Research on Chrysomelidae 6

Edited by

Pierre Jolivet, Jorge Santiago-Blay & Michael Schmitt

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EDITORIAL

A peer-reviewed open-access journal
ZooKeys
Launched to accelerate biodiversity research

Editorial

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The present volume will be the last one bearing the names of us three editors on the cover. Jorge Santiago-Blay has decided to step down from the board of editors (for family reasons), and Pierre Jolivet had done so already last year and returns only to bid farewell to Jorge.

Our co-operative editorship started in 2001, when Pierre Jolivet requisitioned Jorge and me when he planned to edit another volume on leaf beetles. This book grew enormously until the publisher compelled us to terminate further acceptance of contributions. Finally, even two chapters had to appear on a CD ROM that was inserted in the book. These chapters were one on leaf-mining chrysomelids and the extensive treatise on the subfamily Aulacoscelidinae/Aulacoscelinae, both by Jorge Santiago-Blay. "The green book" – *New Developments in the Biology of the Chrysomelidae* – contains 62 chapters by 111 authors from 27 countries. Jorge had been responsible for the majority of the manuscripts, which meant he had to find reviewers, correspond (and often discuss) with the authors, check the English, decide on final acceptance, and proofread. Thus, the biggest and heaviest book on leaf beetles depended for the most part on Jorge's editorial efforts.

We could present the "Green Book" to the attendants of the Sixth International Symposium on the Chrysomelidae in May, 2004 in Bonn (Germany). At the same time, Pierre had already negotiated with Brill publishers about the launch of a new series, shortly after baptised *Research on Chrysomelidae*. As the co-operation of the three

of us ran so harmoniously and productively, Pierre invited again Jorge and me to edit this series jointly. The first two volumes of this series appeared with Brill publishers (Leiden – Boston). Since the selling numbers did not meet the expectations of the publishers, they decided to drop the series after these two volumes. Again it was Pierre who found a solution: During the 9th European Congress of Entomology, held in Budapest (Hungary) in 2010, he agreed with Lyubomir Penev from Pensoft publishers that *Research on Chrysomelidae* should be published as special issues of ZooKeys, again co-edited by the three of us. The present volume is the fourth, but certainly not the last, published by Pensoft. Although the pullout of Pierre Jolivet and Jorge Santiago-Blay marks a crucial cut in the history of *Research on Chrysomelidae,* I understand the reasons of their decision to step down. I hope and wish that the series will prosper and remain accepted as a forum of leaf beetle research by the community of Chrysomelidae enthusiasts all over the world. The next volume of *Research on Chrysomelidae* will contain the proceedings of the 9th International Symposium on Chrysomelidae and will be co-edited by Caroline Chaboo (University of Kansas, Lawrence, Kansas, USA) and myself.

I thank Jorge Santiago-Blay from the bottom of my heart for his tireless engagement in fostering leaf beetle research and his friendship, and wish him All the Best for whatever he may entertain in the future.

Michael Schmitt (Greifswald, Germany)

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RESEARCH ARTICLE

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High-throughput biodiversity analysis: Rapid assessment of species richness and ecological interactions of Chrysomelidae (Coleoptera) in the tropics

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Abstract

Biodiversity assessment has been the focus of intense debate and conceptual and methodological advances in recent years. The cultural, academic and aesthetic impulses to recognise and catalogue the diversity in our surroundings, in this case of living objects, is furthermore propelled by the urgency of understanding that we may be responsible for a dramatic reduction of biodiversity, comparable in magnitude to geological mass extinctions. One of the most important advances in this attempt to characterise biodiversity has been incorporating DNA-based characters and molecular taxonomy tools to achieve faster and more efficient species delimitation and identification, even in hyperdiverse tropical biomes. In this assay we advocate for a broad understanding of Biodiversity as the inventory of species in a given environment, but also the diversity of their interactions, with both aspects being attainable using molecular markers and phylogenetic approaches. We exemplify the suitability and utility of this framework for large-scale biodiversity assessment with the results of our ongoing projects trying to characterise the communities of leaf beetles and their

host plants in several tropical setups. Moreover, we propose that approaches similar to ours, establishing the inventories of two ecologically inter-related and species-rich groups of organisms, such as insect herbivores and their angiosperm host-plants, can serve as the foundational stone to anchor a comprehensive assessment of diversity, also in tropical environments, by subsequent addition of trophic levels.

Keywords
Angiosperms, Biodiversity, Chrysomelidae, insect-plant interaction, molecular ecology, molecular taxonomy, tropics

1. Biodiversity assessment: challenges and approaches

1.1. An all-encompassing view on Biodiversity

Few unifying concepts in Biology are so well established and ingrained in scientific and popular thinking as Biodiversity (Wilson 1988). Yet, the actual definition of Biodiversity is as encompassing and universally accepted, as it is elusive or ambiguous. Biodiversity is the diversity of Life, and by diversity of life we can understand every level of organisation, from the structural elements of genes in a particular genome, to the whole biosphere, past and present. The most intuitive idea of biodiversity has its roots in the enlightened and encyclopaedic inventorying efforts that propelled the voyages of discovery in the XVIIIth Century to collect and catalogue animals, plants and minerals all over the Globe. This inventorying urge promoted in turn the creation of Museums, Zoos and Botanical Gardens in developed countries, places to keep and share with the public the records of the catalogue (institutions still reputed and alive and experimenting today a renaissance of that cataloguing spirit). Following this tradition, the word biodiversity evokes a display of life forms, or a list or catalogue of species names, ideally ranked following some system. In this context, biodiversity is tightly linked to the practice and development of Taxonomy, after all the science in charge of recognising, describing and naming organisms. Biodiversity inventories thus benefit from every conceptual and methodological advance that has contributed to the maturation of Taxonomy, from the consolidation of evolutionary thinking to the debates on species concepts, from ultrastructural analyses under the microscope to the study of gene differences among individuals or metagenomic analyses of complex environments. However, as we said, the concept of biodiversity is an all-encompassing idea that should reflect any possible way in which life is organised, including supra-organismal assemblages, such as antagonist or mutualistic associations and behaviours, food-webs, communities and biomes, their combination in ecosystems, and so on. This is essentially the diversity of ways in which life forms can interact, an aspect of biodiversity traditionally approached from Ecology, with a boost in recent times thanks to the progress made in the field of community ecology. The diversity of interactions is perhaps a less intuitive idea attached to biodiversity than the composition of a community *per se* (i.e., the idea of the inventory), but both are the complementary angles that shape the all-unifying concept of Biodiversity (Novotny and Miller 2014). Thus,

the concept of Biodiversity, certainly the one we will use throughout this assay, merges composition and functioning criteria of diversity in a given environment. These cataloguing and integrative scopes take on their highest relevance when biodiversity assessment is coupled with conservation initiatives, which ideally aim at preserving not only the nominal diversity of life forms but vitally the processes that sustain them too.

1.2. The challenge of Biodiversity assessment

When the emphasis of biodiversity assessment focuses on the inventorying angle, this 'simplified' view on biodiversity is nonetheless generally restricted by taxonomic expertise, sampling techniques, budgetary limitations, but most of all by the sheer diversity of life forms that even the most simple biomes can harbour. A relatively homogeneous, well-delimited environment, such as a high-mountain lagoon or a monoculture crop, can be home to hundreds or thousands of different species, considering seasonality and transient and resident organisms, particularly when micro-fauna, micro-flora and, needless to say, prokaryotes are taken into account. This situation forces most biodiversity assessment plans to narrow their scope to simplified sampling strategies, e.g. canopy fogging of individual trees or deep-sea or soil probing, and typically to a specific group of organisms or habit, e.g. arthropods, insects, trees, benthic fauna, etc. Inventorying is certainly a challenge, but adding the interactions dimension to biodiversity assessment is nearly utopian. When biodiversity is described considering its functional aspects, it generally requires a much more restrictive assessment, taxonomic and for a particular interaction, e.g. pollinators of a particular plant species, community of animals exploiting a certain tree, or microorganisms with specific bioremediation potential.

These simplified approaches are defensible from an academic point of view, and they are also well adjusted to the serious underfunding for most biodiversity assessment initiatives. However, they are clearly inefficient to tackle the biological, cultural and moral problem dubbed as the Biodiversity Crisis (Western 1992; Singh 2002). Again, the challenge remains the inordinate number of species and the combinatory of their interactions, coupled in great part with the ever-declining expertise in recognising (let alone naming) this diversity. The tip of the biodiversity crisis iceberg are groups of organisms such as angiosperms, birds, amphibians or mammals, amenable to relatively deep biodiversity assessment at least in parts of their ranges, even though the most serious concerns relate to less conspicuous but hyperdiverse groups of organisms, such as the insects (Dunn 2005). Moreover, biodiversity follows gradients, whereby the still highly unexplored tropics show the highest species counts and associations (Pianka 1966; Janzen 1973; Dowle et al. 2013), and for the known fraction of biodiversity, precisely the tropics harbour most of biological diversity perceived under significant threat, the so-called biodiversity hotspots (Myers et al. 2000). For hyperdiverse groups in hyperdiverse regions of the Planet we can generalise that our taxonomic knowledge is basic and our insight into the species ecology is merely anecdotic—although there

are, of course, important exceptions (e.g., InBIO Costa Rica; Smithsonian Institution, Barro Colorado, Panama).

The task ahead is titanic. The goal is to unravel the Earth's biodiversity as fast as possible against the ever-growing extinction rates due to habitat disappearance, fragmentation and alteration, the combined effect of climate change, overexploitation and the impact of biological invasions (Dirzo and Raven 2003; Barnosky et al. 2011). And non-trivially, the challenge is against a worrying cultural trend in this field known as the Taxonomic Impediment, the combined effect of the perception of Taxonomy as an old decaying science and the gradual disappearance of taxonomic expertise (Hebert et al. 2003; Lipscomb et al. 2003; Wheeler et al. 2004). All in all, the task is perhaps unapproachable, a mere intellectual chimera, but the scientific challenge, societal responsibility and achievable benefits are solid reasons to continue investing in biodiversity assessment, improving our assessment potential with training and technical developments.

In recent years, and as a reaction to the biodiversity crisis there has been a proliferation of initiatives aiming at large-scale biodiversity assessment. This is just to say initiatives that aim at a comprehensive (with constraints) characterisation of biodiversity, with a large regional, ecological and/or taxonomic scope. Large-scale biodiversity assessment has been a traditional practice in ecology, particularly in tropical ecology, whereby scientists sample more or less indiscriminately certain environments, providing with thousands of specimens to museums and academic laboratories around the globe. In some cases, specimens are prepared and sorted, becoming amenable for identification and cataloguing when taxonomic expertise is available. However, most typically sorting reaches a relatively high taxonomic rank, too high for meaningful community analyses, and detailed biodiversity assessment stretches indefinitely in time, depending on the interest of experts and accessibility to these collections. Today, large-scale biodiversity assessment, particularly in the context of the race against the doom to extinction of many organisms, is intimately associated to what has been referred to as rapid biodiversity assessment, in other words, quickly collecting information on the species present in a given area (Oliver and Beattie 1993; Basset et al. 1998).

1.3. Molecular support to biodiversity assessment

A major boost in rapid and large-scale biodiversity assessment has been possible in the last two decades thanks to the routine implementation of molecular tools as a valuable standard to recognise diversity. The use of DNA for biodiversity assessment has provided with robust solutions for most of the challenges described above. This is a unique character system for all life forms, which is suitable for analysis with standard laboratory methods that require in turn very basic training. Thus, even modest laboratories can engage in the use of this technology for biodiversity assessment without imposing taxonomic restrictions, both in terms of scope and availability of previous knowledge, but also in terms of required taxonomic expertise (Tautz et al. 2003). Also helping

the routinely use of these approaches, the cost associated to DNA-based biodiversity assessment keeps dropping as the methods become more efficient and technology less exclusive (Yu et al. 2012). The basic laboratory steps in this procedure fundamentally require the use of DNA isolation techniques, on an individual or environmental basis (e.g., soil sample, residue of filtered sea water, ...), traditionally followed by PCR-based amplification protocols of specific genome regions, *a priori* defined standards for analysis, and finally sequencing of these markers. The process is facilitated because the latter stage can be handed to a profusion of biotechnology companies that offer sequencing services at very competitive prices. Moreover, the innovative boost of sequencing technologies of the past decade, methods collectively known as next-generation sequencing, all free from the limitations of Sanger technology, has facilitated the analysis of environmental samples and little by little displacing the need for an intermediate PCR step in some applications relevant for biodiversity assessment (Timmermans et al. 2010; Zhou et al. 2013; Andújar et al. 2015).

The use of these affordable, classical and revolutionary methodologies can potentially generate uncountable objective data for analysis, huge numbers of nucleotide characters in DNA sequences only limited by the size of the respective genomes involved, whose variability can inform of species diversity in a sample. While data can easily grow to vast amounts, these are nonetheless amenable for study even with modest computational power, given their suitability for large-scale information technology data storage and analyses. Thanks to the incorporation of molecular tools to the toolkit of taxonomists and ecologists, now the challenge and budgetary needs for biodiversity assessment are not anymore on the generation of raw data, but again on the acquisition of samples, on financing fieldwork and expeditions for biological prospection. There is still an important need for specialisation to some extent, in this case to use and develop methods to extract relevant information from collections of DNA sequences for sound biodiversity assessment. Large-scale biodiversity assessment thus rests on a new pillar as important as taxonomy and ecology: bioinformatics. The bioinformatics for biodiversity assessment has experienced an important development, receiving and exploiting the advances of more than half a century of numerical taxonomy and phylogenetics, but also the suitability of DNA sequence data for digital storage and the availability of an ever growing public database for DNA data generated worldwide.

1.4. Large-scale DNA-based biodiversity assessment

There are several ways to approach the use of DNA sequences for objective species delimitation and/or identification, but they can be divided fundamentally in two main categories. The first type of approach takes advantage of the easiness for computation of differences among DNA sequences and the assumption of a relatively uniform divergence threshold between intraspecific and interspecific DNA sequence variation. These numerical or phenetic approaches to biodiversity assessment evaluate the match of a sequence of unknown origin against comparable sequence information in a refer-

ence database (e.g., via BLAST algorithms; Altschul et al. 1997), or take advantage of more or less sophisticated clustering algorithms to facilitate taxonomic assignment. The most successful initiative following this strategy is the so-called DNA-barcoding (Hebert et al. 2003), which puts the emphasis on species identification. The second type of approaches aims at extracting evolutionary, phylogenetic information from matrices of homologous DNA-sequences to guide species inference (e.g., Wiens and Penkrot 2002). In this case, there are no implicit divergence thresholds, but there is a strong bearing on the concept of monophyly and inference of processes related to the species problem, e.g. gene flow, recombination, incomplete lineage sorting or hybridisation, among others. This field has flourished in the past few years thanks to advances in two areas of research. One is integrative taxonomy (Dayrat 2005; Schlick-Steiner 2010; Andújar et al. 2014), which tries to formalise the procedures to manage multiple sources of data, with a predominant role of molecular data, in defining (and discovering) species. The other encompasses the conceptual and methodological progress on procedures collectively known as species-trees methods, which use coalescence theory to incorporate discordance among multiple gene trees and predict species boundaries (Yang and Ranala 2010; Fujita et al. 2012). In general, phylogenetic methods have found a better use for problems related to species delimitation.

Phenetic approaches are particularly well suited for large-scale biodiversity assessment by virtue of straightforwardness and speed of analysis. However, they have some drawbacks as well. Their hypothetical optimal performance is achieved when there is a complete reference library available for comparisons (Ekrem et al. 2007), and a consistent barcoding gap or species-diagnostic behaviour of the marker of choice (Meyer and Paulay 2005). These criteria may be met for specific groups, but they are not universal. The quality and coverage of reference libraries can improve over time as new data enter the system, but there is a limitation imposed by the Taxonomic Impediment itself in providing solid taxonomies attached to the reference sequences, not to mention the fundamental problem of incompleteness of the inventory of Life. In any case, reliance on a static barcoding gap will always represent a problem, since this is not a universal, intrinsic property of species and DNA data (Meyer and Paulay 2005; Meier et al. 2006). Indeed, some alternatives exist to customise the concept of species thresholds, such as the ABGD method (Puillandre et al. 2012), but there will be always problematic groups for this criterion, e.g. species that hybridise, recent speciation events, convergence and evolutionary stasis or lineage-specific differences in evolutionary rates for the marker of choice. Moreover, taxonomic gaps in the reference library and exceptions to the barcoding gap do not prevent these approaches from producing species inferences even in the absence of true conspecifics in the reference database; these are known as false positives, and constitute one of their most serious limitations (Ross et al. 2008).

In turn, phylogenetic approaches are powerful and can assist both species delimitation and identification when used with a reference. In this case, even if the reference library does not include conspecific data, phylogenetic inference protects against false positives at the expense of taxonomic resolution (Ross et al. 2008; Berger et al. 2011).

Phylogenetic theory and practice have pushed dramatic advances in speed of analysis, both with more efficient and faster algorithms and a better use of computing capacities with parallelisation of complex calculations. However, these methodologies tend to be complex analytically, intense computationally and generally benefit from studying multiple markers, therefore are slower, less intuitive and need more training than their phenetic counterpart. Moreover, the performance of phylogenetic inference varies depending on the markers and underlying assumptions, which advises against blind attempts to conduct biodiversity assessment, without a way to evaluate systematically the robustness of the phylogenetic trees.

Clearly, DNA-based biodiversity assessment in the context of large-scale studies, can benefit of tree-based approaches taken from the field of molecular systematics, but it also requires speed of analysis. Specifically related to the problem of species identification, bacterial molecular taxonomy and current efforts to characterise microbiotas in multiple environments (e.g., Human Microbiome Project or TerraGenome) have built upon this tree-based concept for many years now. Thus, in this field, researchers exploit fast maximum likelihood phylogenetic analyses of query prokaryote 16S sequences against curated taxonomic references for this marker, e.g. workbench of Greengenes, SILVA and others (McDonald et al. 2012). Inspired by the philosophy of bacterial taxonomy, we have recently developed an analogous strategy for any kind of organism adding flexibility for the marker of choice by exploiting real-time taxonomically-tagged sequence availability in public nucleotide sequence databases [see section 2.3], the so-called BAGpipe protocol originally applied to angiosperm identification based on *psbA-trnH* data (Papadopoulou et al. 2015).

2. The contribution of Chrysomelidae to a diverse world

2.1. Leaf beetle communities matter to large-scale biodiversity assessment

The field of conservation biology has relied on bioindicators to monitor the quality of the environment (Noss 1990; Caro and O'Doherty 1999). Rather than attempting massive biodiversity studies on particular environments, perhaps a sound way to enhance biodiversity assessment could find inspiration in the notion of indicators, assessing the biodiversity of certain communities both in terms of taxonomic diversity and their species interactions. The focus would be on a highly diverse group of organisms in a given environment with a range of diverse but representative ecological interactions. Biodiversity assessment on such a group would serve as scaffold to anchor successive complementary studies above and below that particular interaction level, aiming with time at a multitrophic level description of the whole system. In this respect, for terrestrial ecosystems, ubiquitous herbivore insects constitute an excellent focal group to launch large-scale enquiry on the biodiversity and interactions of biomes (Stork and Habel 2014). In our opinion, phytophagous beetles, and leaf beetles in particular, represent a study system with important advantages. Their taxonomic diversity and that

of their food-plants can be staggering in any given tropical environment (Erwin 1982; Wagner 1999; Novotny and Miller 2014), in general they portray a tight ecological relationship with plants in all life cycle stages, and have high endemicity rates, both factors generating a perception of strong relationship with the environment. All in all, by focusing on the inventory and interactions of leaf beetles, it is possible to design research simultaneously on two highly diverse components (=indicators) of biota from most tropical ecosystems—insects and plants—, as well as on one of the predominant ecological interactions, herbivory (Price 2002).

Over the past few years (since 2007) we have thus developed on the notion that we can significantly contribute to an enhancement of biodiversity studies by targeting the fast characterisation of complex leaf beetle (or other herbivore insects) communities in the tropics as well as their ecological associations by using a combination of DNA-barcodes, tree-based species delimitation and forensic characterisation of food plants, with a robust and automatable analytical set-up. As a general proposition, we advocate that, when attempting large-scale biodiversity studies, where both delimitation and identification of species represent a challenge, the most efficient approach involves the use of DNA sequence data (only one or few 'barcodes') and phylogenetic approaches. Thus, our general workflow for large-scale biodiversity assessment of tropical leaf beetle communities includes four distinctive stages: (1) indiscriminate sampling of chrysomelid beetles in a particular environment or region; (2) non-destructive DNA extractions and specimen preparation for future reference; (3) DNA sequencing of at least one beetle mtDNA marker (typically *cox1*) and at least one putative diet marker (either *trnL* or *psbA-trnH*); and (4) phylogenetic inference for beetle species delimitation and host-plant identification.

2.2. Species delimitation and enhanced species discovery

We mentioned above that DNA-enhanced species delimitation has achieved fundamental progress over the past few years in great part thanks to the development of powerful phylogenetic methodologies to deal with gene tree incongruence as well as conceptual advancement on how to integrate taxonomically relevant data. However, these procedures are time and resource consuming, benefiting from the analysis of multiple genes and generally from a good taxonomic knowledge of the group of interest. These tree-based procedures find a good use in systematic research but are impractical for large-scale, rapid biodiversity assessment. Instead, our methods of choice, with a good trade-off between economy and speed of analysis (including data acquisition) and robustness and accuracy of results are the Generalized Mixed Yule-Coalescent model (GMYC; Pons et al. 2006; Fujisawa and Barraclough 2013) and the Bayesian implementation of the Poisson tree processes model for species delimitation (bPTP; Zhang et al. 2013). These are tree-based methods that do not require previous knowledge of species boundaries, making them suitable for the analysis of groups with poor taxonomy, and are specifically designed to work with single locus data (e.g., a DNA-barcode).

For example, GMYC tests changes in branching rates at the species boundary on an ultrametric tree based on the optimisation of a likelihood function with predictions for branching patterns both in speciation and population neutral coalescent processes. In practice, the algorithm scans two types of information on gene trees—waiting times between successive branching events and number of lineages within each interval—to optimise a single or multiple thresholds defining species branches on the tree subtending one or more populations evolving under neutral coalescent diversification processes. bPTP in turn relaxes the need for an ultrametric tree and infers species boundaries based on the so-called Poisson tree processes model (Zhang et al. 2013). Focusing on a single standard DNA-barcode lowers the cost and increases the speed and robustness of data acquisition, and both algorithms are fast and accessible thanks to functions of the R package 'splits' (SPecies LImits by Threshold Statistics; Ezard et al. 2013) in the case of GMYC, and a fully functional web server (http://species.h-its.org/ptp/) in the case of bPTP, both desirable characteristics for rapid biodiversity assessment.

