morphological variability, even not coherent with the separation in two different species. Morphological variability that came out from the analyses confirmed the high variability described already within the tribe, but this variability was found not only on the antennal clubs, as expected in a so modified parasites, but even in other body parts, indicating that evolutionary modification involved all body, not only the structures mostly considered to be more strongly associated in interactions with ants.

Ants could act as a driving selective pressure factor on the morphology of the ant nest beetles, and the force of this selective pressure depends upon the interactions on the structures strictly involved in the interspecific communications between beetle and ants (antennal clubs, head and pronotum) and demonstrated to be proportional with it.

We then analyzed the pattern of directional asymmetry (DA), for demonstrating and quantifying the existence of coevolutionary interactions. The presence of DA could be also associated with the presence of evolutionary plasticity, the capacity of the organisms to respond to the environmental changes.

A certain evolutionary pattern emerged in the analyses of DA in all species, but was stronger in *P. favierei*, which seems to be under a stronger selective pressure than the other two species. Even if we performed the analyses considering body parts as separate, moreover they constitute the body as a whole, and consequently they are developmentally and structurally integrated. In consideration of that we quantified the amount of evolutionary integration among different parts. Again, *P. favierei* was the species with higher integration between parts, demonstrating to be a species with high plasticity and under a strong selective pressure.

Additionally, from our analyses emerged that different body parts have not to be treated together for not misleading any evolutionary difference in the way selective pressure acts, and that could come out from the analyses. Consequently an analysis of integration is necessary in any cases for assessing how linked the different body parts are.

Nevertheless further morphometrics studies are needed to confirm whether a similar morphological adaptation and evolutionary pattern are involved also in other paussine species, with the same or different degrees of integration with their hosts.

The morphometric tool proved its validity for studying evolutionary morphological processes; here we have demonstrated this approach for several examples of ant nest beetles, but it can be used in the analysis of evolutionary pattern in any organism.



## **REFERENCES**

- Adams D.C., Rohlf F.J., Slice D.E. 2004. Geometric morphometrics: ten years of progress following the 'revolution'. *Italian Journal of Zoology*. 71: 5-16.
- Alibert P., Moureau B., Dommergues J-L., David B. 2001. Differentiation at a microgeographical scale within two species of ground beetle, *Carabus auronitens* and *C. nemoralis* (Coleoptera, Carabidae): a geometrical morphometric approach. *The Norwegian Academy of Science and Letters, Zoologica Scripta*. 30: 299-311.
- Anderson R.M., May R.M. 1982. Coevolution of hosts and parasites. *Parasitology*. 85: 411-426.
- Assis L.C.S. 2009. Coherence, correspondence, and the renaissance of morphology in phylogenetic systematics. *Cladistics*. 25: 528-544.
- Barbero F., Thomas J.A., Bonelli S., Balletto E., Schönrogge K. 2009. Queen ants make distinctive sounds that are mimicked by a butterfly social parasite. *Science*. 323: 782-785.
- Bolen G.M., Rothstein S.I., Trost C.H. 2000. Egg recognition in yellow-billed and black-billed magpies in the absence of interspecific parasitism: implications for parasite-host coevolution. *The Condor* 102 (2): 432-438.
- Brown R.P., Suarez N.M., Pestano J. 2002. The Atlas mountains as a biogeographical divide in North-West Africa: evidence from mtDNA evolution in the Agamid lizard *Agama impalearis*. *Molecular Phylogenetics and Evolution*. 24: 324-332.
- Casale A., Sturani M., Vigna Taglianti A. 1982. Coleoptera. Carabidae. I. Introduzione, Paussinae, Carabinae. *Fauna d'Italia*, 18. Edizioni Calderini, Bologna. xii + 499 pp.
- Combes C. 2001. Parasitism, the ecology and evolution of intimate interactions. *The University of Chicago Press*. 552 pp.