The suitability of this approach to investigate well-known leaf beetle communities in temperate regions has been shown recently (Baselga et al. 2015). In addition, we are successfully applying it to several projects studying leaf beetle biodiversity at large in different tropical systems for which there is a deficient taxonomic knowledge on the composition of their respective leaf beetle communities. One such study focuses on the diversity of Eumolpinae in New Caledonia, a group that recent taxonomic work has exposed as highly diverse without a precise estimate of the expected total diversity (Gómez-Zurita 2011; Papadopoulou et al. 2013). In other studies we investigate the communities of leaf beetles in dry tropical forests of Nicaragua and Vietnam with a common aim of evaluating biodiversity parameters that can be eventually used for conservation initiatives targeting this highly threatened tropical biome (Janzen 1988; Miles et al. 2006). In these studies, we sampled hundreds of leaf beetle specimens which were individually characterised for one mtDNA standard locus, an 830 bp fragment of the 3'-end of the first subunit of the cytochrome *c* oxidase (*cox1*), and an additional mtDNA locus in the case of New Caledonian Eumolpinae (a 515 bp fragment of the small rRNA subunit, *rrnS*). In every case, the individuals characterised from a genetic viewpoint were preserved and mounted dry, with their genitalia dissected. Vouchering specimens from such large-scale biodiversity studies is essential for fulfilling the inventorying angle of biodiversity assessment, particularly when the lack of readily available taxonomic expertise or the weak taxonomic knowledge of the focal group, hampers the immediate naming of species. The amount of new species for Science in understudied tropical faunas can be high, and subsequent in-depth taxonomic work to name species usually reveals undescribed diversity. As will be seen below, the non-destructive treatment of samples is crucial to allow for species descriptions and instantly provides with standard type material (besides the DNA sequences used to speed up their discovery). Preparation of our processed specimens has yet another short-term practical advantage, which is allowing for a fast complementary assessment of species diversity based on the concept of morphospecies, i.e. groups of individuals that look alike. A comparison between the two pragmatic strategies for rapid species

assessment, DNA-based GMYC-groups *versus* morphospecies, can assist in the evaluation of performance of the first, objective method (Papadopoulou et al. 2013), as well as the discovery of new species, while drawing attention to interesting biological characteristics of the system, particularly if sample metadata is taken into account (e.g., geography, biome, host-plant information, etc.).

The systematic implementation of GMYC species delimitation to each of our datasets produced consistently species counts compatible with estimates based on morphospecies assessment (Table 1), and disagreements revealed in general a better performance of the molecular tree-based strategy. Essentially identical results have been shown and the same perception championed by Tänzler et al. (2011) based on their rapid-biodiversity assessment exercise centred on a single hyper-diverse weevil genus in New Guinea, *Trigonopterus*. Additionally, these authors formally explored a very interesting aspect of rapid species assessment that we also experienced from a pragmatic viewpoint, adding to the value of molecular approaches: DNA-based species delimitation outperforms sorting skills by trained, but non-expert parataxonomists. In our experience, there are always a few cases of morphospecies misplacements that benefit from reassessment *a posteriori* using phylogenetic information. These misplacements are not necessarily the result of real identification difficulties, but could be simply owing to visual memory limitations, when dealing with hundreds, perhaps thousands of specimens belonging to dozens or hundreds of species, in the context of massive sampling in tropical settings. Of course, DNA-based approaches have shown their strength in revealing hidden, cryptic diversity, externally invisible to expert eyes, let alone to rapid sorting for accelerated biodiversity inventories (e.g., *Astraptes*, Prado et al. 2011; Staphylinidae, Thormann et al. 2011). However, there is an additional important advantage of using DNA for species delimitation, somehow tackling the opposite scenario offered by cryptic diversity. This is the opportunity to sort accurately all life-stages (e.g., Ahrens et al. 2007), species with colour polymorphism (e.g., Rugman-Jones et al. 2013) or sexually dimorphic species (e.g., Smith and Brown 2008), i.e. situations that are challenging for morphospecies-based assessment of diversity, while they are rather common in insects, in particular in certain groups such as butterflies and many beetles, including the Chrysomelidae. Our research on tropical leaf beetle communities has

Table 1. Sampling and sequencing effort, and DNA-based species diversity estimates in three large-scale leaf beetle biodiversity studies in the tropics.

Study	N	Geographic scope	Longest transect	Taxonomic rank	DNA-barcode	GMYC species
New Caledonia	840	Grande Terre	400 km	Eumolpinae	*cox1, rrnS*	107 [94-121][a]
Nicaragua	1270	Pacific and northern provinces	250 km	Cassidinae, Eumolpinae, Galerucinae,	*cox1*	336 [333-347]
Vietnam	494	Núi Chúa Natl. Pk.	5 km	Chrysomelidae	*cox1*	161 [156-165][b]

[a]Averaged data from Papadopoulou et al. (2013).

[b]Taken and averaged from Nguyen and Gómez-Zurita (in prep.)

provided with examples for each of these advantages, matching larvae and adults of the chrysomeline *Plagiodera septemvittata* Stål in Vietnam (Nguyen and Gómez-Zurita, in prep.) or the cassidines *Coptocycla leprosa* (Boheman), *Omocerus caeruleopunctatus* (Boheman) and *Parorectis rugosa* (Boheman) in Nicaragua (Papadopoulou et al. 2015), the very distinctive males and females of several eumolpine species of *Taophila* Heller in New Caledonia (Papadopoulou et al. 2013; Gómez-Zurita and Cardoso 2014), and the highly polymorphic galerucine *Cerotoma atrofasciata* Jacoby in Nicaragua.

Once there is a sound estimate of species numbers resulting from a sampling effort of known intensity, it is possible to investigate how representative the measure of biodiversity is of the total expected diversity. For example, we used a strategy based on rarefaction curves representing accumulation of objectively delimited species across sampling events for New Caledonian Eumolpinae to extrapolate the expected total species richness in the studied environments. From our empirical demonstration of slightly over one hundred species in our ensemble sample, we could analytically propose an expected total number of eumolpine species in New Caledonia between 148 and 210, depending on input data and species richness estimator of choice (Papadopoulou et al. 2013). Preliminary data for three Chrysomelidae subfamilies sampled in Nicaraguan dry forests (Eumolpinae, Cassidinae s.l. and Galerucinae s.l.) or the whole Chrysomelidae community in a National Park in southern Vietnam, both analysed using a similar accumulation-curve approaches as in New Caledonia, reveal that our samples may represent between 53–69% of the total leaf beetle diversity in the studied biomes. Thus, a continued sampling effort should recognise in the order of 500-600 Chrysomelidae species in the abovementioned subfamilies in the dry Pacific side of Nicaragua, or the same number of chrysomelids in a 10 sq. km. forest patch across a slight elevation gradient in southern Vietnam.

The experience gained from this type of studies shows that the main limiting factor for robust diversity assessment is obtaining sampling densities representative of the studied environment always, i.e. fieldwork. Once samples are available, laboratory methods can be optimised in weeks or few months, depending on the number of samples used and smoothness of PCR protocols, and a similar or slightly longer time for standardised analytical procedures.

2.3. Forensic methods for the analysis of species interactions

We stressed already that there is one quantitative advantage of molecular characters to aid biodiversity assessment: speeding up the rate of species delimitation and also diagnosis. Additionally, these characters have at the same time the potential to contribute an extremely important qualitative advantage: the possibility to investigate complex systems and processed samples, which is the door to community ecology and the study of food-webs. In 2009, simultaneously with the studies of Valentini et al. (2009) and Soininen et al. (2009) and the earlier approach of Matheson et al. (2008), we pioneered the investigation of animal-plant interactions using DNA (Jurado-Rivera et al.

2009). In our approach, conversely to the mainstream DNA-barcoding stance of these contemporaneous and other subsequent studies, one of our main motivations was to extract taxonomically relevant information from processed food in the face of an incomplete reference database, by exploiting molecular phylogenies as the most rigorous and powerful tool for taxonomic assessment.

In most studies that target trophic associations, DNA extraction is directed to the most obvious sources for food DNA, including gut contents and faeces. In our case, and in great part motivated by the special characteristics of our study organism, the starting material is always the whole leaf beetle specimen, generally small enough to fit the tubes used for the DNA extraction procedure. The main idea is that when we obtain DNA from the whole specimen, we indeed mostly retrieve nucleic acids from the beetle species, useful for its genetic characterisation. However, with host DNA, we obtain simultaneously a significant proportion of DNA from organisms onto and into the beetle, therefore representing the ecological interactions it sustains, including DNA from all of its symbionts, endosymbionts, phoretics, commensals, parasites, hyperparasites and, of course, food remains. We refer to this condition as the *ecology inside a vial*. In recent years, we have been particularly interested in the analysis of the host trophic ecology, but the same samples are amenable to studies of different trophic levels (see Montagna et al. 2015, for a pioneering study on leaf beetle microbiomes, for instance).

PCR-based molecular characterisation of a predator's food can be challenging, particularly in the case of carnivorous animals, when their food can belong to a closely related taxon, requiring a selective procedure to distinguish (and avoid) template DNA from the host. In a DNA metabarcoding framework, this can be achieved by using primers specifically designed to target a specific taxonomic group of potential diets (e.g., Riaz et al. 2011). One such example is the use of insect-specific mtDNA PCR primers to identify insects preyed by spiders, which takes advantage of the high mtDNA divergence between these two taxonomic Classes, allowing for selective PCR (e.g., Northam et al. 2012; Sint et al. 2015). Yet, even large taxonomic gaps could result in non-trivial technicalities hampering the design of suitable primers. However, the same type of analysis to investigate the diet of a herbivore is much simpler methodologically, since Nature provides already with the best possible tool: plastid DNA (cpDNA), exclusive of plants, and together with ITS sequences, the marker of choice for DNA-based plant identification, as well as for plant DNA-barcoding (Kress et al. 2005). Botanical molecular systematic research has provided through the years with robust universal primers targeting a variety of cpDNA loci to assist plant species diagnosis. Among these, two loci in particular have been selected as the standard for plant DNA-barcoding, the tandem *rbcL* and *matK* (CBOL Plant Working Group 2009). These and other loci are generally easy to amplify with specific and reliable universal primers which are not interfered, by definition, by animal DNA; they produce PCR fragments of suitable size for easy amplification and sequencing; and their continued use by botanists determines a high taxonomic representation in nucleotide sequence databases, which makes them suitable for identification purposes. While

DNA-barcoding has favoured the use of length-invariant, protein coding loci, in our implementation for herbivore diet inferences, we have opted instead for length-variable cpDNA intergenic spacers, specifically the so-called *trnL* intron and most recently the *psbA-trnH* spacer (Jurado-Rivera et al. 2009; Gómez-Zurita and Cardoso 2014; Papadopoulou et al. 2015; De la Cadena et al., 2016). Sequence length differences are a nuisance for similarity assessment and genetic distance estimation and thus impair reliability of fast algorithms for taxonomic assignment. Yet, in our opinion, and specifically from a phylogenetic perspective, sequence length differences can be efficiently treated with current multiple sequence alignment algorithms, and provide with two main advantages: (1) they become an additional source of useful variation to increase the diagnostic value of these markers, and (2) size differences usually enable resolving homologous PCR products from different species by means of agarose gel electrophoresis, allowing to skip expensive and time-consuming cloning steps when studying the diet of leaf beetle individuals that fed upon two or more plant species.

We showed that this methodology is efficient and highly informative based on our extensive study of diets of Australian Chrysomelinae (Jurado-Rivera et al. 2009). In that study, we used *trnL* sequences obtained from whole specimen DNA extractions to infer the diet of 76 species in 24 genera of Chrysomelinae based on individual phylogenetic analyses carefully including all closely related homologous sequences available in GenBank at the time. In this proof-of-principle study, we were able to infer the correct host plant family in every case (for many species we had known host records), although resolution dropped at lower taxonomic levels (83% at tribal, and 51% at generic levels). Robust phylogenetic analyses provided a sound identification shortcut relying on information available in public sequence databases, and despite lower accuracy at infrafamilial taxonomic levels, we could refine our inferences, sometimes down to the species level, thanks to detailed floristic catalogues for the areas where the beetles had been collected. Yet public database incompleteness is a severe problem and inference power greatly benefits from availability of a local reference database for meaningful comparisons (e.g., García-Robledo et al. 2013). Indeed, since 2008 we have been working on setting a standard for this type of analyses whereby the analysis of the leaf beetle community goes hand-in-hand with a systematic compilation of angiosperm sequence data from the biome of interest to provide with a sound reference library for DNA-based inference of ecological associations. In the particular case of Nicaragua, we have sampled, vouchered, sequenced and made available to the scientific community *psbA-trnH* sequence data for some 450 plant species, nearly half of the plant diversity present in the Nicaraguan dry biomes, in an ongoing effort to enhance DNA-based species identification that we can use to characterise these valuable environments (Papadopoulou et al. 2015).

These approaches are becoming standard in many studies of tropical biodiversity, including studies on leaf beetles (Table 2) and other groups of phytophagous beetles, mainly weevils (Pinzón-Navarro et al. 2010; Kitson et al. 2013). But precisely in the context of large-scale and rapid biodiversity assessment, the generalisation of this type of studies is generating a new challenge. In our specific study of dry tropical forest

structure and interactions in Nicaragua, we have analysed some 840 individual leaf beetle specimens, which yielded nearly 1100 sequences of putative diets. Such a large amount of data is not anymore amenable to individualised tree-based inferences, and two alternatives stand out to scale-up accelerated biodiversity assessment: either giving up trees and using fast BLAST-based approaches or, alternatively, automating the inference process. Given our concerns about the unavoidable problem of incomplete reference databases, especially when working at a regional scale or above, we have opted for the latter. Automated taxonomic identification from multiple sequences can be efficiently tackled by splitting the data into phylogenetically robust datasets together with taxonomically-tagged homologs from GenBank and/or a local reference database of known taxonomy. Making this procedure fully automated meets two main challenges: one is extracting this meaningful subset of homologs and their taxonomically relevant information, and the other is parsing phylogenetic trees for taxonomic information. We have developed a dynamic procedure that solves these problems in efficient ways to iteratively generate tree-based taxonomic identifications from large collections of unidentified DNA-barcoding data, which we called BAGpipe ('Pipeline for Biodiversity Assessment using Genbank data'; Papadopoulou et al. 2015). Starting from a collection of sequence data of the selected genetic marker, the procedure uses a combination of local and global similarity searches to pick up all similar and putatively homologous sequences available in the latest Genbank release, recording their taxon ID and associated taxonomic hierarchy. At the same time, sequences are reoriented if needed, their ends trimmed to the length of the marker of choice, and redundant sequence data (i.e., population data) removed. These ensemble data constitutes the basis for subsequent phylogenetic matrix assemblage and phylogenetic inference, the so-called *reference database*. Robust phylogenetic inference is achieved for a certain level of sequence divergence where positional homology assessment is not compromised and homoplasy due to saturation is low (Goldman 1998; Yang 1998). Thus, we solved the problem of data partition for meaningful phylogenetic inferences by first splitting the unidentified *query sequences* in groups of similarity below a custom divergence threshold, each one used in turn to extract similar sequences from the reference database based on the same criterion. Query sequences and taxonomically identified reference database sequences within a predefined divergence threshold are submitted to multiple sequence alignment and maximum likelihood tree inference (and node support assessment). Automatically drawing taxonomic conclusions from trees was a challenge that we met exploiting the taxonomic hierarchy attached to Genbank data (inspired by Hunt and Vogler 2008; Chesters and Vogler 2013). The obtained unrooted trees are secondarily polarised and the most inclusive supported clades including unidentified query sequences are recognised, parsing the common taxonomy from reference sequences (i.e. Genbank taxon IDs and their hierarchy). This taxonomic inference, at the lowest taxonomic level allowed by the reference, is finally linked to the unidentified query sequence(s) using both strict and liberal criteria. In this context, it becomes obvious that coverage and reliability of available barcode reference libraries are critical for a meaningful use of this approach (Jinbo et al. 2011). A tool like BAGpipe

Table 2. Molecular analyses of insect-plant associations for tropical Chrysomelidae.

Leaf beetle	Source	cpDNA marker	Host-plant	Reference
Alagoasa decemguttata	Nicaragua	*psbA-trnH*	Verbenaceae, Bignoniaceae	De la Cadena et al. (2016)
Anadimonia sp.	Borneo	*rbcL*	Lauraceae, Dipterocarpaceae	Kishimoto-Yamada et al. (2013)
Arsipoda geographica	New Caledonia	*trnL*	*Ardisia* (Myrsinaceae)	Gómez-Zurita et al. (2010)
A. isola	New Caledonia	*trnL*	Ericaceae	Gómez-Zurita et al. (2010)
Blepharida suturalis	Nicaragua	*psbA-trnH*	Burseraceae, Boraginaceae	De la Cadena et al. (2016)
Brachycoryna pumila	Nicaragua	*psbA-trnH*	*Sida* and *Triumfetta* (Malvaceae), *Chiococca* (Rubiaceae)	Papadopoulou et al. (2015)
Calligrapha thermalis	Mexico	*psbA-trnH*	*Perymenium* (Asteraceae)	Montelongo and Gómez-Zurita (2013)
Cephaloleia spp.	Costa Rica	ITS2, *rbcL*	Heliconiaceae, Zingiberaceae, Costaceae, Marantaceae, Cannaceae	García-Robledo et al. (2013)
Chelobasis bicolor	Costa Rica	*rbcL*	*Heliconia* (Heliconiaceae)	García-Robledo et al. (2013)
C. perplexa	Costa Rica	*rbcL*	*Heliconia* (Heliconiaceae)	García-Robledo et al. (2013)
Dematochroma cancellata	New Caledonia	*psbA-trnH*	Primulaceae, Lamiaceae, Millettieae (Fabaceae), Cunoniaceae, *Syzygium* (Myrtaceae), Sapindoideae (Sapindaceae)	Gómez-Zurita and Cardoso (2014)
Glenidion sp.	Nicaragua	*psbA-trnH*	Burseraceae, Fabaceae, Lantaneae (Verbenaceae)	De la Cadena et al. (2016)
Heterispa vinula	Nicaragua	*psbA-trnH*	Malvaceae, Cucurbitaceae, Annonaceae, Poaceae, Boraginaceae	Papadopoulou et al. (2015)
Hyphaenia sp.	Borneo	*rbcL*	polyphagous (7 plant families)	Kishimoto-Yamada et al. (2013)
Liroetiella antennata	Borneo	*rbcL*	Acanthaceae, Fabaceae, Fagaceae, Moraceae	Kishimoto-Yamada et al. (2013)
Monolepta spp.	Borneo	*rbcL*	polyphagous	Kishimoto-Yamada et al. (2013)
Omophoita octomaculata	Nicaragua	*psbA-trnH*	*Stachytarpheta* (Verbenaceae), Lamiaceae	De la Cadena et al. (2016)
Parorectis rugosa	Nicaragua	*psbA-trnH*	*Physalis* and *Solanum* (Solanaceae), Lamiaceae, Cucurbitaceae, Scrophulariaceae, Fabaceae	Papadopoulou et al. (2015)
Physonota alutacea	Nicaragua	*psbA-trnH*	*Cordia* (Boraginaceae), Fabaceae	Papadopoulou et al. (2015); De la Cadena et al. (2016)
Platymela cephalotes	Australia	*trnL*	*Acacia* (Fabaceae)	Jurado-Rivera et al. (2009)

Syphrea sp.	Nicaragua	*psbA-trnH*	*Acalypha* (Euphorbiaceae)	De la Cadena et al. (2016)
Taophila (*Jolivetiana*) *mantillerii*	New Caledonia	*psbA-trnH*	Polypodiopsida	Gómez-Zurita and Cardoso (2014)
Taophila (*Lapita*) spp.	New Caledonia	*psbA-trnH*	Cyatheales, Fabaceae, *Syzygium* (Myrtaceae), Rauvolfioideae (Apocynaceae), Oxalidales, Sterculioideae (Malvaceae)	Gómez-Zurita and Cardoso (2014)
Taophila s. str. spp.	New Caledonia	*psbA-trnH*	Polypodiopsida, Primulaceae, Millettieae (Fabaceae)	Gómez-Zurita and Cardoso (2014)
Theopea sp.	Borneo	*rbcL*	polyphagous (10 plant families)	Kishimoto-Yamada et al. (2013)
Walterianella venustula	Nicaragua	*psbA-trnH*	Lamiaceae, *Buddleja* (Scrophulariaceae)	De la Cadena et al. (2016)

(http://www.ibe.upf-csic.es/SOFT/Softwareanddata.html) makes it possible to boost large-scale biodiversity assessment both in its inventorying angle, but also in the study of interactions if applied to the identification of ecology-in-a-vial associations or metabarcoding studies.

2.4. Simultaneous progress in inventory and interactions

From our previous account, it should be clear already that the use of DNA has the potential to enhance simultaneously the study of both species inventories and species interactions, by using a limited number of standard laboratory and analytical techniques. In molecular systematics research, it is routine to use the PCR technique and suitable sets of primers to amplify more than one molecular marker from each sample. These data combined inform on the organisation of diversity and can potentially hint at specific evolutionary processes that shaped this diversity. Based on this common practice, we have easily incorporated to the lab routine the characterisation of a plant cpDNA marker from leaf beetle DNA extractions, in addition to our standard beetle markers. As a result, we systematically add a new ecological dimension to the description of diversity. We described several new tropical leaf beetle species interpreting DNA differences with other known beetle taxa, providing also with a DNA-based diagnosis of plant species for putative diet sequences. These include a southern Nearctic Chrysomelinae, the Mexican *Calligrapha thermalis* Gómez-Zurita associated to the composite *Perymenium mendezii* (Montelongo and Gómez-Zurita 2013), two species of New Caledonian Alticinae in the genus *Arsipoda* with one of them associated to Myrsinaceae (Gómez-Zurita et al. 2010), and two species of the New Caledonian endemic genus *Taophila* (Eumolpinae) together with an assessment of their dietary breadth (Gómez-Zurita and Cardoso 2014).

The above examples do not fall of course in the category of large-scale biodiversity assessment, although at least in the particular case of the study on the genus *Taophila*,

it is a direct consequence, a refinement of findings derived from the wider biodiversity scope facilitated by this methodological approach (Papadopoulou et al. 2013). Nevertheless, each of these studies contributes individually to our understanding of tropical biodiversity and, if this strategy became the standard for systematic research in herbivore beetles, it would represent a fast progress in the complementary analysis of species and interactions. As seen, scaling-up this strategy for community analyses is feasible. Yet, we strongly believe that, even if some steps in species delimitation and identification are facilitated by the use of the described techniques, there will always be a dramatic need of taxonomic expertise to come full circle in any attempt for reliable biodiversity assessment.

3. Concluding remarks

As a short summary of our contribution, we can highlight that biodiversity is more than just species lists, and that biodiversity assessment should not neglect the way in which species are inter-connected in the ecosystems. Cataloguing biodiversity at large is certainly challenging, but it is also feasible, and DNA is possibly the key to fast and as comprehensive as possible inventorying of life forms, but also of their interactions. Phylogenies provide a robust approach to species delimitation and, in the absence of a comprehensive reference for comparison, the most robust approach to DNA-based species identification. Finally, the use of DNA as standard for species delimitation and identification makes these processes fully automatable, which is essential for high-throughput biodiversity assessment.

We tried to be constructive and discuss solutions to some of the current challenges in large-scale biodiversity assessment, however some fundamental problems remain and are not exclusively conditioned by technological or conceptual advancement. Rather, societal awareness (which is in great part our responsibility as professionals of biodiversity) and commitment of politicians and funding agencies alone can provide already a quantitative advantage for biodiversity research. As noted before, the emphasis for effective biodiversity research needs to be put again on funding expeditions and environmental sampling, pretty much with the same spirit as in the original voyages of discovery, but with the benefits of technology and trained specialists in different groups. Initiatives of this kind exist, most notably targeting insular systems, e.g. SANTO 2006, targeting the island of Espiritu Santo, the largest in the archipelago of Vanuatu (http://www.santo2006.org), or the Mo'orea Biocode Project, on the homonym island in the Tahiti archipelago (http://mooreabiocode.org). While these initiatives exceptionally mobilise millions of dollars and hundreds of scientists for comprehensive biological prospection, and are built with the right spirit, they typically yield a very modest global output. The reason is the currently existing bottleneck of available taxonomic expertise for extracting meaningful biodiversity information from these surveys, which remains the most serious challenge for large-scale biodiversity research (Kim and Byrne 2006). This towering limitation impairs not only the rigor-

ous assessment of biodiversity in classical ways, but also our chances to count with a reliable taxonomy attached to public sequence databases, one of the most valuable resources for improving and speeding-up biodiversity assessment. Again, this challenge can be in part solved by restoring the importance and value of taxonomic research and allocating resources to taxonomic training, coupled with commitment and pedagogy for and from taxonomists to reinforce and expand available expertise.

Besides these fundamental limitations, there are still others of technical and conceptual nature which need to be dealt with, such as devising creative and efficient ways to incorporate new technologies for the improvement of large-scale biodiversity assessment. These should include for instance the use of next-generation sequencing technologies and environmental metagenomics, or more specifically in the case of insects the recently developed 'metagenome skimming' approach (Andújar et al. 2015; Linard et al. 2015), which promises to transform the standards of DNA-based biodiversity assessment by eliminating the PCR step and associated biases. Additionally, new automated procedures are required to democratise both species delimitation and identification (through reliable publicly available references). Of course, as conveyed in this assay from the start, there must be also a dedicated effort to routinely integrate ideas of inventory and interactions in biodiversity surveys, with an ever larger and more integrative scope. These and many more ideas are in the agenda of biodiversity researchers, as evidenced by many international Biodiversity Initiatives throughout the world and at different scales, of which these with global scope are the paradigm for large-scale biodiversity assessment, e.g. Global Ocean Biodiversity Initiative (http://www.gobi.org), Center for Tropical Forest Science (http://www.ctfs.edu), or the Global Genome Biodiversity Network (http://data.ggbn.org/index.php), among others. These initiatives address some of their objectives by bringing genomics, taxonomy and ecology together through the combination of strategic sampling and massive sequencing technologies, when possible.

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RESEARCH ARTICLE

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Barcoding Chrysomelidae: a resource for taxonomy and biodiversity conservation in the Mediterranean Region

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Abstract

The Mediterranean Region is one of the world's biodiversity hot-spots, which is also characterized by high level of endemism. Approximately 2100 species of leaf beetle (Coleoptera; Chrysomelidae) are known from this area, a number that increases year after year and represents 5/6% of the known species. These features, associated with the urgent need to develop a DNA-based species identification approach for a broad spectrum of leaf beetle species, prompted us to develop a database of nucleotide sequences, with a solid taxonomic background, for all the Chrysomelidae Latreille, 1802 sensu latu inhabiting the Mediterranean region. The Mediterranean Chrysomelidae Barcoding project, which has started in 2009, involves more than fifty entomologists and molecular biologists from different European countries. Numerous collecting campaigns have been organized during the first seven years of the project, which led to the col-

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lection of more than 5000 leaf beetle specimens. In addition, during these collecting campaigns two new allochthonous species for Europe, namely *Ophraella communa* LeSage, 1986 and *Colasposoma dauricum* Mannerheim, 1849, were intercepted and some species new to science were discovered (e.g., *Pachybrachis sassii* Montagna, 2011 and *Pachybrachis holerorum* Montagna et al., 2013). DNA was extracted from 1006 specimens (~13% of the species inhabiting the Mediterranean region) and a total of 910 cox1 gene sequences were obtained (PCR amplification efficiency of 93.8%). Here we report the list of the barcoded subfamilies, genera and the number of species for which cox1 gene sequences were obtained; the metadata associated with each specimen and a list of problematic species for which marker amplification failed. In addition, the nucleotide divergence within and between species and genera was estimated and values of intraspecific nucleotide divergence greater than the average have been discussed. *Cryptocephalus quadripunctatus* G. A. Olivier, 1808, *Cryptocephalus rugicollis* G. A. Olivier, 1791 and *Exosoma lusitanicum* Linnaeus, 1767) are representatives of these cases.

Keywords
Leaf beetles, molecular taxonomy, DNA barcoding, Cytochrome c oxidase subunit 1, C-bar project

Introduction

In the last decades we have witnessed what has been defined as the "taxonomy impediment" (Rodman and Cody 2003) indicating the crisis in taxonomic studies due primarily to a shortage of time and taxonomists (Wheeler 2004, Wheeler et al. 2004, Wilson 2004), a situation that is made even more critical due to the decrease in the funding of natural history studies. The causes of the taxonomy crisis are many and complex, and a comprehensive analysis of this situation is beyond our purpose (see as example Boero 2001, Tautz et al. 2003). In our view, the causes can be described by the sentence …*a lack of prestige and resources that is crippling the continuing cataloguing of biodiversity* (Godfray 2002). If we consider the increased rate of species extinction (Thomas et al. 2004) amplified by climate change and habitat erosion due to exploitation by human beings the situation is worsened. A DNA-based strategy, which plays a central role in modern taxonomic studies, has been proposed by different authors as a methodology to overcome the identified problems (Tautz et al. 2002, Tautz et al. 2003, Hebert et al. 2004, Goldstein and DeSalle 2010) whilst maintaining the importance of a traditional approach mainly based on morphology. Interestingly, in a survey conducted among Coleopteran taxonomists, taxonomic initiatives based on DNA have been regarded of potential utility in solving the "taxonomy impediment", even if a few consider it absolutely useless (Löbl 2005). Currently, in the scientific world, an agreement on the correct approach to be adopted has not yet been reached. The "gold standard" for species identification studies based on molecular markers (e.g. mitochondrial cytochrome oxidase subunit I–cox1, or the nuclear small ribosomal subunit–SSU 18S rRNA) is to develop sequence databases used as a reference, beginning with DNA extracted from type and type series specimens preserved in Museum dry collections. The main problem with this strategy is related to the conservation status of the old dry specimens; 18th and 19th century specimens have fragmented DNA (not easily amplified through standard PCR approaches targeting

fragments of 500-700 bp) and are often infested by fungal hypha, which contaminate the insect's genomic DNA. Even with the advent of high-throughput sequencing technologies to solve the problem of fragmented sequences, the contamination due to fungal DNA remains. Developing strategies for the acquisition and storage of molecular data to address molecular taxonomy purposes, we face another problem, which affects the DNA sequences deposited in publicly available databases, i.e. the accuracy of specimen identification. In light of these issues, an alternative strategy has been adopted in the Mediterranean Chrysomelidae Barcoding project (C-Bar). The aim of the C-Bar project is to develop a reference database of cox1 gene sequences for all the Chrysomelidae (excluding Bruchinae Latreille, 1802), the Megalopodidae Latreille, 1802 and the Orsodacnidae Thomson, 1859 (hereafter indicated as Chrysomelidae or leaf beetles sensu latu – s. l.) inhabiting the Mediterranean region. The study area of C-Bar includes all the states that possess coastline on the Mediterranean Sea or territories characterized by Mediterranean-type habitat plus Romania and Switzerland (Figure 1). Starting from the Catalogue of Palaearctic Coleoptera (Löbl and Smetana 2010), about 2100 species of Chrysomelidae s. l. (corresponding to an estimated 5/6% of all described species) are present in this area. The Mediterranean Region is one of the world's biodiversity "hot-spots" (Myers et al. 2000, Cuttelod et al. 2008), which is characterized by exceptional concentrations of species with high levels of endemism that inhabit one of the most populated areas. The assumption of high levels of endemic species inhabiting the Mediterranean Region is also valid for leaf beetles (Biondi et al. 2013, Sassi 2006). Although the Mediterranean region has been the subject of investigation by generations of entomologists, knowledge of Chrysomelidae inhabiting this area is far from being fully known. The number of leaf beetle species new to science described from the Mediterranean region in the last decades, associated with the fact that they are widespread among different genera, confirms the need to increase the effort in biodiversity-based studies (e.g. *Cryptocephalus* O.F. Muller, 1764, *Chrysolina* Motschulsky, 1860, *Gonioctena* Motschulsky, 1860, *Longitarsus* Berthold, 1827, *Psylliodes* Berthold, 1827, *Colaspidea* Laporte de Castelnau, 1833; Bastazo 1997, Biondi 1997, Sassi 2001, Leonardi 2007, Daccordi and Ruffo 2005, Baviera 2007, Vela and Bastazo 2012, Zoia 2014).

In this project are involved taxonomists, specialized in different leaf beetle clades, in order to guarantee the accurate specimen identification. In our view, the adoption of this strategy is a way to bring together traditional (intended as based on morphology) and molecular taxonomy in order to tentatively overcome the "taxonomy impediment" (Rodman and Cody 2003).

The purpose of this paper is to report the preliminary results achieved during the first seven years of the project in order to show the potential of a cooperation between molecular biologists and traditional taxonomists. In particular, we report: *i*) the method adopted and issues arisen in the development of the sequence dataset; *ii*) the list of subfamilies, genera and the number of species for which cox1 gene sequences were obtained; *iii*) the metadata associated with the processed organisms; *iv*) mean values of intraspecific and interspecific nucleotide divergence *v*) the new species described and the important faunistic findings.

Figure 1. Area investigated by the Chrysomelidae Barcoding project. The countries in which were performed the collecting campaigns are reported in dark grey. The percentage of the total processed specimens is reported for each country.

Materials and methods

Specimen collection and identification

More than 50 entomologists, from different European Countries, have joined the C-Bar project and have actively participated in samples collection. During the first seven years of the project (from 2009 to 2015) numerous collecting campaigns were organized from March to September of each year. The specimens were collected using different methods: from the vegetation by sweep net or by beating sheet, and directly by hand in specific habitats (e.g. under stones or digging the host plant roots). All the collected specimens were placed in 5 ml vials filled with absolute ethanol in order to preserve the genomic DNA. Within an hour of specimen collection, the mixture in the vials was replaced with fresh absolute ethanol in order to obtain better sample dehydration and preservation for long-term storage. Each vial was preserved at -20°C and was labeled by a unique identifier plus other metadata related to the sampling locality (i.e. Country, Province, Region, exact site, latitude, longitude and elevation), the date of collection, the collector/s and other ecological information related to the specimens.

Specimen manipulation and dissection (when necessary) were completed with the auxiliary use of a stereomicroscope. Images of the specimen habitus were acquired by a reflex camera (Canon EOS 450D, macro objective 60 mm or 100 mm with a set of

macro extension tubes) or with Axiocam 506 mounted on Zeiss Axio Zoom V16. The specimens were morphologically identified by Italian taxonomists expert in different leaf beetle clades (most of them are listed among the authors of the present article). The nomenclature adopted in the C-bar project follows the work of Bouchard et al. (2011) at the levels of family and subfamily, while at the levels of genus and species was adopted the recently published Catalogue of Palaearctic Coleoptera–Chrysomeloidea (Löbl and Smetana 2010).

DNA extraction, PCRs and sequence quality control

DNA extraction was performed in two different ways since it took place in different laboratories (Biodiversity Institute of Ontario, University of Guelph and Department of Agricultural and Environmental Sciences, Università degli Studi di Milano): for 950 samples the DNA was extracted from one hind leg while for the 56 remaining samples the DNA was extracted from the whole specimen, after the removal of the abdomen. The latter procedure ensures to keep specimen morphology intact. In both cases, DNA was purified using the Qiagen DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). Here we describe the adopted non-destructive procedure: the specimen was taken off from absolute ethanol and dried in single 1.5 ml vials for 45 minutes at 30°C; after the removal of the abdomen with the use of sterile pins and tweezers the specimen was placed in 180 µL of ATL lysis buffer (Qiagen) with 200 ng/mL proteinase K (Sigma Aldrich, St. Louis, MO, USA) at 56°C for 12 hours. The following steps of the DNA extraction were performed according to the manufacturer's instructions of Qiagen DNeasy Blood and Tissue Kit. After DNA extraction, the specimens were dry mounted on pins together with genitalia and kept for future reference. A quote of the extracted DNA was preserved in the C-bar DNA library at -80°C for long term storage and a rate was preserved at -20° in order to perform the following amplifications. A fragment of 658 bp at the 5'-end of the mitochondrial cytochrome c oxidase subunit 1 gene (cox1) was amplified with primers LCO1490 5'-GGT CAA CAA ATC ATA AAG ATA TTG G / HCO2198 5'-TAA ACT TCA GGG TGA CCA AAA AAT CA (Folmer et al. 1994). When this pair of primers resulted in unsuccessful amplification of the target marker, other primers amplifying the same gene region were used, i.e. LepF1 5'-ATT CAA CCA ATC ATA AAG ATA TTG G / LepR1 5'-TAA ACT TCT GGA TGT CCA AAA AAT CA (Hebert et al. 2004). Successful amplifications were determined by gel electrophoresis. PCR products were directly sequenced on both strands using the marker-specific primers from ABI technology (Applied Biosystems, Foster City, CA, USA). The obtained sequences were edited using Geneious R8 (Biomatters Ltd., Auckland, New Zealand) and primers, pseudogenes and contaminations removed. Finally, they were deposited in the Bold Systems (Ratnasingham and Hebert 2007) and in the European Nucleotide Archive (Montagna et al. under revision).

Intraspecific and intrageneric nucleotide divergence

The obtained cox1 gene sequences were aligned at codon level using MUSCLE (Edgar 2004) with default parameters. A pairwise nucleotide distance matrix was estimated starting from the aligned sequences implementing the Kimura-two-parameter (K2P) model (Kimura 1980), considered as an adequate evolutionary nucleotide model when p-distances between sequences are low (Nei and Kumar 2000). The nucleotide distance matrix was used for the calculation of the mean intraspecific and interspecific nucleotide distances and for the calculation of mean intrageneric distance; these analyses were performed using the R package Spider (Brown et al. 2012). We also calculated nucleotide intraspecific distances for some species with a wide range of distribution.

Results and discussion

Until now, C-Bar collecting campaigns have investigated some areas of Bulgaria, France, Greece, Italy, Morocco, Romania, Spain, Switzerland, Turkey and Tunisia (Figure 1). The sampling efforts that have been accomplished until now led to the collection of more than 5000 Chrysomelidae specimens. During the identification process, some specimens of previously unknown species were recognized, these samples were used for the description of the following species: *Pachybrachis sassii* (Montagna 2011) from the Giglio Island in the Tuscan Archipelago; *Pachybrachis holerorum* (Montagna et al. 2013) from the Northern Apennines and *Oulema mauroi* Bezděk & Baselga, 2015, from Northen Italy. Other samples collected during the C-Bar collecting campaigns were used in a revision of Colaspidea genus that led to the description of seven new species (Zoia 2014). All these new taxa were formally described by a traditional morphological approach, in some cases molecular data were added to confirm the existence of the new species. Besides the discovery of new taxa, two allochthonous species new to Europe, namely *Ophraella communa* (Boriani et al. 2013) and *Colasposoma dauricum* (Montagna et al. in press), were intercepted. *O. communa* is a leaf beetle of Nearctic origin accidentally introduced in 1996 in Taiwan (Wang and Chiang 1998) and Japan (Takizawa et al. 1999); the species rapidly spread in East Asia and few years ago we intercepted it in the Northern part of Italy (Boriani et al. 2013). *C. dauricum* is a species originally present in the North and Central-East of Asia, it has never been observed out of its original range until our interception in 2011 in Piedmont (North of Italy).

Among the collected samples, the DNA was extracted from 1006 specimens and PCRs targeting a fragment of the cox1 gene performed. PCRs with the selected primer pairs lead to successful amplification in 93.8% of the cases (62 specimens failed the amplification). Among the specimens for which the amplification failed, 43 specimens belong to the subfamily Cryptocephalinae Gyllenhaal, 1813: 18 species of *Cryptocephalus* (40 specimens); interestingly cox1 sequences have never been obtained for *Cryptocephalus therondi* Franz, 1949, *Cryptocephalus cantabricus* Franz, 1958 and *Cryptocephalus etruscus* Sassi, 1995. We can hypothesize the presence of mutations in the

annealing region of the used primers. Sequences obtained from *Clytra laeviuscula* Ratzeburg, 1837, *Clytra quadripunctata* Linnaeus, 1758, *Cryptocephalus cristula* Dufour, 1843, *Cryptocephalus octoguttatus* Linnaeus, 1767, *Lachnaia tristigma* Lacordaire, 1848 and *Oomorphus concolor* Sturm, 1807 did not possess an open reading frame and were thus considered as nuclear pseudogenes. Twenty-seven sequences were discarded because of contamination from exogenous DNA. A total of 910 cox1 sequences (267 species corresponding to ~13% of those inhabiting the Mediterranean region) were obtained, the size of the sequences was > 400 bp in ~99% of the cases.

We observed that only two species, namely *Cryptocephalus violaceus* Laicharting, 1781 and *Cryptocephalus duplicatus* Suffrian, 1845, sharing the same haplotype can not be discriminated through DNA barcoding. In this and in similar cases a barcoding failure can be confirmed only ensuring the correct identification of the samples by expert taxonomists. Therefore 99.3% of the species (265) for which we obtained cox1 sequences possessed unique haplotypes, allowing their molecular identification. The mean intraspecific nucleotide distance value is of 2%, while the mean interspecific and intrageneric distances result of, respectively, 25.2% and of 19.8%. The obtained intraspecific value are higher than that inferred in a previous study on Coleoptera (Pentinsaari et al. 2014). This results might be the effect of geographical distances among localities of collection of co-specific specimens; a possible alternative explanation is the presence of cryptic species. Among the species showing high intraspecific nucleotide distance noteworthy are the cases of *Cryptocephalus rugicollis* (2.8% [0%, 5.5%]), *Exosoma lusitanicum* (6.7% [0.2%, 9.2%]) and *Cryptocephalus quadripunctatus* that shows a mean intraspecific distance (3% [0%, 4.9%]). To test the formulated hypotheses further analyses, including the use of other mitochondrial and nuclear markers as well as a wider sample of specimens, are required.

Among the nine subfamilies for which cox1 sequences were obtained (Table 1), Cryptocephalinae and Galerucinae Latreille, 1802 were better represented. In the first subfamily are listed 111 species (83 species of Cryptocephalini Gyllenhaal, 1813 and 28 of Clytrini Lacordaire, 1848, 426 specimens in total) while the second counts 88 species (24 species of Galerucini Latreille, 1802 and 64 of Alticini Spinola, 1844, 274 specimens in total). The unbalanced sampling towards Cryptocephalini, which in some way might affect the obtained results, could be explained by the fact that most of the C-bar specimens have been collected by Sassi and Montagna, which mainly work on this clade and are likely to have developed collecting strategies that increase their sampling (Figure 1).

The metadata related to the specimens (i.e., specimen identification, collection identifier, collecting date, state, province, exact site of collection, latitude, longitude, elevation and collector/s) from which cox1 gene sequences were obtained, are available in a web site dedicated to the project (http://www.c-bar.org). Regarding the specimens collected within Italian administrative boundaries the metadata associated with the specimens are also available in the Biodiversity Database and GIS platform of the Italian National Network of Biodiversity. These faunistic data are useful because increase the awareness of species presence and distribution in the sampled area.

Table 1. List of the barcoded subfamilies and genera with the number of species and specimens belonging to each taxon.

Subfamily	Genus	N_s^a	$^bN_{spec}$	N^b
Zeugophorinae Böving and Craighead, 1931	*Zeugophora* Kunze, 1818	1	1	1
Orsodacninae Thomson, 1859	*Orsodacne* Latreille, 1802	3	7	2.3
Donacinae Kirby, 1837	*Donacia* Fabricius, 1775	2	6	3
Criocerinae Latreille, 1804	*Crioceris* Muller, 1764	3	18	3
	Lilioceris Reitter, 1912	1		
	Lema Fabricius, 1798	1		
	Oulema Gozis, 1886	1		
Cassidinae Gyllenhal, 1813	*Cassida* Linnaeus, 1758	14	61	3.4
	Hypocassida Weise, 1893	2		
	Hispa Linnaeus, 1767	1		
	Dicladispa Gestro, 1897	1		
Chrysomelinae Latreille, 1802	*Chrysolina* Motschulsky, 1860	13	117	3.4
	Chrysomela Linnaeus, 1758	3		
	Entomoscelis Chevrolat, 1836	1		
	Gastrophysa Chevrolat, 1836	1		
	Gonioctena Motschulsky, 1860	3		
	Oreina Chevrolat, 1836	6		
	Plagiosterna Motschulsky, 1860	1		
	Phratora Chevrolat, 1836	1		
	Plagiodera Chevrolat, 1836	1		
	Prasocuris Latreille, 1802	1		
	Timarcha Latreille, 1829	3		
Galerucinae Latreille, 1802	*Agelastica* Chevrolat, 1836	1	274	3.1
	Arima Chapuis, 1875	1		
	Calomicrus Stephens, 1831	3		
	Exosoma Jacoby, 1903	2		
	Diabrotica Chevrolat, 1836	1		
	Galeruca Geoffroy, 1762	5		
	Galerucella Crotch, 1873	3		
	Lochmaea Weise, 1883	2		
	Luperus Geoffroy, 1762	6		
	Nymphius Weise, 1900	2		
	Sermylassa Reitter, 1913	1		
	Altica Muller, 1764	4		
	Aphthona Chevrolat, 1842	6		
	Argopus Fischer von Waldheim, 1824	1		
	Arrhenocoela Foudras, 1860	1		
	Chaetocnema Stephens, 1831	2		
	Crepidodera Chevrolat, 1836	5		
	Derocrepis Weise, 1886	2		
	Dibolia Latreille, 1829	2		
	Epitrix Foudras, 1860	1		
	Hermaeophaga Foudras, 1860	1		
	Hippuriphila Foudras, 1860	1		

Subfamily	Genus	N_s^a	$^bN_{spec}$	N^b
	Longitarsus Berthold, 1827	9		
	Lythraria Bedel, 1897	1		
	Neocrepidodera Heikertinger, 1911	6		
	Phyllotreta Chevrolat, 1836	4		
	Podagrica Chevrolat, 1836	1		
	Psylliodes Berthold, 1827	12		
	Sphaeroderma Stephens, 1831	2		
Cryptocephalinae Gyllenhal, 1813	*Cryptocephalus* Geoffroy, 1762	73	426	3.8
	Pachybarchis Chevrolat, 1836	8		
	Stylosomus Suffrian, 1848	2		
	Clytra Laicharting, 1781	4		
	Coptocephala Chevrolat, 1836	3		
	Labidostomis Chevrolat, 1836	10		
	Lachnaia Chevrolat, 1836	3		
	Macrolenes Chevrolat, 1836	1		
	Smaragdina Chevrolat, 1836	7		
	Tituboea Lacordaire, 1848	1		
Eumolpinae Hope, 1840	*Chrysochus* Chevrolat, 1836	1	5	1.7
	Colaspidea Laporte de Castelnau, 1833	1		
	Macrocoma Chapuis, 1874	1		

[a]N_s indicates the number of barcoded species; [b]N_{spec} and N indicates respectively the total number and the average number of barcoded specimens belonging to each subfamily

Conclusion

In this paper, we report that C-Bar project, besides having produced useful data for molecular taxonomy (cox1 sequences were obtained for about 13% of the species inhabiting the investigated area), has obtained important results also from the viewpoint of the classical taxonomy leading to the morphological description of same new species of Chrysomelidae. A further important achievement has been the interception of allochthonous species. These results have been obtained only thanks to the cooperation amongst the taxonomists specialized in different leaf beetle clades, which have ensured the correct identification of samples, the people involved in the extensive collecting campaigns and the molecular biologists.