- Darlington P.J. Jr. 1950. Paussid Beetles. Transactions of the American Entomological Society. 76 (2): 47-142.
- Davies N.B., Bourke A.F.G., de L Brooke M. 1989. Trends in Ecology & Evolution. 4: 274-278.
- Dawkins R. and Krebs J.R. 1979. Arms Races between and within Species. Proceedings of the Royal Society of London. 205: 489-511.
- Debat V., Alibert P., David P., Paradis E., Auffray J-C. 2000. Independence between developmental stability and canalization in the skull of the house mouse. Proceedings of the Royal Society of London. B. Biol. Sci. 267: 423-430.
- Di Giulio A., Fattorini S., Kaupp A., Vigna Taglianti A., Nagel, P., 2003. Review of competing hypotheses of phylogenetic relationships of Paussinae (Coleoptera: Carabidae) based on larval characters. Systematic Entomology. (28): 509–537.
- Di Giulio A., Kaupp A., Fattorini S., Vigna Taglianti A., Nagel P. 2007. Pupal morphology in the subfamily Paussinae (Coleoptera: Carabidae). Revue Suisse de Zoologie. 114 (1): 33-48.
- Di Giulio A., Maurizi E., Hlaváč P. and Moore W., 2011. The long-awaited first instar larva of *Paussus favieri* (Coleoptera: Carabidae: Paussini). European Journal of Entomology. (108): 127–138.
- Di Giulio A., Maurizi E., Rossi Stacconi M.V., Romani R. 2012. Functional structure of antennal sensilla in the myrmecophilous beetle *Paussus favieri* (Coleoptera, Carabidae, Paussini). Micron. 43: 705-719.
- Di Giulio A. and Moore W. 2004. The first-instar larva of the genus *Arthropterus* (Coleoptera: Carabidae: Paussinae): implications for evolution of myrmecophily and phylogenetic relationships within the subfamily. Invertebrate Systematics. (18): 101–115.
- Di Giulio A., Rossi Stacconi M.V., Romani R. 2009. Fine structure of the antennal glands of the ant nest beetle *Paussus favieri* (Coleoptera, Carabidae, Paussini). Arthropod Structure & Development. 38: 293-302.



- Di Giulio A. and Vigna Taglianti A. 2001. Biological observations on *Pachyteles* larvae (Coleoptera: Carabidae: Paussinae). Tropical Zoology. 14: 157–173.
- Drake A.G., Klingenberg C.P. 2010. Large-scale diversification of skull shape in domestic dogs: disparity and modularity. The American Naturalist 2010. 175: 289-301.
- Escherich K. 1899. Zur Naturgeschichte von *Paussus favieri* Fairm. Verhandlungen des Zoologisch-Botanischen Vereins in Wien. 49: 278–283.
- Figueirido B., Serrano-Alarcón F.J., Palmqvist P. 2012. Geometric morphometrics shows differences and similarities in skull shape between the red and giant pandas. Journal of Zoology. 286: 293-302.
- Foitzik S., Achenbach A., Brandt M. 2009. Locally adapted social parasite affects density, social structure, and life history of its ant hosts. Ecology. 90 (5): 1195-1206.
- Foitzik S., DeHeer C.J., Hunjan D.N., Herbers J.M. 2001. Coevolution in host-parasite systems: behavioural strategies of slave-making ants and their hosts. Proceedings of the Royal Society B. 268: 1139-1146.
- Foitzik S., Fischer B., Heinze J. 2003. Arms races between social parasites and their hosts: geographic patterns of manipulation and resistance. Behavioral Ecology. 14 (1): 80–88.
- Geiselhardt S.F., Peschke K., Nagel P. 2007. A review of myrmecophily in ant nest beetles (Coleoptera: Carabidae: Paussinae): linking early observations with recent findings. Naturwissenschaften. 94 (11): 871-894.
- Harris D.J., Perera A., Barata M., Tarroso P., Salvi D. 2010. New distribution notes for terrestrial herpetofauna from Morocco. North-Western Journal of Zoology. 6 (2): 309-315.