The promising preliminary results that have been obtained encourage us to continue with this project since they strongly confirm the urgent need to increase the efforts in faunistic studies to uncover the real biodiversity of leaf beetles inhabiting the Mediterranean region. For these reasons, we are confident that the aim of C-bar project of developing a repository of cox1 sequences for the majority of the species of Chrysomelidae s. l. inhabiting the Mediterranean region may be achieved in the near future.

In conclusion, as demonstrated by the relevant results obtained during the first years of the project, we believe that DNA barcoding projects, when developed with the participation of taxonomists and molecular biologists, represent an opportunity

to bring together two different worlds and may be considered the driving force able to revive interest in what can be regarded as the milestone of biological studies that is a-taxonomy, helping to fill the "taxonomy impediment".

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Natural history of *Javeta pallida* Baly, 1858 on *Phoenix* palms in India (Chrysomelidae, Cassidinae, Coelaenomenoderini)

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Abstract

Members of the Old World hispine tribe, Coelaenomenoderini, are documented on host plants of Arecaceae, Cyperaceae, and Zingiberales. A few species are renowned pests of oil palm, especially in Africa. The host plants and natural history of *Javeta pallida* Baly, 1858, the only Indian species of the tribe, is reported for the first time. These beetles can densely infest indigenous wild date palms, *Phoenix sylvestris* (L.) Roxb. (Arecaceae), and also use the introduced date palm, *Phoenix dactylifera* L., which is an expanding crop in India. *Javeta* females lay single eggs and cover each with an ootheca. All larval stages mine the leaves and pupation occurs within the larval mine. Adults are exophagous, leaving linear feeding trenches. Natural and induced infestations of *J. pallida* on these two palms were observed and the potential of *J. pallida* as a pest of date palm in India is discussed. *Javeta pallida* completed development on *Phoenix* palms in 52–88 days (mean 66.38 days) with egg period 11–15 days (mean 12.8 days), larval period 21–54 days (mean 33.02 days) and pupal period 17–23 days (mean 20.52 days). *Elasmus longiventris* Verma and Hayat and *Pediobius imbreus* Walker (Hymenoptera: Eulophidae) parasitize the larva and pupa of *J. pallida*.

Keywords

Leaf beetles, leaf miner, pest, Arecaceae, Eulophidae, oil palm

Introduction

The palm genus *Phoenix* L. (Arecaceae: Phoeniceae) comprises 15 species which are grown as ornamentals and for food and beverage. The sweet fruit of several species are eaten and sap is tapped to make various fermented drinks and vinegar. Nine *Phoenix* species occur in southern Asia (Henderson 2009; Govaerts et al. 2015). *Phoenix sylvestris* (L.) Roxb., the silver date palm, the wild date palm or the date sugar palm, is a medium-sized palm with solitary stems up to 20 m in height (Fig. 1) (Henderson 2009). According to Krishnamurthi et al. (1969), about 29 million palms of *P. sylvestris* exist in India; they summarized its biology, cultivation practices and myriad local uses in the encyclopedic Wealth of India Series. Banerji (2012) discussed the wild date palm and the near-mythical status of the palm sugar in Bengali gastronomy (West Bengal state in India and the adjoining area of Bangladesh that form the erstwhile Bengal). Thirteen species of insect herbivores have been documented on *P. sylvestris* (Mathur and Singh 1961; Howard et al. 2001).

The date palm, *Phoenix dactylifera* L. is one of the first cultivated tree crops, being grown since early Bronze Age (late 4th/early 3rd millenia B.C.) (Tengberg 2012). Date palm is commercially grown in Gujarat and Rajasthan in India (Radha and Mathew 2007). Despite the popularity of its fruit, date palm is not cultivated in Kerala, India (where the outbreak of the insect was noticed), due to unfavorable climatic conditions. Stray seedlings, which germinate from the seeds discarded after eating the flesh, are rarely observed in Kerala. Carpenter and Elmer (1978) reviewed pests and diseases of *P. dactylifera* globally. In India, about 21 insect pests are associated with the species (Mathur et al. 1958; Mathur and Singh 1961; Wadhi and Batra 1964; Batra 1972; Bindra and Varma 1972; Sohi and Batra 1972; Batra and Sohi 1974; Sachan 1976; Muralidharan 1993; Radha and Mathew 2007).

The Old World "hispine" tribe Coelaenomenoderini comprises nine genera and 88 species (Gressitt and Kimoto 1963; Gressitt and Samuelson 1990; Würmli 1975; Staines 2012b). The limited data indicates Arecaceae, Cyperaceae, Pandanales and Zingiberales as host plants (Staines 2004, 2012b). Juvenile stages (larva or pupa) are known for just two species—*Coelaenomenodera* (*Coelaenomenodera*) *elaeidis* Maulik (Maulik 1920; Cox 1988, 1994) and *Cyperispa hyloytri* Gressitt (Cox 1996).

Some species are pests of oil palm, *Elaeis guineensis* Jacq. (Rajagopalan and Alderungboye 1970; Calvez 1976; Godfray and Chan 1990; Mariau and associates 1972–2004; Cochard et al. 2005). *Coelaenomenodera* Maulik is by far the best-known genus because three species are significant pests of oil palm in Africa and have received much research attention, especially by the French agro-entomologist, Dominique Mariau. Mariau and colleagues intensely studied *C.* (*C.*) *elaeidis* Maulik for over 10 years as it was considered the most important pest of oil palm in West Africa (Morin and Mariau 1970). Due to the mining behavior, palm leaflets are severely damaged and produce lower yields (Ruer 1964) by as much as a 30% reduction (Simmonds 1970). The biology, life cycle and enemy complex are well-documented for *C.* (*C.*) *elaeidis* (see Maulik 1920; Cotterell 1925; Waterston 1925; Cachan 1957; Morin and Mariau 1970, 1971, 1974; Mariau and Morin 1971, 1972, 1974; Mariau 1976, 1999; Mariau et al. 1978; Bernon and

Figures 1, 2. *Phoenix* palms in India. **1** Naturally growing *P. sylvestris* in Mount Abu, Rajasthan **2** *Javeta pallida* infested *P. sylvestris*, Tirurangadi, Kerala.

Graves 1979; Philippe et al. 1979; Mariau and Philippe 1983; Philippe 1990; Timti 1991; Mariau et al. 1999a), *Coelaenomenodera* (*Coelaenomenodera*) *lameensis* Berti and Mariau (see Berti and Mariau 1999; Mariau and Lecoustre 2000, 2004; Mariau 2001), *Coelaenomenodera* (*Coelaenomenodera*) *perrieri* Fairmaire (Mariau 1988, 2001; Lecoustre et al. 1980), and *Coelaenomenodera* (*Coelaenomenodera*) *speciosa* Gestro (Uhmann 1961; Santiago-Blay 2004). These provide a model for research on other coelaenomenoderine species which might pose pests of economically-valuable palms.

Javeta pallida Baly, 1858, the type species of the genus, is the only species of Coelaenomenoderini known from India (Maulik 1919). *Javeta* Baly, 1858 comprises 19 species found in Asia (Staines 2012a, b). The biology of *Javeta* is poorly known but records indicate host associations of three species with Arecaceae (Jolivet 1989; Jolivet and Hawkeswood 1995; Santiago-Blay 2004)—*Javeta arecae* Uhmann, 1943 on *Areca catechu* (Uhmann, 1943) and *Areca* sp. (pinang; Kalshoven 1981); *Javeta corporaali* Weise, 1924 on *Pinanga kuhlii* Blume (Uhmann 1955); and *Javeta thoracica* Uhmann, 1955 on *Areca* sp. (Uhmann 1955) and *Metroxylon* sp. (Kalshoven 1957). Steiner (2001) listed undetermined species of *Javeta* amongst the insects associated with the rattan palms, *Daemonorops hirsuta* Blume and *Calamus manan* Miq. (Arecaceae: Calameae). Data on *Javeta* juvenile stages is limited to the described pupa of *J. corporaali* by Uhmann (1955) and the mining larva of *J. arecae* (Kalshoven, 1981). The only information on *Javeta* life history is a short remark by Kalshoven (1951: 759; 1981: 456) about *J. arecae*, reported from an outbreak in Sumatra: "The larvae make long mines in the leaves and feeding by the beetles produces brown stripes". No information is available on the egg, oviposition and pupation sites.

The goal of this paper is to report the host plants and natural history of *J. pallida* for the first time, taking advantage of a heavy infestation on *Phoenix sylvestris* in southern India (Fig. 2). *Javeta pallida* was originally described from the Nilgiri Hills, southern India, and is known today to extend to West Bengal and Uttar Pradesh in north India (Basu 1999). Our discovery of the heavy infestation has implications for the cultivation

of two regional palm food resources, both the indigenous local host and the date palm, *P. dactylifera* introduced to India. Thus, the propensity of coelaenomenoderine species to become significant pests of major palm crops in tropical countries and the lack of information on the biology of *J. pallida* motivates this research contribution. We use natural populations and transfer experiments to: 1) study the life cycle and assemble a specimen collection for morphological study, 2) explore the potential of *J. pallida* to become a pest of the date palm in India, and 3) compare beetle development on the two hosts.

Material and methods

The study is based on field observations of live populations of *J. pallida* at Malappuram District, north Kerala, India, led by authors KMS, PKD, and MN. To document the life cycle and biology, beetles were reared on date palm, *P. dactylifera* and on the wild date palm, *P. sylvestris.*

Field sites

(i) The initial infestation of *J. pallida* was observed on three stray palms of *P. sylvestris* during December, 2014 (Fig. 2). The plants are ornamentals in a 30 m wide "garden" between a concrete building and a road joining the National Highway 17 at Tirurangadi (N11°02'12.0", E75°56'12.6", 47m above msl).
(ii) Remnants of natural infestation was observed on a stray date palm of about ten years old at Tirurangadi (N11°02'17.24", E75°55'40.61", 35m above msl) in April, 2015.
(iii) Rearing of *J. pallida* on *P. dactylifera* was carried out at Tirurangadi (N11°02'31.60", E75°55'8.72", 23m above msl) on a three-year old stray date palm.
(iv) Rearing of *J. pallida* on *P. sylvestris* was carried out at the Botanical Garden of the Calicut University, Kerala (N11°07'59.01", E75°53'22.83", 77m above msl) on a 10–12 year old, 2.25m tall palm (excluding crown).

Rearing of *J. pallida* on *P. dactylifera*

Dry season. Nine adults were released and confined with pieces of nylon net (mesh size 0.701mm–0.827 x 0.628–0.686mm; Nylon Maharani Net http://www.indiamart.com/goldfinchcreators/fabrics.html) on a frond on 23.IV 2015 for two days. On a second frond of the same palm, five adults were confined on 27.IV 2015 and a sixth adult was added on 30.IV.2015. The beetles were maintained on the frond until 3.V.2015.

Rainy season. During the rainy season, seven adults were confined on a third frond of the same palm of *P. dactylifera* on 10.VI.2015 and five more were added on the next day. All of them were retained on the frond till the eighth day. On a fourth frond, seven adults were confined on 17.VI.2015, and were retained till 20.VI.2015.

Rearing of *J. pallida* on *P. sylvestris*

Rearing was carried out only during rainy season on *P. sylvestris*. Three young fronds were selected and 12 adults were used in the study. On the first frond, 12 adults were confined with nylon net for five days from 10.VI.2015. The same adults were shifted to the second frond on 15.VI.2015 and confined for two days. They were again shifted to a third frond on 17.VI.2015 and confined on it for a single day.

All adults used in rearing experiments were collected from the wild population of *J. pallida* on *P. sylvestris* at the first field site in Tirurangadi.

Individual eggs were counted and marked on the leaflets every day and the development was followed through larva and pupa till the emergence of adult. Developmental periods such as egg, larval and pupal duration of all individuals, which could be tracked, were recorded. The date of hatching of the eggs was determined by observing the beginning of the leaf mine (Fig. 5). Similarly the end of the larval period was determined by observing cessation of feeding followed by the withdrawal of the mature larva from the leading end of the mine. After pupation, the leaflets holding the pupa in larval mine, were removed from the leaf rachis and were placed individually inside the bottles for emergence of adults.

Mean values of developmental periods of individuals reared during dry season (eggs laid in April, 2015) and rainy season (eggs laid in June, 2015) on *P. dactylifera* were compared using t-test of significance (Panse and Sukhateme 1985). Similarly, the developmental periods of individuals reared during rainy season on *P. dactylifera* and *P. sylvestris* were compared using the same tool to find out possible statistical difference in developmental periods on the two host species of *Phoenix*.

Visits to commercial plantations. Two visits, during January and April, 2015, were made to the commercial plantations of date palms in Dharmapuri, Tamil Nadu, southern India.

Collection of natural enemies. Naturally infested leaves of *P. sylvestris* from the first field site were brought to the laboratory and kept in plastic containers of about 5 L capacity for emergence of adult parasitoids.

Specimen collection. A total of 173 adults, 81 pupae, 41 larvae, and nine eggs were collected at Tirurangadi on 12, 14, and 28.XII.2014 (KMS and KDP); one adult was collected at Jakkur Lake, Bangalore on 12.VIII.2012 (KDP), and eight adults were collected on 9–11.XI.2014 at Bangalore (H. M. Yeshwanth) on *P. sylvestris*. Voucher specimens of *J. pallida* are deposited in the Kansas Natural History Museum, KS, USA, National Bureau of Agriculturally Important Insects, Bangalore, and the Travancore Insect Collection, Kerala Agricultural University, Vellayani, India. Vouchers of the parasitoids are deposited in the Zoological Survey of India, Western Ghats Regional Station, Kozhikode. A plant voucher of *P. sylvestris* (accession no. 6863) is deposited in the Calicut University Herbarium, India.

Results

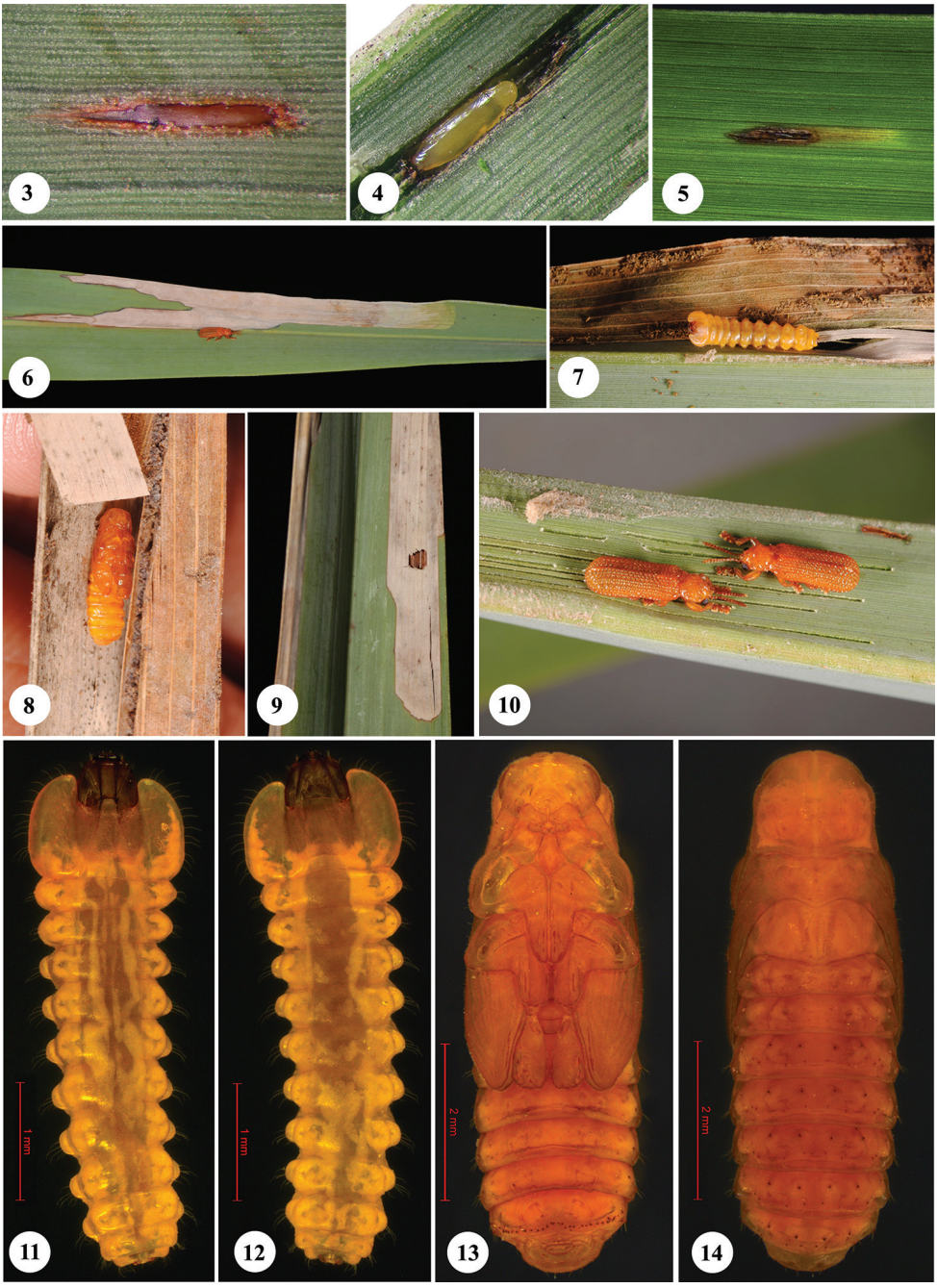
Life cycle of *J. pallida*. At Tirurangadi (field site 1), three palms of *Phoenix sylvestris* were observed heavily infested (Fig. 2) and with dried up older leaves. Eggs are laid singly mostly on the abaxial surface of leaves in longitudinal slits and are covered with a yellow secretion that turns reddish brown and forms an ootheca of about 1.8–2.3 mm length and 0.14–0.19 mm width (n=4) (Fig. 3). Freshly laid eggs, extracted from the slit of leaves, measured 1.35–1.38 mm in length and 0.25–0.28 mm in width (n=2), and were translucent yellow (Fig. 4). After the larva hatches, it bores into the mesophyll adjacent to the leaf cavity and initiates a leaf mine starting from the point of the egg insertion (Fig. 5). The leaf mines appear like elongate blotches of about 8.5–15.5 cm length and 0.5–1.1 cm width (n=18) (Fig. 6). Generally a single larva (Figs 7, 11, 12) occupies a mine, however, two or more larvae were also observed inside the mine when the adjacent larval mines of two or more individual larvae coalesce. A single leaflet of *P. sylvestris* could support the development of up to four individuals. Pupation occurs inside the mine (Fig. 8). Fully mature larva, prior to pupation withdrew from the leading end of the leaf mine, where it is feeding, towards the middle of the mine and pupated. The pupa (Figs 8, 13, 14) exhibits little movement inside the hollow leaf mine, even when disturbed. However, when exposed by opening the leaf mine, it moves its abdomen vigorously and is also able to move forward and backward by applying the apex of its abdomen on the substrate, albeit to a limited extent. The adult emerged through an irregular hole of about 2.0–3.5 mm width (n=22) (Fig. 13). Adult emergence holes could be seen on either adaxial or abaxial surface of the leaf. Adults mostly feed on the abaxial surface of the leaflet making linear feeding trenches (Fig. 14). Generally they moved towards the tip of the leaves and feeding started from the apex to the base. Thus drying of leaves due to feeding starts from the apex of leaflets towards the petiole.

We observed the presence of larval mines and adult exit holes on 21 leaflets of the single *P. dactylifera* at Tirurangadi (field site 2) in April, 2015, proving the occurrence of natural infestation of *J. pallida* on the date palm in Kerala, where it is of little commercial importance. A dead larva and pupal cases were recovered from the leaf mines, though no live insect was observed.

Adults confined on the first frond of *P. dactylifera* during dry season at Tirurangadi (field site 3), laid 14 eggs. Twelve out of the 14 eggs hatched. Of the 12 larvae, nine pupated and finally emerged into adults. On the second frond 12 eggs were laid, however, only four of them hatched. Only one of the four larvae pupated and reached adulthood.

During the rainy season on the third frond of *P. dactylifera*, 22 eggs were laid and 18 of them hatched. Five of them reached pupal stage and all five emerged as adults. On the fourth frond, nine eggs were laid and all of them hatched. Of these nine larvae, five pupated and all emerged as adults.

On a wild date palm, *P. sylvestris*, in the Botanical Garden of the University of Calicut (field site 4), we observed 21 eggs on a first frond during rainy season (second



Figures 3–14. Life stages of *Javeta pallida*. **3** Egg covered with ootheca **4** Egg, ootheca removed **5** Beginning of leaf-mine **6** Leaf mine and adult of *J. pallida* **7** Larva in leaf-mine, exposed **8** Pupa in leaf-mine, exposed **9** Adult exit hole **10** Adults and feeding trenches **11** Larva, ventral view **12** Larva, dorsal view **13** Pupa, ventral view **14** Pupa, dorsal view. (Figs 3–10 on *P. sylvestris*, except 9 on *P. dactylifera*).

Table 1. Developmental periods of *Javeta pallida* on *Phoenix dactylifera* and *P. sylvestris* during dry and rainy seasons.

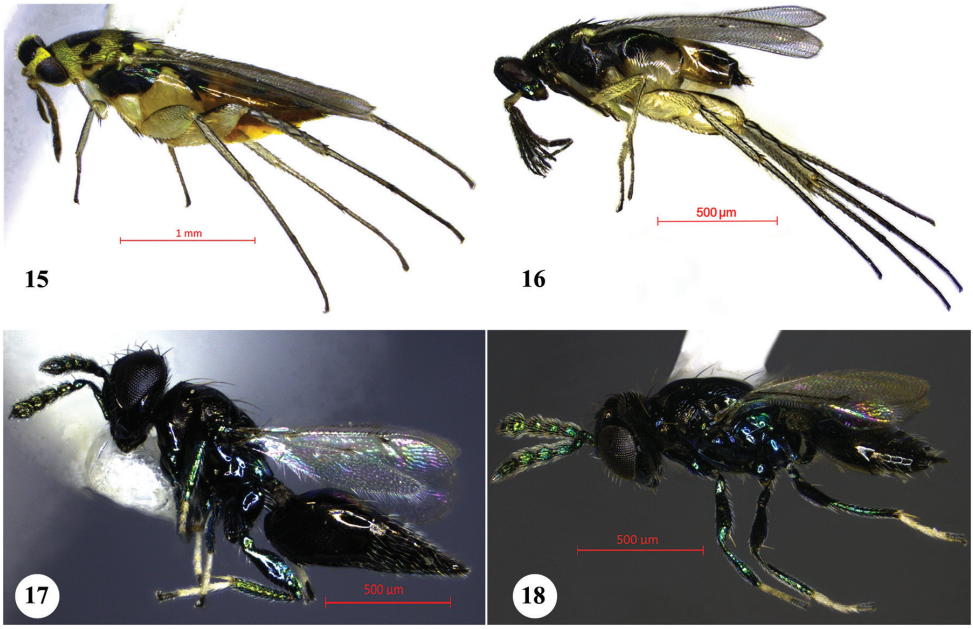
Host	Season	No. of individuals tracked up to adulthood	Egg period (days)		Larval period (days)		Pupal period (days)		Total developmental period (days)	
			Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation	Mean	Standard deviation
Phoenix dactylifera	Dry	10	12.2*	0.63	26.3*	3.59	20.2	1.32	58.7*	4.08
	Rainy	8	13*	0.93	37.5*	4.47	21.13	0.99	71.63*	5.15
Phoenix sylvestris	Rainy	24	13.04	1.0	34.33	5.85	20.45	1.32	67.83	6.53
t test	Dry vs Rainy seasons on *P. dactylifera*	t value	2.18		5.90		1.645		5.946	
	P. dactylifera vs *P. sylvestris* in rainy season	t value	0.103		1.31		1.306		1.490	

*significantly different (5%)

week of June, 2014 onwards). Nineteen of the 21 eggs hatched; 12 larvae pupated, and 12 adults emerged. On a second frond, we observed 29 eggs of which 24 hatched, 15 larvae pupated and 14 adults emerged; one pupa was observed dead inside the leaf mine. On a third frond, we observed 21 eggs; 19 hatched, and eventually 12 larvae reached pupal stage and adulthood.

Mature larvae and pupae often exited when the leaf mines were ruptured and such larvae pupated normally inside the glass beaker or nylon mesh in which they were confined and adults emerged.

A total of 58 adults were reared on *P. dactylifera* and *P. sylvestris*. However, the duration of all life stages from egg to adult could be tracked only in the case of 42 individuals, as at times the mines merged. Data on the developmental periods of *J. pallida* (based on the above 42 individuals), on *P. dactylifera* during dry and rainy season as well as on both *P. dactylifera* and *P. sylvestris* during rainy season are presented in Table 1. Egg period on *P. dactylifera* during dry season was significantly shorter than the same during the rainy season. Larval period also showed a similar trend, being highly significantly longer during rainy season than during the dry period. The pupal period was longer during rainy season, than during the dry season. However, the duration of pupal stage during dry and rainy seasons did not differ significantly on statistical comparison. The total developmental period was significantly longer on *P. dactylifera* during rainy season (mean 71.63 days) compared to dry season (mean 58.7 days).



Figures 15–18. Parasitoids of *Javeta pallida*. **15** *Elasmus longiventris*, female **16** *E. longiventris*, male **17** *Pediobius imbreus*, female **18** *P. imbreus*, male.

During rainy season, egg, larval, pupal and total developmental periods of *J. pallida* on both *P. sylvestris* and *P. dactylifera* were statistically on par with each other.

In short, *Javeta pallida* completed development on *Phoenix* palms in 52–88 days (mean 66.38 days) with egg period 11–15 days (mean 12.8 days), larval period 21–54 days (mean 33.02 days) and pupal period 17–23 days (mean 20.52 days).

No beetles or signs of infestation were observed in commercial plantations of date palm in Dharmapuri, Tamil Nadu, during January or April, 2015.

Natural enemies of *J. pallida*. Two species of chalcidoid parasitoids emerged from the larvae and/or pupae of *J. pallida* collected at Tirurangadi. Six females and six males of *Elasmus longiventris* Verma and Hayat (Figs 15, 16) and five females and 12 males of *Pediobius imbreus* Walker (Figs 17, 18) (both Eulophidae) emerged from larvae and/ or pupae in the laboratory.

Discussion

The trophic selection of *J. pallida*, within Arecaceae, corresponds to that in other known members of the genus as well as most Coelaenomenoderini, as host plants of three *Javeta* species are previously known (Uhmann 1943, 1955; Kalshoven 1957, 1981).

The fundamental features of the life cycle of *J. pallida* follow the pattern in Coelaenomenoderini: single egg deposition, mining larvae with up to four instars, endogenous solitary pupation, and heavy infestation on the appropriate hosts. The female's repertoire of making linear slits in the leaf, laying eggs singly within the slits, and then covering the egg firmly with brown colored material appears to be unique to *Javeta* within the tribe. In the most intensively studied *Coelaenomenodera* (*Coelaenomenodera*) *elaeidis*, females lay eggs in clusters at the ends of adult feeding scars and cover them with regurgitated leaf fibre (Cachan 1957; Howard et al. 2001; Mariau 2004). In *C.* (*C.*) *lameensis* eggs are laid in clusters inside cavities dugout on the leaf lamina and covered with faeces (Berti and Mariau 1999). We did not find groups of eggs, as has been noted for other Coelaenomenoderini — *C.* (*C.*) *elaeidis* (Cachan 1957; Morin and Mariau 1970, 1971, 1974; Mariau and Morin 1972, 1974) and *C.* (*C.*) *lameensis* (Berti and Mariau 1999; Mariau and Lecoustre 2000, 2004). In *J. pallida*, the slits in which eggs are laid, are independent of the adult feeding scars. There was no apparent additional covering, over ootheca, like frass as in other coelaenomenoderines. Laying single eggs probably is a better mechanism of defense against egg parasitoids than laying clusters of eggs in adult feeding scars. However, Kalshoven (1951) reported that 70% of the eggs of *J. arecae* were parasitized during an outbreak in April, 1937 in Sumatra, but he did not report the mode of oviposition in this species. The size of leaflet in *P. sylvestris* (15–46 cm long, 2–2.5 cm wide) is less than that in oil palm (60–120 cm long, 3.5–5 cm wide). Smaller leaflet size in *P. sylvestris* could be yet another driving factor behind *J. pallida* choosing solitary egg laying over egg clusters as this would ensure optimum availability of food resources for the larvae. In *Coelaenomenodera* spp., as a result of laying eggs in clusters, several larvae can occur within a single leaf mine

(Cachan 1957). In *J. pallida*, a single larva per leaf mine is the norm, unless adjacent leaf mines coalesce. All of the Coelaenomenoderini life cycles documented to date indicate four larval instars. This is interesting as most Cassidinae have five instars and a few particular species have up to nine instars (Chaboo 2007).Coelaenomenoderine instar 1 appears to lack egg bursters (Cox 1988, 1994).

Drying of leaves due to adult feeding starts from the apex of leaflets towards the petiole. This appears to conserve the leaf as feeding near the base of the leaf lamina would result in drying up of the entire leaflet that could otherwise have been consumed.

At 74–97 days from egg to adult, the development of *Coelaenomenodera* spp. is relatively long among Cassidinae (Cotterell 1925; Morin and Mariau 1970; Appiah et al. 2007). These beetles appear to have high fecundity with females laying >70 eggs per week (Morin and Mariau 1971, 1974; Mariau and Bescombes 1972). Incubation is about 15–28 days, four larval stages last about 40–50 days, and pupation lasts up to 10–22 days (Cotterell 1925; Morin and Mariau 1970; Appiah et al. 2007). *Javeta pallida* that completes development in 52–88 days, too have a similar duration of life cycle. The data on duration of development of *J. pallida* during dry and rainy seasons on *P. dactylifera* as well as during the rainy season on *P. dactylifera* and *P. sylvestris* present interesting patterns. The total developmental period and egg and larval periods were significantly longer during the rainy season than during the dry season, which indicates that dry climate is probably better for the growth and development of *J. pallida*. Similarly the near identical pattern of development of all life stages on both *P. dactylifera* and *P. sylvestris* indicates equal suitability of both host plants for beetle development. This suggests that outbreaks of *J. pallida* on the date palm is possible, as has happened on the wild date palm in Bangalore (Yeswanth H. M., personal communication) and Tirurangadi. Thus our rearing experiments have established the potential of *J. pallida* as a serious pest on the cultivated date palm.

Hymenoptera parasitoids belonging to the families Eulophidae and Trichogrammatidae act as the most important natural enemies of Coelaenomenoderini (Waterston 1925; Kerrich 1970, 1974; Boucek 1976; Viggiani 1980; Cox 1994; Mariau and Lecoustre 2004; Aneni 2014a, b). Morin and Mariau (1971) studied parasites and predators of the egg while Mariau et al. (1978) uncovered the parasites in each of the four larval instars. Discovery of two eulophid parasitoids on *Javeta pallida* reveal the same pattern of host-parasite relationship.

Although some papers have been titled "morphology" they give only minimal information about morphological structures. Therefore detailed comparative study of all life stages, including scanning electron microscopy, is needed both to uncover many more taxonomic and phylogenetic characters to strengthen understanding of systematics and evolution and to better manage a notorious economically-important pest.

One of the most remarkable aspects of Coelaenomenoderini life cycles is the alternation of mixed populations of different stages with synchronized populations in outbreak periods. This has been described for *C.* (*C.*) *elaeidis* (Mariau and Morin 1972; Bernon and Graves 1979) and *C.* (*C.*) *lameensis* (Mariau and Lecoustre 2004). There are many such sporadic pests, such as the rice caseworm, *Nymphula depunctalis* (Guenee) (Lepidoptera: Pyralidae), rice swarming caterpillar, *Spodoptera mauritia* (Boisduval)

(Lepidoptera: Noctuidae), and locusts having periodic swarms and outbreaks. It is unclear at this time what factors trigger the changes in life cycles (from asynchronous to synchronous) and what might be any behavioral, morphological and physiological changes. Despite the serious pest status of these species, very little has been written about the natural history of the adults.

Several factors contribute to their success. The females have very high fecundity (for Cassidinae) and there can be up to four generations per year (Timti 1991). Distinct cycles with periodic outbreaks have been documented for *C.* (*C.*) *elaeidis* (Morin and Mariau 1970; Bernon and Graves 1979; Mariau et al. 1999a; Mariau and Lecoustre 2004), and *C.* (*C.*) *lameensis* (Berti and Mariau 1999). The scraping and mining behavior of feeding produces severely damaged leaves and defoliated trees; this results in costly lower yields of fruit and oil. Chemical (Jover 1950; Mariau et al. 1973, 1979; Philippe and Diarrassouba 1980; Mariau and Philippe 1983; Philippe 1990), host plant resistance (Mariau et al. 1999b) and parasitoid (Mariau and Morin 1972; Mariau et al. 1978; Lecoustre et al. 1980) control measures must be well-timed for this phasic pattern in population explosions.

The severity of infestation observed on *P. sylvestris* suggests that *J. pallida* poses a potential pest of any species of *Phoenix*, including *P. dactylifera*. Our study foresees a native leaf beetle becoming a serious pest on an exotic crop of immense economic potential in India.

Chemical control with sprays and injection of trees have been used to control *Coelaenomenodera* pests (Jover 1950; Philippe 1990; Mariau et al. 1973, 1979; Mariau and Genty 1992). However, Timti (1991) indicated that years of chemical sprays had little effect in controlling infestations of *C.* (*C.*) *elaeidis* in West and Central Africa. Chemical measures may also have limited use against the larvae concealed inside mines. Alternative control measures must be developed for long term control. Limited data suggests that palm hybrids with different leaf mechanical properties can impede larval development (e.g. Mariau et al. 1999a).

World-wide interest in more sustainable and healthier harvesting and processing of food is stimulating shifts to organic farming, including in date-palm and wild date palms (Mahmoudi et al. 2008). This change of attitude and its economic implications suggest that biological control measures that exploit the predators and parasitoid complex of each life stage of Coelaenomenoderini may be the most economical, most effective, and most sustainable long-term control. Eulophidae and Trichogrammatidae can parasitize all life stages, including eggs and larvae that are encased within the leaf. Timti's (1991) study with *C.* (*C.*) *elaeidis* populations in Cameroon revealed that ants can also act as biocontrols. These studies carried out in Africa more than 30 years ago may provide a model to pursue knowledge about the Indian parasitoid complex if *Javeta* becomes a major pest of expanding indigenous and introduced palm crops.

Conclusion. Comparative study of morphology and biology across Coelaenomenoderini will certainly yield many novel phylogenetic characters. Our study here suggests that the oviposition (number and coverage of eggs), number of instars and morphology, pupation site, and eruptive population behaviors might be considered as

character complexes. Palms are one of the most important crops in the world and their insect fauna needs further study. Within Cassidinae, we also need to understand the evolutionary relationship of certain tribes with palms. Furthermore, study of the insect milieu—the predator and parasitoid complex—can help us understand their impacts on the beetles' evolution and provide models for sustainable biocontrols of palm resources. We plan to continue documenting the biology, pest status, and insect enemy complex of *J. pallida* in the field. Our next step is also a detailed morphological study of the juvenile and adult stages.

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Revision of the legume-feeding leaf beetle genus *Madurasia* Jacoby, including a new species description (Coleoptera, Chrysomelidae, Galerucinae, Galerucini)

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Abstract

Madurasia Jacoby is revised and *M. andamanica* **sp. n.**, endemic to the Andaman Islands in the Indian Ocean, is described and illustrated. *Madurasia obscurella* Jacoby, **syn. n.**, is a new junior synonym of *Madurasia undulatovittata* (Motschulsky), **comb. n.** A lectotype is designated for *M. obscurella*. Literature on the biology and management of *M. undulatovittata* is reviewed.

Keywords

Asia, Africa, biology, pest, pulses, taxonomy

Introduction

The monotypic galerucine genus *Madurasia* was described by Jacoby (1886) for a new species, *Madurasia obscurella*, from southern India. Aslam (1972) synonymized *Neorudolphia bedfordi* Laboissière, 1926, the only species in this monotypic genus from Sudan, with *M. obscurella* Jacoby. Examination of the type of *Monolepta undulatovittata* (Motschulsky 1866) (originally described in *Teinodactyla* Chevrolat = *Longitarsus* Latreille) from Sri Lanka has shown that *Madurasia obscurella* is a junior synonym of Motschulsky's species. The genus is here revised and a new species is described from

the Andaman Islands in the Indian Ocean. Information on the biology, pest status and management of *M. undulatovittata* comb. n., which is a significant pest of various legume crops in south-east Asia and Africa, is reviewed.

Materials and methods

Dissecting techniques and descriptive terminology follow Konstantinov (1998). Label data for holotypes, lectotypes, and paralectotypes has been recorded verbatim, with lines on the same label separated by "/" and labels separated by ";". Material examined is from the following collections:

BMNH	Natural History Museum, London
INPC	National Pusa Collection, Indian Agricultural Research Institute, New Delhi
JBC	Personal collection of Jan Bezděk, Czech Republic
KAU	Travancore Insect Collection, Kerala Agricultural University, Vellayani
NBAIR	National Bureau of Agricultural Insect Resources, Bangalore
UASB	University of Agricultural Sciences, Bengaluru
USNM	National Museum of Natural History, Smithsonian Institution, Washington D.C.
ZMUH	Zoologisches Institut und Zoologisches Museum, Universität von Hamburg, Hamburg, Germany
ZMUM	Zoological Museum, Moscow State University, Moscow

Determination of the gender of the undissected specimens is provisional as sexually dimorphic characteristics are often not clearly discernible externally.

Systematics

Madurasia Jacoby, 1886

Madurasia Jacoby, 1886: 280 (Type species: *Madurasia obscurella* Jacoby, 1886, southern India, by monotypy)–Maulik 1936: 72–Wilcox 1973: 435–Seeno and Wilcox 1982: 107–Jolivet and Hawkeswood 1995: 101 (host plants)–Medvedev and Sprecher–Uebersax 2005: 316 (key)–Beenen 2010: 481.

Neorudolphia Laboissière, 1926: 190 (Type species: *Neorudolphia bedfordi* Laboissière, 1926, Sudan, by monotypy)–Wilcox 1973: 435–Aslam 1972: 500 (= *Madurasia* Jacoby 1886: 280).

Description. Body: length 2.0–3.0 mm; 1.8–2.3 times longer than wide. Moderately small, oblong, flattened in lateral view, length 3.1–3.4 times height. General color

straw brown to dark brown with a characteristic, more or less distinct, dark, broad longitudinal stripe on each elytron (Figs 1, 3, 5–7, 21); mesal margin of stripe nearly straight; each stripe nearer to suture than to lateral margin of elytron; stripe narrowing laterally posteriorly of humerus and in distal 2/3 of elytron.

Head (Fig. 8) hypognathous with frontal view slightly longer than wide. In lateral view anterior margin forms a moderately convex line with a notch where vertex meets antennal calli and a second notch at anterior end of frontal ridge. Supraorbital pore represented by a large setaceous pore adjacent to orbital sulcus near eye. Seta in supraorbital pore upcurved. Vertex shiny, indistinctly wrinkled, nearly impunctate. Antennal calli trapezoidal, longer than wide, moderately convex, raised above adjacent border of vertex, separated from each other by a deep midfrontal sulcus; anterior ends acutely triangular, enter into interantennal space, reaching well below midlevel of antennal socket. Orbital sulcus short, deep, represented by supraorbital pore and adjacent area. Supracallinal sulcus represented by punctures arranged in an irregular transverse row, each puncture in supracallinal row bearing a short down-curved seta. Midcranial suture absent. Supraorbital sulcus less distinct than midfrontal sulcus. Suprafrontal and supraantennal sulci well defined. Subgenal suture distinct. Transverse diameter of eye 5.2–8.8 times distance between eye and antennal socket, 2.9–4.4 times distance between antennal sockets, 1.7–1.9 times width of antennal socket, 0.6–0.7 times distance between eyes. Eyes lateral, medium sized, convex, inner margins indistinctly concave, and ventrally divergent. Frontal ridge narrowest between antennal sockets, joins anterofrontal ridge anteriorly. Anterofrontal ridge transverse, gently curved. Frontal ridge together with antero-frontal ridge forms T–shaped ridge. Anterofrontal ridge lower than frontal ridge. Frontolateral area coarsely punctate, each puncture bearing a long seta. Frontoclypeal suture with a row of eight setae. Clypeus narrow. Visible part of labrum much wider than long, with a transverse row of eight pores; all eight pores in *M. andamanica* sp. n. with a well-developed seta; while only six pores, excluding third pore from either end, with seta in *M. undulatovittata*. Labrum (Fig. 13) with anterior margin incised medially; about seven sensillae on either side of incision, arranged along anterior margin of labrum's inner surface; tormae longer than width of labrum. Mandible (Fig. 12) palmate with six sharp denticles. Maxilla (Fig. 11) with four palpomeres: first shortest, second and third subequal, both longer than first, but shorter than apical palpomere, apical longest; lacinia wider than galea. Labial palpi (Fig. 10) with three palpomeres, basal two wider than long, middle widest, apical palpomere longer than wide and longest of three. Antenna (Fig. 9) reaches more or less middle of elytron. First antennomere longest, club shaped; second smallest; third a little longer than second; fourth distinctly longer than third; 4–10 subequal in length; eleventh longer than all except first antennomere; five to six distal antennomeres wider than preceding three or four (Fig. 9).

Dorsum glabrous. Pronotum (Fig. 14) 1.2–1.3 times wider than long; greatest width slightly anterior of middle. Posterior margin 1.1–1.2 times wider than anterior margin, lateral margin gently convex, posterior margin nearly straight in middle, curved laterally, and narrowly margined. Anterolateral callosity longer than wide, setigerous pore posterolaterally situated, not forming denticle at pore; posterolateral

callosity protruding slightly laterally, setigerous pore laterally situated. Disc without impressions, shiny, uniformly punctate, punctures small, smaller than those on elytra. Anterior coxal cavity open behind (Figs 4, 16); intercoxal prosternal process short, acutely pointed, not reaching midlevel of procoxa (Fig. 16); procoxae longer than wide and closely associated; shortest distance from anterior margin of prosternum to procoxal cavity about 1/4–1/5 of longitudinal procoxal diameter. Mesoscutellum triangular, about two times wider than long, flat, impunctate to minutely punctate. Intercoxal mesosternal process short, not reaching midlevel of mesocoxa (Figs 4, 17). Mesepisternum broader than mesepimeron (Fig. 17). Metasternum no longer than first two abdominal ventrites combined (Fig. 4).

Elytra broader than pronotum basally, maximum width posterior of middle. Humeral callus well developed; elytral border narrow, becoming indistinct towards apex; elytral apex broadly rounded; epipleuron (Fig. 4) oblique, maximum width near anterior 1/4 of elytron, maximum width subequal to about 1.5 times maximum width of mid-femur, narrows abruptly before middle and then continues very narrowly, becoming indistinct towards the elytral apex. Hind wings present. Metanotum (Fig. 15) well developed with full complement of internal ridges.

All femora oblong in cross section; all tibiae subcylindrical, subcircular in cross section with a minute apical spur; metatibial spur subequal to claw in length; proportionate length of femur–tibia–tarsomeres 1–4 as follows: 1: 1.0–1.1 : 0.2–0.3 : 0.1–0.2 : 0.1–0.2 : 0.2–0.3 (foreleg); 1: 0.9–1.0 : 0.3 : 0.1–0.3 : 0.1–0.2 : 0.2–0.3 (midleg); 1: 1.1–1.2 : 0.4 : 0.1–0.2 : 0.1 : 0.2 (hindleg); joint where metatibia and first metatarsomere meet, black; third tarsomere always bilobed; claws simple and appendiculate, appendix small and basal. Abdomen (Fig. 4) with five distinct ventrites; ventrites 2–4 becoming progressively slightly shorter; fifth ventrite slightly longer than fourth; intercoxal projection of first abdominal ventrite acute; apical abdominal tergite (Fig. 19, 20) without a median longitudinal groove, posterior margin slightly concave medially in male of *M. undulatovittata* (Fig. 20) and distinctly emarginate in *M. andamanica* sp. n.; posterior margin of apical tergite broadly convex (Fig. 19) in females of both species; posterior margin of apical ventrite more or less lobed medially in male (Figs 22, 23), entire in female.

Female genitalia with receptacle of spermatheca (Figs 28, 29) pot-shaped, wider than long; pump curved, longer than receptacle and enlarged distally, appendix well developed; spermathecal duct shorter than receptacle, glandular duct beyond middle of spermathecal duct. Tignum (Figs 32, 33) gently curved near middle, grooved medially, with long setae near distal margin of broad membranous apex. Vaginal palpi (Figs 30, 31) fused from proximal end to a short distance beyond middle, separate distally, each palpus narrowing towards rounded apex, lateral margin concave preapically, with long distal setae. Median lobe of aedeagus strongly curved in lateral view (Figs 26, 27), acutely pointed. Tegmen with stem much longer than arms.