- Hermesen J.E. and Hendricks J.R. 2008. W(h)ither fossils? Studying morphological character evolution in the age of molecular sequences. *Annals of the Missouri Botanical Garden*. 95(1): 72-100.
- Hölldobler B. and Wilson E.O. 1990. *The ants*. Cambridge MA: Harvard University Press. 732pp.
- Holub E.B. 2001. The arms race is an ancient history in *Arabidopsis*, the wildflower. *Nature Reviews Genetics*. 2: 516-527.
- Klingenberg C.P. 2003. A developmental perspective on developmental instability: theory, models and mechanisms. In *Developmental instability: causes and consequences*. Edited by: Polak M. New York. Oxford University Press. pp 14-34.
- Klingenberg CP. 2008. Morphological Integration and Developmental Modularity. *The Annual Review of Ecology, Evolution, and Systematics*. 39: 115-132.
- Klingenberg C.P. 2011. MorphoJ an integrated software package for geometric morphometrics. *Molecular Ecology Resources*. 11: 353-357.
- Klingenberg CP, Barluenga M, Meyer A. 2002. Shape analysis of symmetric structures: quantifying variation among individuals and asymmetry. *Evolution* 56:1909–1920.
- Klingenberg C.P., Debat V., Roff D.A. 2010a. Quantitative genetics of shape in cricket wings: developmental integration in a functional structure. *Evolution*. 64: 2935-2951.
- Klingenberg C.P, McIntyre G.S, Zaklan S.D. 1998. Left–right asymmetry of fly wings and the evolution of body axes. *Proc. R. Soc. Lond B*. 265:1255-1259.
- Klingenberg C.P., Zaklan S.D. 2000. Morphological integration between developmental compartments in the *Drosophila* wing. *Evolution*. 54: 1273-1285.
- Klingenberg C.P., Wetherill L., Rogers J., Moored E., Ward R., Autti-Rämö I., Fagerlundh Å., Jacobson S.W., Robinson L.K., Hoyme H.E.,

- Mattson S.N., Li T.K., Riley E.P., Foroud T., and the CIFASD Consortium. 2010b. Prenatal alcohol exposure alters the patterns of facial asymmetry. *Alcohol* 44: 649-657.
- Krijgsman W., Hilgen F.J., Raffi I., Sierro F.J., Wolson D.S. 1999. Chronology, causes and progression of the Messinian salinity crisis. *Nature*. 400: 652-655.
- Lauder G.V. 1990. Functional morphology and systematics: studying functional patterns in a hystorical context. *Annual Review of Ecology and Systematics*. 21: 317-340.
- Le Masne G. 1961a. Observations sur le comportement de *Paussus favieri* Fairm., hôte de la fourmi *Pheidole pallidula* Nyl. *Annales de la Faculte des Sciences de Marseille*. Marseilles & Paris. 31: 111–130.
- Le Masne G. 1961b. Recherches sur la biologie des animaux myrmécophiles I: L'adoption des *Paussus favieri* Fairm. par une nouvelle société de *Pheidole pallidula* Nyl. *Comptes Rendus Hebdomadaires des Seances de l'Academie des Sciences*. 253: 1621–1623.
- Le Masne G. 1961c. Recherches sur la biologie des animaux myrmécophiles: Observations sur le régime alimentaire de *Paussus favieri* Fairm., hôte de la fourmi *Pheidole pallidula* Nyl. *Comptes Rendus Hebdomadaires des Seances de l'Academie des Sciences*. 253: 1356–1357.
- Lorenz W. 2005. Systematic list of extant ground beetles of the world. Insecta Coleoptera “Geadephaga”: Trachypachidae and Carabidae incl. Paussinae, Cicindelinae, Rhysodinae). Second edition. Published by the author, Tutzing, Germany.
- Luna de Carvalho E. 1989. Essai monographique des Coléoptères Protopaussines et Paussines. *Memórias do Instituto de Investigação Científica Tropical*. 70: 1–1028.
- May R.M. and Anderson R.M. 1990. Parasite-host coevolution. *Parasitology*. 100: 89-101.