Host plants. Fabaceae.

Distribution. Asia, Africa (Sudan).

Remarks. *Madurasia* closely resembles *Medythia* Jacoby, 1887, and species of both genera are pests of legumes. The general morphology, including the structure of the

head, female genitalia, and even the presence of elytral stripes in some species of *Medythia,* are similar to those in *Madurasia*, making differentiation of these genera difficult. *Madurasia* can be separated from *Medythia* by the structure of the pronotum. The pronotum in *Medythia* is elongate and narrows posteriorly, whereas the pronotum is transverse and a little wider posteriorly in *Madurasia*. The elytral epipleuron is short in *Madurasia*, hardly extending beyond middle of the elytron. In *Medythia quadrimaculata* Jacoby, type species of the genus, the elytral epipleuron is longer, extending beyond the middle of the elytron. However, the epipleura are identical to those of *Madurasia* in a few Indian *Medythia* species examined. In *Madurasia*, the distal antennomeres are darker, while antennomeres 8–10 are whitish in most *Medythia* species, including the type species.

Adults are attracted to light.

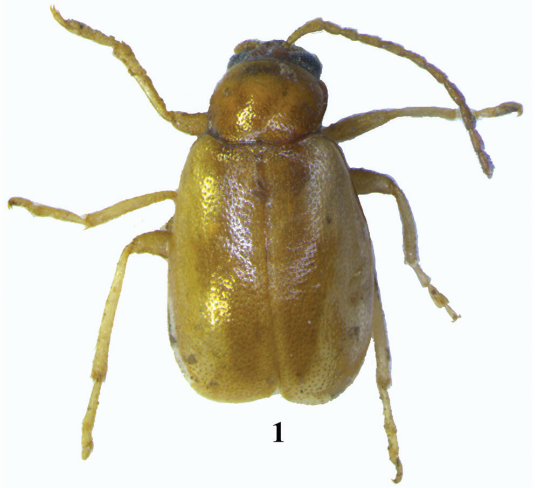
Madurasia andamanica sp. n.

http://zoobank.org/3D810CFF-3113-43FE-8E67-BD8335505E90
Figs 21, 22, 24, 26, 28, 30, 32, 33

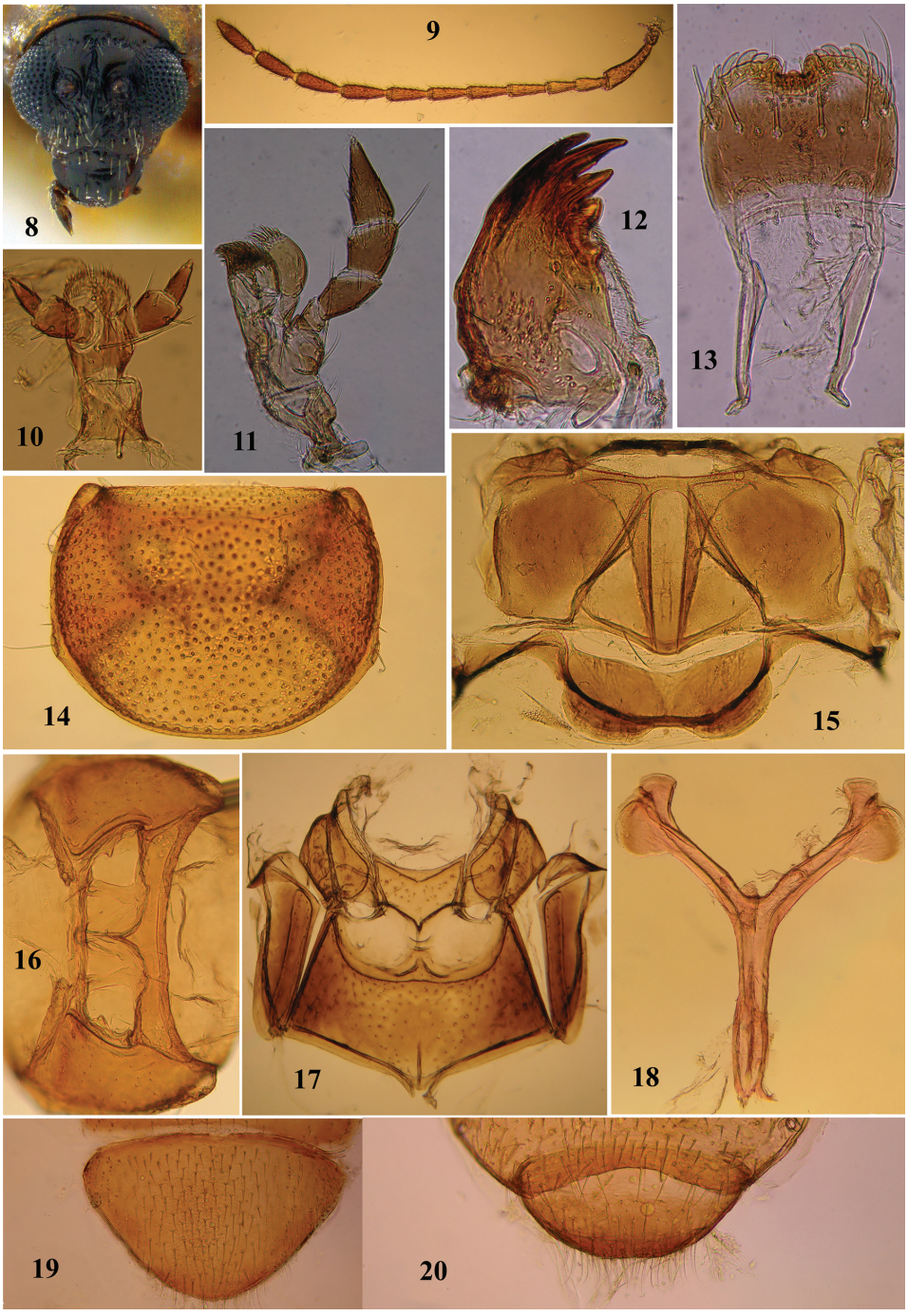
Diagnosis. The new species can be recognized by the following characters: 1) elytral stripe not reaching the elytral apex, narrowing in distal 1/4; 2) labrum with a transverse row of eight well developed setae; 3) posterior margin of apical ventrite in male distinctly lobed medially; 4) apex of aedeagus in lateral view curved like a parrot's beak with an acute tip; 5) ventral side of aedeagus depressed in basal 1/2, then distally raised in the form of a narrow ridge which reaches the apex.

Description. Body: length 2.1–2.6 mm; width 1.1–1.2 mm; 1.8–2.1 times longer than wide (Fig. 21). Dorsum straw colored. Head dark brown. Antenna with basal three or four antennomeres a pale straw color, distal antennomeres becoming progressively darker. Mandible, maxilla, and labium paler than labrum and anterior aspect. Pronotum with pale orange hue. Elytra a pale straw color, the dark elytral stripe not reaching the elytral apex (Fig. 21), widest anteriad of middle, narrowing distinctly posterior of humerus as well as in distal 1/4. Thoracic sternites and pronotum concolorous, metathoracic sternite often a slightly darker laterally. Abdominal ventrites pale brown, with lateral margins and apical abdominal ventrite darker in many specimens. Legs straw colored, tibia and first two tarsomeres often a slightly darker than femur. Antenna reaching slightly beyond middle of elytron. Proportionate length of antennomeres 1–11: 1: 0.48: 0.45–0.50: 0.63–0.67: 0.63: 0.61–0.62: 0.66–0.67: 0.62–0.70: 0.63–0.69: 0.62–0.63: 0.75–0.88. Transverse diameter of eye 6.3–8.0 times width of orbit, 3.3–3.8 times width of interantennal space, 1.7–1.9 times width of antennal socket, 0.7 times distance between eyes. Pronotum 1.2–1.3 times wider than long, posterior width 1.1–1.2 times wider than anterior width.

Proportionate length of femur:tibia:tarsomeres 1–4 as follows: 1: 1.0–1.1 : 0.2–0.3 : 0.2 : 0.1–0.2 : 0.2–0.3 (foreleg); 1: 0.9–1.0 : 0.3 : 0.1–0.3 : 0.1–0.2 : 0.2–0.3 (midleg); 1: 1.0–1.1 : 0.4 : 0.1–0.2 : 0.1 : 0.2 (hindleg).

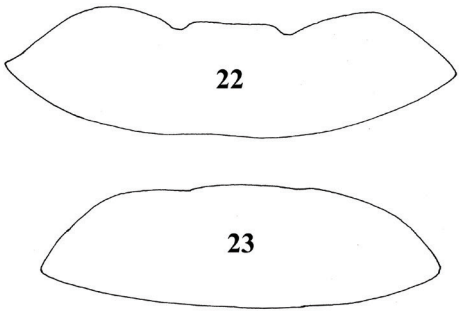


Figures 1–7. *Madurasia undulatovittata*. **1** Lectotype (specimen on card, photograph edited) **2** labels on lectotype **3** and **5–7** dorsal view, color variation **4** ventral view.



Figures 8–20. *Madurasia undulatovittata*. **8** head, frontal view **9** antenna **10** labium **11** maxilla **12** mandible **13** labrum **14** pronotum **15** meso– and metanotum **16** prosternum **17** meso– and metasternum and pleurites **18** metendosternite **19** apical visible tergite, female **20** apical visible tergite, male (all specimens, except head, have been macerated).

21



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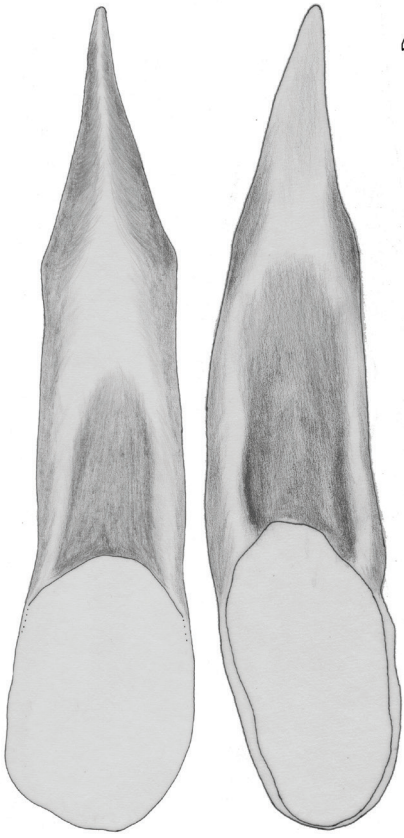

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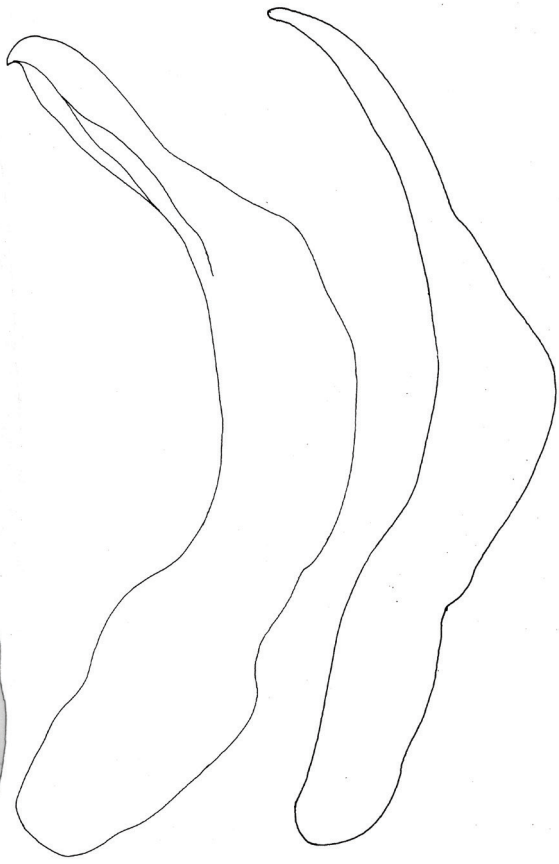

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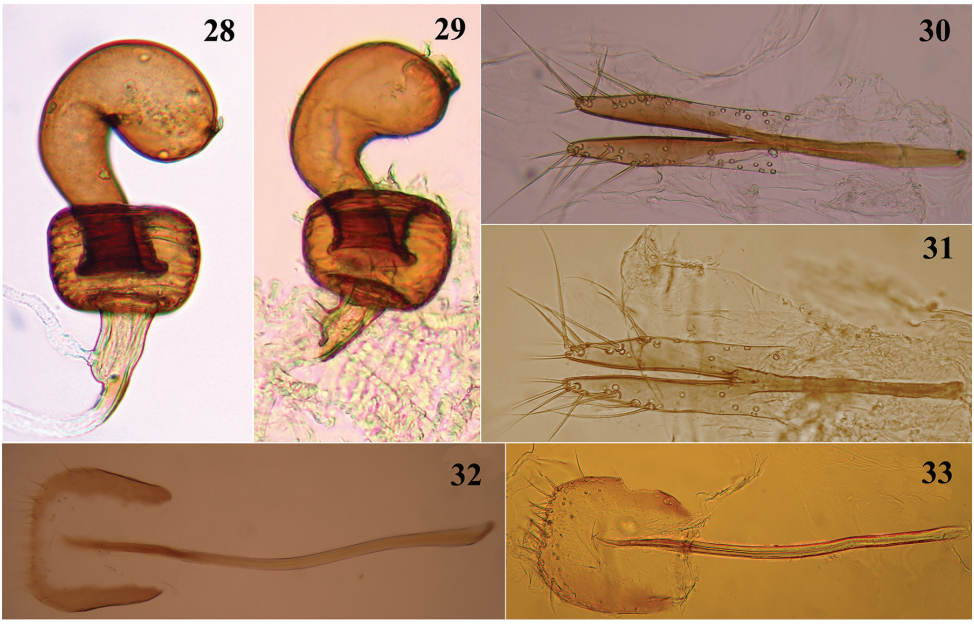
Figures 21–27. *Madurasia andamanica* sp. n. **21** dorsal habitus **22** apical ventrite of *M. andamanica* sp. n. male **23** apical ventrite of *M. undulatovittata* male **24** median lobe of aedeagus in *M. andamanica* sp. n., ventral view **25** median lobe of aedeagus in *M. undulatovittata*, ventral view (bilaterally symmetrical, specimen tilted) **26** median lobe of aedeagus in *M. andamanica* sp. n., lateral view **27** median lobe of aedeagus in *M. undulatovittata*, lateral view.



Figures 28–33. Spermatheca in **28** *M. andamanica* sp. n. **29** *M. undulatovittata*; vaginal palpi of **30** *M. andamanica* sp. n. **31** *M. undulatovittata*; tigna in **32** *M. andamanica* sp. n. **33** *M. undulatovittata*.

Posterior margin of apical ventrite in male distinctly lobed medially (Fig. 22). Receptacle of spermatheca 2.4 times wider than long (Fig. 28). Tignum widened proximally; membranous apex wider towards posterior (Fig. 32).

Aedeagus in lateral view (Fig. 26) with greatest width near middle, narrow in proximal 1/4, apex curved like a parrot's beak with an acute tip. In ventral view (Fig. 24), greatest width at base, narrowing abruptly in apical 1/3; ventral aspect depressed in basal 1/2, then distinctly raised in the form of a narrow ridge which reaches the apex.

Etymology. Named after the Andaman Islands, where the new species occurs.

Material examined. Holotype ♂ "INDIA: Andaman & Nicobar / North Andaman: Diglipur / 13°14'53.9"N, 92°58'37.5"E, / 15 mts. 24.iv.2014. At light / Yeshwanth H. M." (white label); "HOLOTYPE / Madurasia andamanica / Prathapan sp. nov., 2015" (red label) (BMNH).

Paratypes (104). 5♂, 8♀ same data as holotype; 7♂, 19♀ same data as holotype, but 23.iv.2014; 2♀ INDIA: Andaman & Nicobar / South Andaman: Sippighat / 11°67'26"N, 92°67'12"E, / 44 mts. 18.iv.2014, Light trap / Yeshwanth H. M.; 1♂, 16♀ India: South Andaman / Garacharama / 12.xi.2014 / Bharathimeena Coll. / Ex Redgram; 16♂, 7♀, 1 unsexed same data but 8.I.2015; 2♂, 20♀ same data but 4.XII.2014 and collector Krishnaveni (5 BMNH, 5 USNM, 5 JBC, 5 KAU, 5 UASB, 40 NBAIR, 39 INPC).

Distribution. India (Andaman Islands) (Fig. 34).

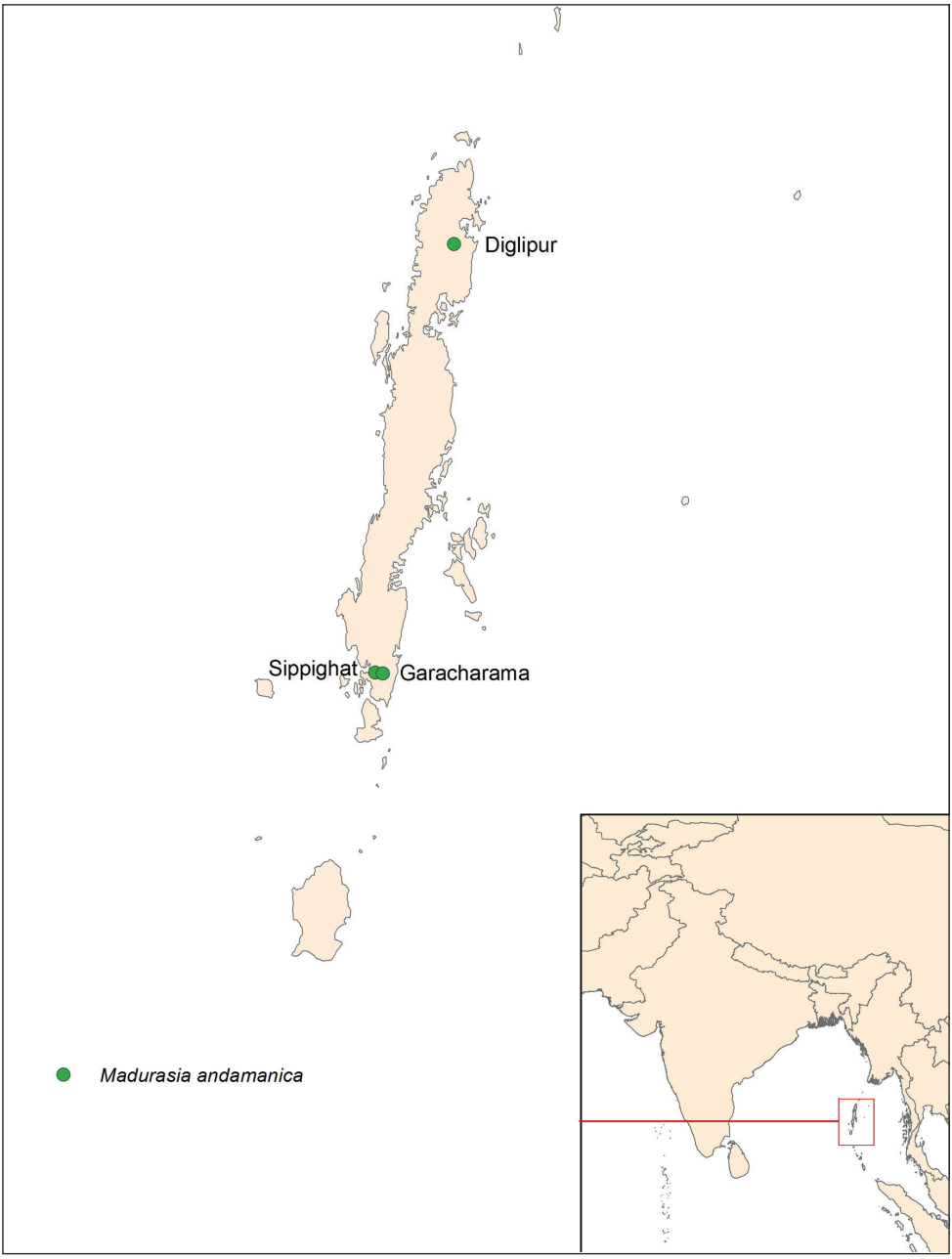


Figure 34. Distribution of *M. andamanica* sp. n. on the Andaman Islands.

Remarks. Color pattern in *M. andamanica* sp. n. (Fig. 21) appears to be consistent and less variable compared to that in *M. undulatovittata* (Motschulsky, 1866), where the color of specimens collected on the same host at the same locality on the same day

varied greatly. *Madurasia andamanica* sp. n. resembles *M. undulatovittata* externally. However, it can be distinguished based on the structure of the aedeagus and the number of labral setae, as described under *M. undulatovittata*.

Host. *Cajanus cajan* (L.) Millsp. (Fabaceae) (red gram or pigeon pea) (Bharathimeena T., pers. comm. 2015).

***Madurasia undulatovittata* (Motschulsky), comb. n.**
Figs 1, 3–20, 23, 25, 27, 29, 31, 33, 35

Teinodactyla undulatovittata Motschulsky, 1866: 417 [Sri Lanka, Lectotype (ZMUM)]–Wagner and Bieneck 2012: 214–215.
Longitarsus undulatovittatus: Gemminger and Harold 1876: 3509–Maulik 1926: 361.
Monolepta undulattovittata: Ogloblin 1930: 112.
Madurasia obscurella Jacoby, 1886: 381 ["Madura, Madras Presidency", Southern India– Lectotype (BMNH)]–Maulik 1936: 74–Wilcox 1973: 435–Takizawa 1987: 39–Takizawa and Kimoto 1990: 8–Takizawa 1990: 281–Mohamedsaid 1997: 5–Medvedev and Sprecher 1999: 310 (catalogue)–Mohamedsaid 2000: 370–Kimoto 2005: 58–Beenen 2010: 481–Bezděk 2012: 422, 424. **New synonym.**
Neorudolphia bedfordi Laboissière, 1926: 191 [Brit. Sudan, on *Cajanus indicus,* Syntype (ZMUH, Hamburg)]–Aslam 1972: 500 (synonymized with *Madurasia obscurella* Jacoby)–Wilcox 1973: 435–Weidner 1976: 229.

Description. Body: length 2.0–3.0 mm; width 1.0–1.3 mm; 2.0–2.3 times longer than wide. General color pattern consistent but highly variable in intensity (Figs 1, 3, 5–7). Head dark brown to pale brown, often darker than pronotum. Basal antennomeres 3–6 pale straw brown, distal antennomeres becoming progressively darker. Pronotum more or less pale brown, generally paler than head. Background color of elytron paler than pronotum. Lateral margin of dark elytral stripe emarginate in anterior 1/3 and posterior 1/3; stripe broadening posteriorly, covering width of elytral apex. In some specimens, elytra darker laterally giving the impression of a pale, medially narrowed line on a dark elytron. Intensity of stripe's darkness varies from pale straw brown (Fig. 1) to dark brown. In type of *M. undulatovittata*, elytral stripes are hardly visible (Fig. 1). In some specimens, widest region in middle of stripe extends to lateral elytral margin, thus dividing pale colored lateral area into anterior and posterior spots (Figs 6, 7). Ventral aspect (Fig. 4) generally paler than head. Metasternum slightly darker than pro– or mesosternum. Metepisternum darker than metasternum. Abdomen darker laterally and posteriorly in many specimens. In darkest specimens, ventral side dark brown to piceous. Legs pale brown, all femora nearly concolorous with abdominal ventrites; metafemora darker distally in some specimens. All tibiae paler than femora. Metatibia and first metatarsomere whitish in some specimens. Claw tarsomere and bilobed tarsomere often darker than preceding ones.

Antenna (Fig. 9) reaches middle of elytron or a little beyond. Proportionate length of antennomeres 1–11: 1: 0.54–0.57 : 0.44–52 : 0.65–0.69 : 0.59: 0.66: 0.66–0.69: 0.62–0.75: 0.73–0.75: 0.69–0.72: 0.69–0.71: 0.81–0.91. Transverse diameter of eye 5.3–8.8 times width of orbit, 2.9–4.4 times width of interantennal space, 1.7–1.8 times width of antennal socket, 0.6 times distance between eyes. Pronotum (Fig. 14) 1.3 times wider than long, posterior 1.1 times wider than anterior.

Proportionate length of femur–tibia–tarsomeres 1–4 as follows: 1: 1.0–1.1 : 0.3 : 0.1–0.2 : 0.1–0.2 : 0.3 (foreleg); 1: 0.9–1.0 : 0.3 : 0.1–0.2 : 0.1–0.2 : 0.2–0.3 (midleg); 1: 1.1–1.2 : 0.4 : 0.1–0.2 : 0.1 : 0.2 (hindleg). Two visible apical tergites completely exposed in most females, particularly when killed in alcohol.

Posterior margin of apical ventrite in male (Fig. 23) indistinctly lobed medially. Receptacle of spermatheca 1.6 times wider than long (Fig. 29). Tignum not widened proximally (Fig. 33); membranous distal region widest medially.

Aedeagus in lateral view (Fig. 27) strongly curved after basal 1/2, acutely narrowed in proximal 1/3, with weakly curved apex. In ventral view (Fig. 25), widest in proximal 1/3, narrowing sharply towards apex in apical 1/3, lateral margin a little abruptly narrowed preapically. Ventral aspect of aedeagus depressed with a convex portion in middle.

Material examined. Types. *Madurasia undulatovittata*: Lectotype ♀. "Teinodactila / undulato / vittata Motch / Ceylon"; "Monolepta / undulatovittata Mots. / 1926 D. Ogloblin det."; "LECTOTYPUS / des Döberl 2005" (ZMUM).

Madurasia obscurella: Lectotype ♀. "Type" (rectangular red label); "Andrewes / Bequest. / B. M. 1922–221."; "Madura","738" (3 in 738 is not legible as pierced by pin); "Madurasia obscurella Jac. /Type"; "SYNTYPE" (white circular disc with sky blue margin); "Lectotype / Madurasia obscurella Jacoby / des. K. D. Prathapan, 2015" (here designated, specimen on card, right antenna missing) (BMNH).

Paralectotype ♀. "Type / H. T." (white circular disc with red border); "Madura"; "Jacoby Coll. 1909–28a."; "Madurasia / obscurella / Jac. Type" (Blue label); "SYNTYPE" (white circular disc with sky blue margin); "Paralectotype / Madurasia obscurella Jacoby / des. K. D. Prathapan, 2015" (BMNH).

Non-type material. AFRICA: Sudan: ♀ British Sudan, S. R. J. Madani, 22.ix.1923, H. M. Bedford, feeding on 'adis' (illegible) sudani leaves / Blue Nile A 3024 / Pres by Imp. Bur. Ent. Brit. Mus. 1925–228 / standing as Neorudolfia (sic) bedfordi; 1 unsexed R. F. Wadmedanai J. W. Cowland 21/9/32 Shotholing seedlings of Phaseolus mungo / Ent. Coll. C 12147 / AFRICA 250,000 55–G Map / Pres. by Imp. Inst. Ent. BM 1933–415 / Standing as Neorudolphia bedfordi / SUDAN Govt.; 1 unsexed Blue Nile 5429 / Aenk H. H. & D. King 26.5.13 On boot / Pres. by Imp. Bur. Ent. Brit. Mus. 1927–103 / Neorudolphia bedfordi V. Laboissière–Dèt. (all BMNH).

ASIA: Bangladesh: ♀ (India) Dacca, 2.vi.1945, D. Leston; ♀ (India) Dacca, 10.v.1945, D. Leston (both BMNH); **India:** *Andhra Pradesh:* 3 unsexed Vizagapatnam Dist., Chipurupalli, B.M. 1924–7; *Gujarat*: 2♀ Baruch, 10.xii.1987, Pigeon pea, CIE A19617; Navasari, 15.iii.1992, Assoc with cowpea, IIE 22432, Madurasia obscurella Jac det. M. L. Cox 1992 (all BMNH); *Karnataka*: 1 macerated specimen Belgaum, 1–2.viii. 2008, at light, K. Swamy; 2♀, 1♂ Chikkaballapur, 13°25'48"N,

7°43'12"E 694 mt., 29.viii.2010, Nirmala P., at light (all UASB); *Kerala:* 7♂, 2 ♀ Vellayani, N 08°25'47.5"E, 76°59'8.3", 21.vii.2015, 18 m, Prathapan KD; 19 ♀ , same data except for the date 5.vii. 2015 and ex Green gram (NBAIR, JBC, INPC, BMNH); *Maharashtra*: 3♀ Bandra, Jayakumar, 1905–152; 2 unsexed Bombay (Mumbai), 79.15 ; 1♀ Bombay, G. Bryant, 1919–147; 1 unsexed, Poona (Pune), 27.viii.1944, D. Leston, BM 1946–365; 1♀ 21.x.1944, D. Leston, BM 1945–86 (all BMNH); *Meghalaya*: 1♂, 1 ♀ SW of Cherapunjee, 23°13'15"N/ 91°40'E, 500–900 m, 11–12.v.2004, R. Businsky (all JBC); *New Delhi*: 4♀, 6 unsexed 21.viii.1968, on cowpea, Phaseolus and urd (all BMNH); *Rajasthan*: 1♀Jodhpur N 26°21'4.6"E, 73°2'39"5.VIII.2015 255 m, Prathapan K. D. (KAU); 10 unsexed Banswara 24.ix.2015, S. Ramesh Babu (KAU); *Uttar Pradesh*: Saharanpur Div., Siwalik Hills, 8.iv.1928, H. G. Champion, B.M. 1928–518 (BMNH); *Uttarakhand*: 1 ♀ Dehra Dun, 8.ix.'16, H. G.Champion, BM. 1953– 156; 1 ♀ Ranikhet, 6–8. '16, H. G.Champion, BM. 1953–156 (both BMNH); *West Bengal*: 2 unsexed Sarda, H. G. Champion, B.M. 1953–156; 2 unsexed Sunderbans, H. G. Champion, B.M. 1953–156 (all BMNH); **Sri Lanka:** 2 unsexed, 1♀ Girandurukotte no. 68, 16.xii.86 on cowpea, CIE A18795; 2♀ Maha Illupallama, 1976, R. W. Fellowes, R. W. Fellowes, on Glycine & Vigna, CIE A9047 (all BMNH); **Yemen:** 2 ♀ Al Hudaydah gov., Jabal Bura Valley forest N. P., (stream valley), 240–350 m, 15°52.4–5'N, 43°24.6–25.2'E, J. Bezděk, 4.xi.2010; 1♂, 2♀ Socotra Island, wadi Ayhaft, 12°36.5'N, 53°58.9'E, 200 m, J. Bezděk, 7–8.xi.2010 (all JBC).

Distribution. Africa (Sudan); Asia (Bangladesh, India [Andhra Pradesh, Bihar, Gujarat, Haryana, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Meghalaya, New Delhi, Orissa, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh, Uttarakhand, West Bengal], Nepal, Sri Lanka, Yemen) (Fig. 35).

Remarks. *Madurasia undulatovittata* and *M. andamanica* sp. n. are very similar. However, they can be separated as follows: eight labral setae present in *M. andamanica* sp. n. (only six labral setae visible in *M. undulatovittata*, though eight pores are present); elytral stripes are highly variable in *M. undulatovittata*, even in specimens from the same locality, collected during the same season and on the same host. The elytral pattern in *M. andamanica* sp. n. is rather consistent. The stripe in *M. undulatovittata* is wider apically in specimens where it is well defined, while in *M. andamanica* sp. n., it is narrowed apically. In *M. andamanica* sp. n., the stripe is distinct and well defined against the pale background color. Verma (1995) recorded variation in elytral color pattern. Lobe in the middle of the posterior margin of the apical abdominal ventrite in males distinct in *M. andamanica* sp. n., but poorly distinguishable in *M. undulatovittata*. The two species can easily be separated by the structure of the aedeagus. In lateral view, the apex of aedeagus of *M. andamanica* sp. n. is curved and pointed, like the beak of a parrot (Fig. 26), while the same in *M. undulatovittata* is narrowly rounded, and smoothly curved in apical 1/3 (Fig. 27). The sharply raised ridge on the ventral aspect of the aedeagus in *M. andamanica* sp. n. (Fig. 24) is characteristic, however, this ridge is absent in *M. undulatovittata* (Fig. 25). *Madurasia andamanica* sp. n. is confined to the Andaman Islands and reported to feed on pigeon pea, while *M. undulatovittata* is

transcontinental in distribution and a significant pest of a number of species of pulses in southern Asia and Africa (Sudan).

A photograph of the labels provided by Wagner & Bieneck (Fig. 38a in Wagner and Bieneck 2012) shows three labels, two of which show different information for *M. undulatovittata* (Fig. 2). Labels currently on the specimen indicate that M. Döberl designated the lectotype in 2005. However, no publication by Döberl could be traced in which this specimen is mentioned. According to Wagner and Bieneck (2012), the lectotype was designated by Wagner, and they provide photographs of both the lectotype and its labels. The photograph (Fig. 38b) in Wagner and Bieneck (2012), confirms that the specimen examined by me is the one designated as lectotype by Wagner (Fig. 1). Moreover, Wagner and Bieneck (2012) also mention that the only other specimen, a paralectotype in Motschulsky's collection, is a male from which the aedeagus has been dissected and subsequently lost. Dr Wagner's lectotype designation stands valid as that alone is published (Wagner and Bieneck 2012). Dr Döberl designated the same specimen as lectotype in 2005 as there was a long gap of nearly a decade between the lectotype designation by Dr Wagner and the publication of the same in Wagner and Bieneck 2012 (T. Wagner and M. Döberl, pers. comm., 2016). The specimen collected by Bedford on 22.ix.1923, identified as *Neorudolphia bedfordi* by Laboissière from the BMNH, probably belongs to the type series of *N. bedfordi*. The lectotype for *M. obscurella* is here designated, to have a unique name bearer and standard for its application.

Host plants. Fabaceae: *Cajanus cajan* (L.) Millsp. (red gram or pigeon pea); *Glycine max* (L.) Merr. (soybean); *Lablab purpureus* (L.) Sweet (= *Dolichos lablab* L.) (lablab bean); *Vigna aconitifolia* (Jacq.) Marechal (moth bean); *Vigna mungo* (L.) Hepper (= *P. mungo* L. = *P. radiatus* Roxb. non L.) (black gram); *Vigna radiata* (L.) R. Wilczek (= *Phaseolus aureus* Roxb. = *P. radiatus* L.) (green gram or moong); *Vigna radiata* (L.) Wilczek var. *sublobata* (Roxb.) (= *Phaseolus sublobatus* Roxb.); *Vigna umbellata* (Thunb.) Ohwi & Ohashi (rice bean) and *Vigna unguiculata* (L.) Walp. (= *Vigna sinensis* (L.) Savi ex Hausskn.) (cowpea).

Biology and management. Information on the host plants and biology of *M. undulatovittata* was generated by agricultural entomologists in India, under the name *M. obscurella*, where it is a widely distributed pest of legume crops across many agro climatic zones. The first record of this species as a pest of pulses is that by Menon and Saxena (1970). According to Naresh and Thakkur (1972), it was reported as a major pest of black gram by Naresh and Nene in 1968. However, there is no mention of this leaf beetle in Naresh and Nene (1968). Saxena et al. (1971) described it as a pest of cowpea, green gram or moong and black gram or urd, indicating that it made holes in the leaf lamina. Other recorded host plants include *Glycine* (CAB International Institute of Entomology 1990), moth bean (Pareek et al. 1983), lablab bean (Gupta and Singh 1984a, b), pigeon pea (Saxena 1977, Mishra and Saxena 1983), rice bean (Satyanarayana et al. 1995a, b) and *Vigna radiata* (L.) Wilczek var. *sublobata* (Roxb.) (=*Phaseolus sublobatus* Roxb.) (Kalaichelvan and Verma 2005).

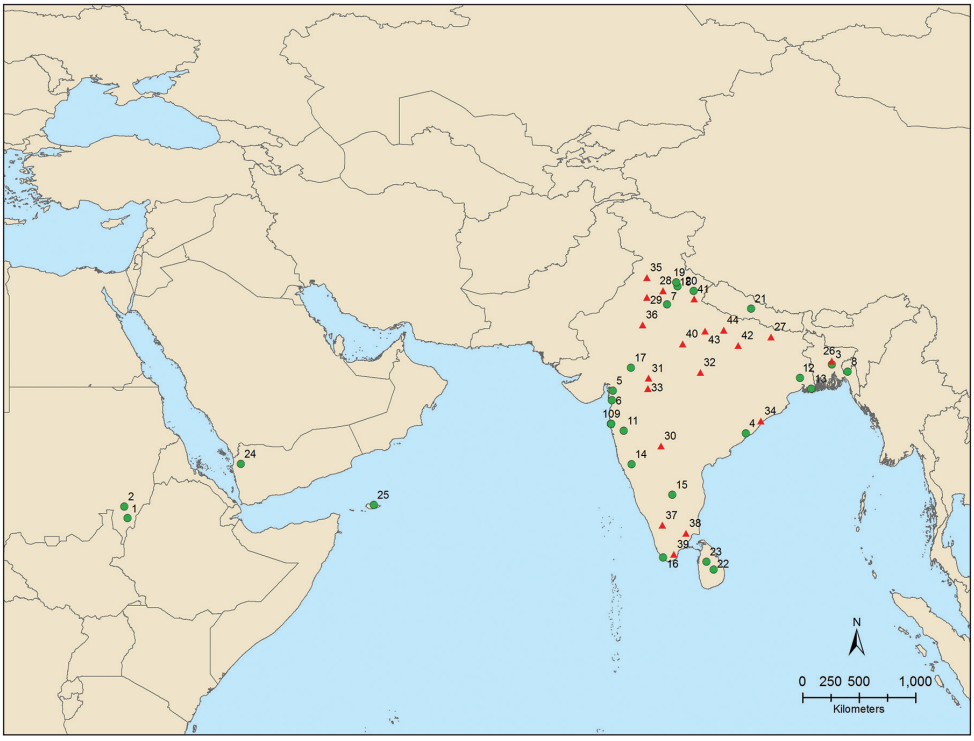


Figure 35. Distribution of *M. undulatovittata* (Motschulsky) in the Afrotropical and Oriental regions (red triangles = literature records).

Gupta and Singh (1984a, b) provided the first account of its life cycle. They recorded the total life cycle as varying between 32 and 44 days and that it completes two generations a year on green gram. A second, more detailed study of the life history was reported by Oza et al. (1996) on cowpea. Eggs were laid singly on soil near the root zone of the plant. The total duration of the life cycle, from egg to death of adult, varied between 35 and 48 days in males and 43 to 58 days in females.

The growth of plants is retarded by severe foliage injury, especially in young plants (Srivastava and Singh 1976). Leaf damage on green gram in summer and rainy season crops ranged between 5–10% and 15–50% respectively (Sinha et al. 1985). Larvae are soil dwelling and feed on root hairs (Srivastava and Singh 1976; Gupta and Singh 1981). Odak and Thakur (1978) reported larval feeding on the root nodules. Gowda and Kaul (1982) recorded adult feeding on leaves, buds and flowers. Gowda et al. (2006) also observed feeding damage by adults on the buds and flowers of pigeon pea. Reddy and Varma (1986) established transmission of southern bean mosaic virus in cowpea by *M. undulatovittata*. The success in transmission varied from 25 to 43%.

The extent of damage on black gram, green gram and cowpea was 20–60% (Srivastava and Singh 1976). This is a common pest of mung bean in the first crop season

(*kharif*) in India, coinciding with the southwest monsoon (June to October) (Tiwari 1978). Singh and Gupta (1982) estimated damage to the leaves of green gram and black gram. Infestation was more pronounced in black gram than in green gram. Infestation starts when the plants are in the two leaf-stage and the insects remain active until flowering (Dhuri and Singh 1983, Nayak et al. 2005).

In Haryana, Yadav and Yadav (1983) recorded it from cowpea and Mrig and Singh (1985) observed maximum damage on *D. lablab* during the third week of September, with the pest disappearing after the first week of November. Feleiro and Singh (1985) carried out yield–infestation studies to fix the critical stages of crops requiring protection. They observed that infestation in summer resulted in heavy yield losses, while the pest attack during the rainy season had no significant effect on yield.

Lal (1985) reviewed information on the biology and control of insect pests of mung bean, including *M. undulatovittata*, in India.

According to Faleiro et al. (1986), *M. undulatovittata* is a sporadic, but major pest of cowpea. A peak population of 10.0–10.25 beetles/10 plants in summer and 29.50–30.25 beetles/10 plants in the rainy season were recorded by Gupta and Singh (1993) in green gram. Sahoo and Patnaik (1994) recorded the incidence of insect pests in green and black gram, and their seasonal activity and the extent of damage in Orissa. *Madurasia undulatovittata* was severe on both the crops in the seedling and vegetative stages, and was the first pest to appear at seedling stage on rice bean, continuing to occur until flowering (Satyanarayana et al. 1995b). Ganapathy and Durairaj (1995) reported it as an important pest on black gram and green gram in drought prone Pudukottai District, Tamil Nadu. There was more damage in black gram (9.78%) than in green gram (1.45%). However, there are also reports of *M. undulatovittata* only being a minor pest (Devesthali and Joshi 1994, Kumar et al. 1998).

Dhuri et al. (1984) observed population buildup of *M. undulatovittata* under ambient temperature of about 32°C, longer duration of bright sunshine and high relative humidity coupled with intermittent rainfall. Sardana and Verma (1986) showed that maximum temperature and sunshine were negatively, but significantly, correlated with the population of the pest, while rainfall showed a significantly positive correlation. Maximum temperature, minimum temperature, sunshine hours and wind velocity had a significantly negative correlation with damage (Irulandi and Balasubramanian 1999). Nayak et al. (2004) reported a significantly negative correlation with minimum temperature and relative humidity during population buildup on black gram. The population did not show any correlation with maximum temperature, relative humidity and rainfall, but it was highly and significantly correlated with minimum temperature (Kumar et al. 2007).

Various cultivars of green gram (Srivastava et al. 1975, Sahoo et al. 1989, Sahoo and Hota 1991) and black gram (Sahoo et al. 1989) vary significantly in their susceptibility to the pest. Pandey et al. (1995) reported that varieties with thicker leaves were preferred by the pest.

Chemical control remains the most effective option against this pest. Several broad spectrum insecticides have been tried against *M. undulatovittata,* with varying degrees of success (Saxena et al. 1971, Naresh and Thakur 1972, Saxena et al. 1975, Verma and

Pant 1975, Verma and Lal 1976, Vyas and Saxena 1977, Verma and Lal 1978, Yadav et al. 1979, Vyas and Saxena 1981, Chaudhary et al. 1981, Rajendran et al. 1981, Vyas and Saxena 1982, Mishra and Saxena 1983, Singh et al. 1983, Singh and Gupta 1984, Sinha 1985, Gattoria and Singh 1988, Rahman 1988, 1991a, b, Chander and Singh 1989, Sinha and Sharma 1989, Verma and Dikshit 1990, Logiswaran and Gopalan 1993, Uddin et al. 1994, Das 1999, Gowda et al. 2006 and Pandey et al. 2007). Application of neem seed kernel extract had no significant effect to increase yield in mung bean (Yadav et al. 1979). Soundararajan and Chitra (2011) tried biological control agents such as *Pseudomonas flourescens* and *Beauveria bassiana* and reported that intercropping black gram with sorghum reduced infestation (Soundararajan and Chitra 2012).

Discussion

A revision of the genus *Medythia* is required to define the boundaries between it and the genus *Madurasia*. *Medythia* and *Madurasia* share the same ecological niche and are often collected together on the same host plants, as well as at light. It is likely that economic entomologists have often misidentified one for the other. *Medythia bukit* and *Medythia marginicollis*, described by Mohamedsaid (1999) from Malaysia, with pronotum broader than wide, as well as a little narrower anteriorly than posteriorly, appear atypical for the genus. Reports of a 6.05mm, ovate beetle as *M. obscurella* from Pakistan (Rizvi et al. 2012, Kamaluddin et al. 2012) are incorrect.

Madurasia andamanica sp. n. is a significant pest of red gram or pigeon pea (*C. cajan*) in the Great Andaman Islands (Bharathimeena T., pers. comm. 2015), similar to the pest status of *M. undulatovittata* elsewhere. This endemic pest of the islands, in case of accidental introduction to the mainland India, is likely to become a pest of various pulses and spread far and wide, as in the case of *M. undulatovittata.*

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RESEARCH ARTICLE

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Spatial and environmental correlates of species richness and turnover patterns in European cryptocephaline and chrysomeline beetles

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Abstract

Despite some general concordant patterns (i.e. the latitudinal richness gradient), species richness and composition of different European beetle taxa varies in different ways according to their dispersal and ecological traits. Here, the patterns of variation in species richness, composition and spatial turnover are analysed in European cryptocephaline and chrysomeline leaf beetles, assessing their environmental and spatial correlates. The underlying rationale to use environmental and spatial variables of diversity patterns is to assess the relative support for niche- and dispersal-driven hypotheses. Our results show that despite a broad congruence in the factors correlated with cryptocephaline and chrysomeline richness, environmental variables (particularly temperature) were more relevant in cryptocephalines, whereas spatial variables were more relevant in chrysomelines (that showed a significant longitudinal gradient besides the latitudinal one), in line with the higher proportion of flightless species within chrysomelines. The variation in species composition was also related to environmental and spatial factors, but this pattern was better predicted by spatial variables in both groups, suggesting that species composition is more linked to dispersal and historical contingencies than species richness, which would be more controlled by environmental limitations. Among historical factors, Pleistocene glaciations appear as the most plausible explanation for the steeper decay in assemblage similarity with spatial distance, both in cryptocephalines and chrysomelines.