- Maor R., Shirasu K. 2005. The arms race continues: battle strategies between plants and fungal pathogens. *Current Opinion in Microbiology*. 8 (4): 399-404.
- Maurizi E., Fattorini S., Moore W., Di Giulio A. 2012. Behavior of *Paussus favieri* (Coleoptera, Carabidae, Paussini): A Myrmecophilous Beetle Associated with *Pheidole pallidula* (Hymenoptera, Formicidae). *Psyche*. Volume 2012, Article ID 940315, 9 pages. doi:10.1155/2012/940315.
- Moore W. and Di Giulio A. 2006. Description and behaviour of *Goniotropis kuntzeni* larvae (Coleoptera: Carabidae: Paussinae: Ozaenini) and a key to genera of Paussinae larvae. *Zootaxa*. (1111): 1– 19.
- Moore W., Xiao-bin S., Di Giulio A. 2011. The larva of *Eustra* (Coleoptera, Paussinae, Ozaenini): a facultative associate of ants. *Zookeys*. (90): 63–82.
- Nagel P. 1983. Contribution to the knowledge of African ant nest beetle (Coleoptera, Carabidae, Paussinae). *Rev Zool. Afr.* 97: 73-93.
- Nagel P. 1987. Arealsystemanalyse afrikanischer Fühlerkäfer (Coleoptera, Carabidae, Paussinae) vol. 21. Franz Steiner Verlag Wiesbaden GmbH Stuttgart.
- Nagel P. 2003. Carabidae: Paussinae. In: Löbl, I. and Smetana, A. (Eds). *Catalogue of palaearctic Coleoptera*. Apollo Books, Stenstrup. 1: 208–211.
- Pizzo A., Roggero A., Palestini C., Moczek A.P., Rolando A. 2008. Rapid shape divergences between natural and introduced populations of a horned beetle partly mirror divergences between species. *Evolution and Development*. 10 (2): 166-175.
- Poulin R. 2006. *Evolutionary Ecology of Parasites*. 342 pp.
- Pretorius E. and Scholtz C.H. 2001. Geometric morphometrics and the analysis of higher taxa: a case study based on the metendosternite of the Scarabaeoidea (Coleoptera). *Biological Journal of the Linnean Society*. 74: 35-50.

- Price P.W., Westoby M., Rice B., Atsatt P.R., Fritz R.S., Thompson J.N., Mobley K. 1986. Parasite Mediation in Ecological Interactions. *Annual Review of Ecology and Systematics* 17: 487-505.
- Rasa O.A.E. 1996. Interspecific association in desert tenebrionid beetles: a cleptoparasite does not affect the host's reproductive success, but that of its offspring. *Naturwissenschaften*. 83: 575-577.
- Rohlf F.J., and Marcus L.F. 1993. A revolution in morphometrics. *Trends in Ecology and Evolution*. 8: 129-132.
- Savriama Y., Gómez J.M., Perfectti F., Klingenberg C.P. 2012. Geometric morphometrics of corolla shape: dissecting components of symmetric and asymmetric variation in *Erysimum mediohispanicum* (Brassicaceae). *New Phytologist*. 196 (3): 945-954.
- Thomas J.A., Schönrogge K., Bonelli S., Barbero F., Balletto E. 2010. Corruption of ant acoustical signals by mimetic social parasites. *Maculeia* butterflies achieve elevated status in host societies by mimicking the acoustics of queen ants. *Communicative & Integrative Biology* 3 (2): 169-171.
- Thomas J.A. and Settle J. 2004. Butterfly mimics of ants. *Nature*. 7015 (432): 283-284.
- Thompson N.J. 2001. Coevolution. eLS.
- Thompson N.J. 2009. The Coevolving Web of Life (American Society of Naturalists Presidential Address). *The American Naturalist*. 173 (2): 125-140.
- Toft C.A. Karter A.J. 1990. Parasite-host coevolution. *Trends in Ecology & Evolution*. 5 (10): 326-329.
- West-Eberhard M.J. 2009. Darwinism in the twenty-first century. *Rendiconti Lincei* 20: 297-299.

- Westwood J.O. 1850. Descriptions of two new species of Paussidae from Australasia in the collection of the Jardin des Plantes de Paris. Proceedings of the Linnean Society of London. 2: 100-101.
- Wilson E.O. 1971. The Insect Societies. Belknap Press (Harvard University Press) 548 pp.
- Zelditch M.L., Swiderski D.L., Sheets H.D. 2012. Geometric morphometrics for biologist: a primer. Second Edition. Elsevier. 478 pp.
- Zerche L. 1990: Book review. Beitr. Entomol. 40: 267–268.



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*I love fools' experiments.  
I am always making them.*  
(Charles Darwin)