Keywords

Beta diversity, biogeography, Chrysomelidae, Chrysomelinae, Cryptocephalinae, species richness

Introduction

The assessment of large-scale biogeographic patterns has proven a fruitful research discipline for understanding the ecological, evolutionary and historical processes that have determined the current distribution of biological diversity (Brown and Maurer 1989; Farrell 1998; Gaston and Blackburn 2000). In recent years, several contributions have considerably advanced in our search for a general explanation about the factors driving species distributions and clade diversification, using both empirical (Hawkins et al. 2003; Svenning and Skov 2007a; Wiens 2007) and theoretical approaches (Hubbell 2001; McGill 2010). However, probably because of the complexity of the problem and the difficulty to perform controlled experiments due to the large temporal and spatial scale of the processes potentially involved, a general comprehensive theoretical framework to explain biological diversity and its distribution on Earth is still missing. From the empirical point of view, one important shortfall is the under-representation of invertebrates in biogeographical studies, caused by the difficulty to sample and identify invertebrate species, compared to vertebrates or plants. This has led to a marked scarcity of invertebrate distribution data that prevented biogeographers to assess invertebrate biodiversity patterns. As a result, most biogeographical and macroecological theories have been tested using vertebrate and plant data, a circumstance that does not fit very well with the fact that invertebrates are the largest fraction of local, regional or global biodiversity (Erwin 1982; Gaston 1991; Odegaard 2000; Storck 1997). Therefore, a potential avenue for progressing towards a full understanding of biodiversity patterns would be to gather invertebrate distribution data and assess large-scale biodiversity patterns.

Recent examples of this approach have proven particularly successful. A few invertebrate taxa (e.g., diurnal Lepidoptera) have been particularly well sampled, so diversity patterns are relatively well known (Hawkins 2010; Hawkins and Porter 2003). In other cases, the acquisition of reliable distributional data requires considerable sampling effort (e.g., Baselga et al. 2013; Papadopoulou et al. 2011), but an alternative solution to go around the impediment caused by the lack of accurate distributional data is to use country level inventories. Despite its limitations derived from the coarse spatial resolution and the unequal country area (Keil and Hawkins 2009), this kind of data has proven robust enough to unveil the major correlates of species richness and turnover patterns, as shown for water beetles (Ribera et al. 2003), longhorn beetles (Baselga 2008), ground beetles (Schuldt and Assmann 2009; Schuldt et al. 2009), springtails (Ulrich and Fiera 2009), Odonata and Lepidoptera (Keil and Hawkins 2009), and darkling beetles (Fattorini and Baselga 2012). Regarding beetles, the important contributions of databases as Fauna Europaea (Fauna Europaea version 2.6, available online at http://www.faunaeur.org) and catalogues as the Catalogue of Palaearctic Coleoptera (Löbl and Smetana 2003; 2004; 2006; 2010) have been crucial for making possible the aforementioned analyses, including the comparative assessment of biodiversity patterns across multiple beetle taxa (Baselga et al. 2012b; Gómez-Rodríguez et al. 2015).

The studies cited above have shown that different beetle taxa have different diversity patterns across continental Europe. Regarding species richness, the steepness of the negative latitudinal richness gradient has been shown to be related to the dispersal capacity of taxa (Baselga et al. 2012b), suggesting a causal link between the loss of species richness to the North and the incomplete re-colonization of northern regions after Pleistocene glaciations (Baselga et al. 2012a; Svenning et al. 2008; Svenning and Skov 2007a). Regarding the variation in species composition (i.e. beta diversity), available evidence suggests that both dispersal capacity and niche traits are responsible for differences in beta diversity patterns among beetle taxa (Gómez-Rodríguez et al. 2015). Within this context, the aim of this study is to assess the biodiversity patterns across continental Europe in two major clades of leaf beetles: Cryptocephalinae (excluding clytrines) and Chrysomelinae. To this end, the variation in species richness, species composition and species spatial turnover has been assessed in both leaf beetle groups, and their relationship with two sets of environmental and spatial variables. The rationale behind is to confront the explanatory capacity of current environmental factors that would reflect the effect of niche-based processes (Hawkins et al. 2003) with purely spatial variables that could reflect alternative dispersal-based processes, as historical colonization events or neutral dynamics (Lobo et al. 2001; Svenning and Skov 2007a).

Methods

Data

The study area included continental Europe. Thirty-six inventories of Cryptocephalinae and Chrysomelinae were obtained from Löbl and Smetana (2010). Delimitations of both subfamilies also follow Löbl and Smetana (2010), so, for example, clytrines were not included within Cryptocephalinae. Most of the 36 inventories refer to European countries, but European Russia was divided in three separate territories (northern, central and southern) due to its extremely large area, while Bosnia and Croatia were included in a single checklist. Finally, only the European portion of Turkey was considered here. For simplicity, all these territorial units are hereafter referred to as "countries." Islands were excluded from this study to avoid insularity effects, which could confound general continental patterns of diversity. In total, 257 cryptocephaline and 328 chrysomeline species (or subspecies) were considered in this study.

Three sets of variables were obtained for each country: (i) area; (ii) spatial position: mean, minimum and maximum longitude (Long, $Long_{min}$, $Long_{max}$), mean, minimum and maximum latitude (Lat, Lat_{min}, Lat_{max}) and longitudinal and latitudinal range ($Long_{ran}$ and Lat_{ran}); and (iii) environmental factors: mean altitude (Alt); altitudinal range (Alt_{ran}); annual mean temperature (T_{ann}); spatial range of T_{ann} (T_{ran}); maximum temperature of the warmest month (T_{max}); minimum temperature of the coldest month (T_{min}); annual precipitation (P_{ann}); spatial range of P_{ann} (P_{ran}); precipitation of

driest quarter (P_{dri}); and spatial range of P_{dri} (P_{drn}). Topographic and climatic variables were obtained from WorldClim 1.4 layers (Hijmans et al. 2005). Thereafter, mean or range values for each country were extracted from a European GIS database (0.08 degrees resolution) using IDRISI (Clark Labs 2000), together with their respective areas (km^2) and geographical coordinates (lat/long).

Analytical methods

The relationship between diversity attributes (species richness, species composition and spatial turnover) and the aforementioned variables was independently assessed for cryptocephalines and chrysomelines:

1. *Variation in species richness*. Multiple relationships between species richness and the explanatory variables were analysed using regression modelling (Legendre and Legendre 1998) performed with Statistica 7.0 (StatSoft 2004). Linear, quadratic and cubic functions of the variables were independently regressed against each response variable to determine significant relationships. Significant terms for each set (i.e. area [A], environment [E], and spatial variables [S]) were selected by means of a backward stepwise procedure. Finally, to partition the variation in species composition among A, E and S sets of variables, models including all the possible combinations of sets were performed (i.e. A+E, A+S, E+S and A+E+S). This allowed to quantify the relative importance of the unique contributions of area (A), environment (E) and spatial variables (S), and their respective shared variances (Legendre and Legendre 1998). Such an approach allows non-independent explanatory variables to be dealt with, as it is explicitly designed to identify the portions of variation that are jointly accounted for by different sets of variables and those portions that are independently accounted for (Borcard et al. 1992). Area is included as a covariable in order to control for the effect of differences in area among sampling units.
2. *Variation in species composition*. The variation in species composition among countries was analysed with the Simpson's index of dissimilarity (β_{sim}) (Lennon et al. 2001; Simpson 1943). This index quantifies the spatial turnover component of beta diversity, i.e. the dissimilarity caused by the substitution of some species by others, removing the effect of richness differences on beta diversity (Baselga 2010; 2012). A pair-wise dissimilarity matrix based on β_{sim} was computed using command *beta.pair* in R package *betapart* (Baselga and Orme 2012). The patterns of variation in species composition were visually inspected by means of an agglomerative hierarchical clustering analysis based on the β_{sim} dissimilarity matrix, using command *hclust* (R Development Core Team 2013) with average linkage, in order to identify groups of territories with similar fauna. Thereafter, we explored the spatial and environmental correlates of the turnover pattern. A Constrained Analysis of Principal Coordinates

(CAP) was computed in R using the vegan package (Oksanen et al. 2007) to examine the relationship between variation in the table of species occurrences and the three sets of predictor variables. CAP was selected because it can be computed with any dissimilarity index with ecological significance and, therefore, β_{sim} dissimilarity was preserved in the constrained ordination. Area, the nine aforementioned environmental variables, and spatial variables (the nine terms of a third degree polynomial of mean latitude and longitude, i.e. Trend Surface Analysis, see Legendre and Legendre 1998) were used as variables to perform constrained ordinations yielding respectively A, E and S models. Since the order of inclusion in the model affects the significance computed by the permutation tests (Borcard et al. 1992; Oksanen et al. 2013), the amount by which the explained variation was reduced due to the elimination of a single variable (compared with the complete model) was tested prior to the final analysis. This allowed the individual variables to be ranked in order of their independent contribution to the total variation in the response variable (from greatest to least), and the variables were included in the significance test in this order. Only significant variables were retained ($p < 0.05$) to avoid overfitting due to the inclusion of non-significant terms. Finally, variation partitioning among sets of variables was used to quantify the relative importance of the unique contributions of area (A), environment (E) and spatial variables (S), and their respective shared variances (Legendre and Legendre 1998).

3. *Variation in spatial turnover*. Given the observed patterns of spatial variation in species composition (i.e. spatial turnover), we assessed whether the rates of turnover with spatial distance were significantly different in northern and southern Europe. To do this, we transformed the pair-wise dissimilarity matrix based on β_{sim} on similarities (i.e. $1-\beta_{sim}$), and split the data into two groups: northern European countries, with mean latitude higher than 48 degrees (n=19), and southern European countries (n=17). Thereafter, we assessed the decay of assemblage similarity with spatial distance in Northern and Southern European datasets, using non-linear regression on similarity matrices to fit exponential decay curves expressed as $y=a^*e^{-bx}$, where y is similarity at distance x, a initial similarity and $-b$ the rate of distance decay. Spatial distance was computed in km as the Euclidian distance between the UTM centroids of countries. Finally, to assess for significant differences in distance decay slopes between Northern and Southern regions, the frequency distributions of the parameters were estimated by bootstrapping. A frequency distribution of 1,000 slopes was retrieved by bootstrapping, using the boot package (Canty and Ripley 2008). When assessing the significance of one slope being larger in one region than in the other, the probability of obtaining the opposite result by chance was empirically computed by comparing the estimated distributions of parameters. The slopes found for Cryptocephalinae and Chrysomelinae were also compared to those of the European Cerambycidae, for which the diversity patterns were previously investigated (Baselga 2007; 2008; 2010).

Results

Variation in species richness

The assessment of variation in species richness revealed a clear latitudinal gradient, with a significant reduction of diversity to the North in both groups (Figure 1a–b, Tables 1–2). In Cryptocephalinae, species richness was also significantly and positively related to country area, altitudinal range, annual mean temperature, maximum temperature of the warmest month, and spatial range of precipitation of driest quarter. In the case of mean altitude, longitudinal and latitudinal range and spatial range of annual precipitation the relationships were curvilinear, with maximum richness at intermediate values. After removing redundant variables, the spatial model for Cryptocephalinae comprised minimum latitude, and the quadratic function of longitudinal range, explaining 51.3% of the variance in species richness. The environmental model included maximum temperature of the warmest month, the spatial ranges of annual precipitation and precipitation of the driest quarter, and the quadratic function of altitudinal range, explaining 74.5% of the variance in richness. Variance partitioning showed that the largest portion of variation in richness (38.0%) was jointly explained by the environmental and spatial models, with a small contribution of country area (Figure 2a). The unique contribution of the environmental model (30.9%) was much larger than the unique contribution of the spatial model (1.7%).

In Chrysomelinae, in addition to the negative relationship with latitude, species richness was also significantly and negatively related to minimum longitude and significantly and positively related to country area, mean altitude, annual mean temperature and spatial range of precipitation of driest quarter. In the case of longitudinal, latitudinal and altitudinal range and spatial range of annual precipitation the relationships were curvilinear, with maximum richness at intermediate values. After excluding collinear variables, the spatial model for Chrysomelinae consisted of mean latitude, latitudinal range, minimum longitude and the quadratic function of longitudinal range, explaining 71.2% of the variance in species richness. The environmental model comprised the spatial range of precipitation of the driest quarter, and the quadratic function of spatial range of annual precipitation, explaining 59.3% of the variance in richness. Variance partitioning showed that the largest portion of variation in richness (34.5%) was jointly explained by the environmental and spatial models, with a small contribution of country area (Figure 2b). The unique contribution of the spatial model (23.5%) was much larger than the unique contribution of the environmental model (13.9%).

Variation in species composition

The assessment of variation in species composition (beta diversity) revealed the existence of similar patterns in both subfamilies, with the presence of singular faunas in

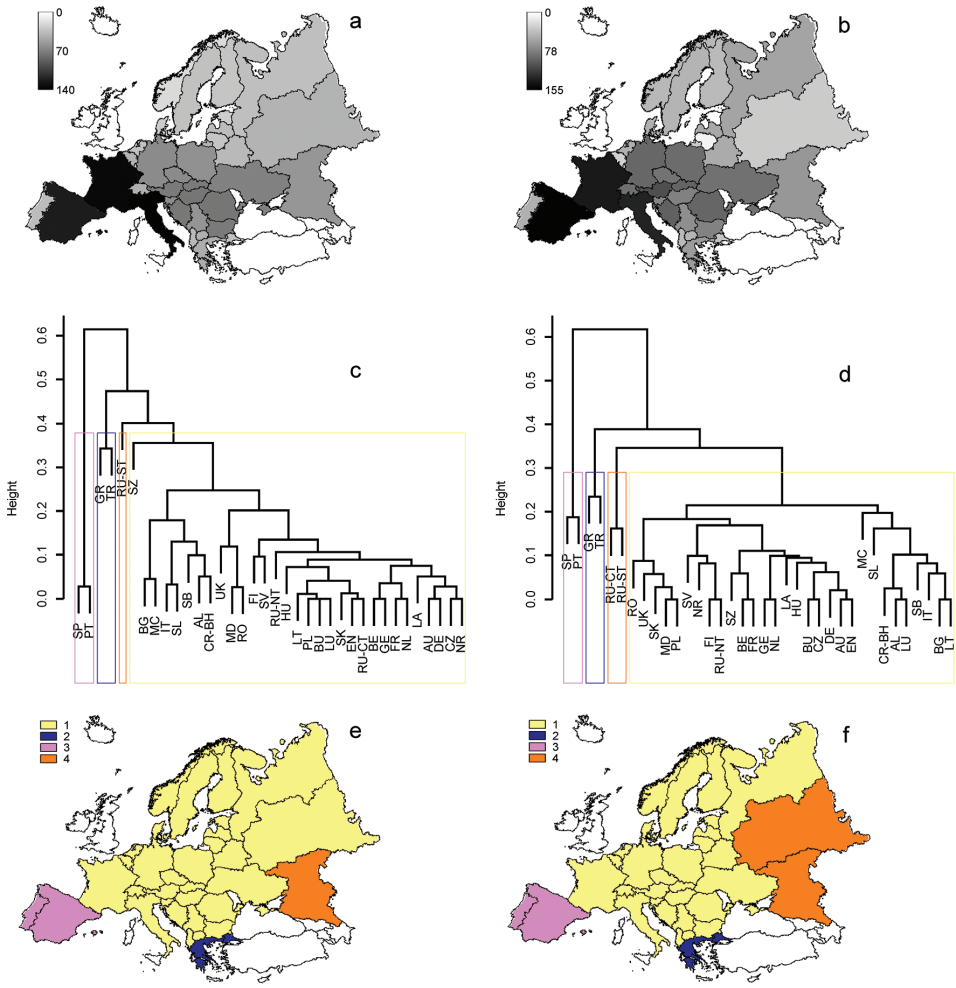

Figure 1. Patterns of variation in species richness (**a, b**), hierarchical clustering based in β_{sim} (**c, d**) and mapping of 4 major clusters (**e, f**) for cryptocephalines (left column: **a, c, e**) and chrysomelines (right column: **b, d, f**). Colours correspond to the 4 major clusters. Countries' abbreviations follow those of Löbl and Smetana (2010).

the Iberian Peninsula, the Greek Peninsula, and Southern Russia, while the remaining European regions formed a relatively uniform cluster of countries with similar composition (Figure 1c–f). When the correlates of these patterns of variation in species composition were assessed, it turned out that faunal composition showed no significant relationship with species richness (pseudo-$F_{1,34}$ = 1.10, p = 0.33 and pseudo-$F_{1,34}$ = 0.66, p = 0.72, for Cryptocephalinae and Chrysomelinae, respectively). The same lack of significant relationship was found in both groups between species composition and the logarithm of country area (pseudo-$F_{1,34}$ = 1.23, p = 0.29 and pseudo-$F_{1,34}$ = 1.63, p = 0.11, respectively). In contrast, the assessment of the contribution of environmental

Table 1. Relationships between cryptocephaline species richness and variables, and models for each group of variables. The sign of the relationships and percentage of explained variance (%) are shown, with their respective *F* parameters, degrees of freedom (d.f.) and p-values. A, S and E are the area, spatial and environmental models, respectively. f is the second order polynomial of the variable considered.

Variable	Function	Variance (%)	F	d.f.	p
Area	logarithmic (+)	12.6	4.9	1, 34	0.034
Long	ns	6.0	2.2	1, 34	0.150
$Long_{min}$	ns	10.0	3.8	1, 34	0.060
$Long_{max}$	ns	4.0	1.4	1, 34	0.244
$Long_{ran}$	quadratic (+,-)	21.9	4.6	2, 33	0.017
Lat	linear (-)	22.2	9.7	1, 34	0.004
Lat_{min}	linear (-)	29.7	14.4	1, 34	0.001
Lat_{max}	linear (-)	15.4	6.2	1, 34	0.018
Lat_{ran}	quadratic (+,-)	16.7	3.3	2, 33	0.050
Alt	quadratic (+,-)	19.6	4.0	2, 33	0.027
Alt_{ran}	linear (+)	28.3	13.4	1, 34	0.001
T_{ann}	linear (+)	16.9	6.9	1, 34	0.013
T_{ran}	linear (+)	30.1	14.6	1, 34	0.001
T_{max}	linear (+)	16.5	6.7	1, 34	0.014
T_{min}	ns	10.8	4.1	1, 34	0.050
P_{ann}	ns	0.1	0.0	1, 34	0.894
P_{ran}	quadratic (+,-)	31.8	7.7	2, 33	0.002
P_{dri}	ns	0.7	0.3	1, 34	0.619
P_{drn}	linear (+)	34.7	18.1	1, 34	<0.001
Model for A	log(Area)	12.6	4.9	1, 34	0.034
Model for E	$T_{max} + P_{ran} + P_{drn} + f$ Alt	76.1	19.2	5, 30	<0.001
Model for S	$f\ Long_{ran} + Lat_{min}$	51.3	11.3	3, 32	<0.001
Model S + A	$f\ Long_{ran} + Lat_{min}$ + log(Area)	52.3	8.5	4, 31	<0.001
Model E + S	$T_{max} + P_{ran} + P_{drn} + f$ Alt + $f\ Long_{ran} + Lat_{min}$	82.0	15.3	8, 27	<0.001
Model E + A	$T_{max} + P_{ran} + P_{drn} + f$ Alt + log(Area)	81.5	21.3	6, 29	<0.001
Model E + S +A	$T_{max} + P_{ran} + P_{drn} + f$ Alt + $f\ Long_{ran} + Lat_{min}$ + log(Area)	83.2	14.3	9, 26	<0.001

and spatial variables to explaining beta diversity yielded significant contributions of both sets of variables (E and S models).

In Cryptocephalinae, the environmental model for variation in species composition included mean altitude, mean annual temperature, minimum temperature of the coldest month, and precipitation of the driest quarter as significant variables (pseudo-$F_{4,31}$ = 6.73, p < 0.001) and explained 46.5% of the variation (Table 3). The spatial model consisted of the cubic polynomial of longitude, the quadratic polynomial of latitude and the interaction between longitude and latitude. To keep the number of variables balanced, only the first four terms with larger independent contribution (Lat, Long, Lat*Long and $Long^2$) were included in the final spatial model (pseudo-$F_{4,31}$ = 9.08, p < 0.001), which explained 54.0% of the variation. Partitioning of the variation in species composition among sets of variables showed that the largest fraction of variation

Table 2. Relationships between chrysomeline species richness and variables, and models for each group of variables. The sign of the relationships and percentage of explained variance (%) are shown, with their respective *F* parameters, degrees of freedom (d.f.) and p-values. A, S and E are the area, spatial and environmental models, respectively. *f* is the second order polynomial of the variable considered.

Variable	Function	Variance (%)	F	d.f.	p
Area	logarithmic (+)	17.1	7.0	1, 34	0.012
Long	ns	9.9	3.7	1, 34	0.062
$Long_{min}$	linear (-)	17.0	6.9	1, 34	0.013
$Long_{max}$	ns	5.2	1.9	1, 34	0.179
$Long_{ran}$	quadratic (+,-)	24.6	5.4	2, 33	0.009
Lat	linear (-)	14.3	5.7	1, 34	0.023
Lat_{min}	linear (-)	19.4	8.2	1, 34	0.007
Lat_{max}	ns	8.8	3.3	1, 34	0.079
Lat_{ran}	quadratic (+,-)	23.3	5.0	2, 33	0.013
Alt	linear (+)	18.0	7.4	1, 34	0.010
Alt_{ran}	quadratic (+,-)	41.1	11.5	2, 33	<0.001
T_{ann}	ns	7.3	2.7	1, 34	0.110
T_{ran}	linear (+)	33.9	17.4	1, 34	<0.001
T_{max}	ns	5.0	1.8	1, 34	0.191
T_{min}	ns	6.6	2.4	1, 34	0.129
P_{ann}	ns	2.2	0.8	1, 34	0.385
P_{ran}	quadratic (+,-)	43.0	12.4	2, 33	<0.001
P_{dri}	ns	2.8	1.0	1, 34	0.331
P_{drn}	linear (+)	44.5	27.3	1, 34	<0.001
Model for A	log(Area)	17.1	7.0	1, 34	0.012
Model for E	$P_{dri} + f\ P_{ran}$	60.0	16.0	3, 32	<0.001
Model for S	$Long_{min} + f\ Long_{ran} + Lat + Lat_{ran}$	71.2	14.8	5, 30	<0.001
Model S + A	$Long_{min} + f\ Long_{ran} + Lat + Lat_{ran} + log(Area)$	75.1	14.6	6, 29	<0.001
Model E + S	$P_{dri} + f\ P_{ran} + Long_{min} + f\ Long_{ran} + Lat + Lat_{ran}$	88.0	24.8	8, 27	<0.001
Model E + A	$P_{dri} + f\ P_{ran} + log(Area)$	65.6	14.8	4, 31	<0.001
Model E + S +A	$P_{dri} + f\ P_{ran} + Long_{min} + f\ Long_{ran} + Lat + Lat_{ran} + log(Area)$	89.1	23.5	9, 26	<0.001

(34.2%) was jointly explained by both models, and that the unique contribution of the spatial model (19.8%) was larger than the unique contribution of the environmental model (12.3%).

In Chrysomelinae, the environmental model for variation in species composition included mean altitude, mean annual temperature, maximum temperature of the warmest month and minimum temperature of the coldest month (pseudo-$F_{4,31} = 4.13$, $p < 0.001$), explaining 34.7% of total variability (Table 4). The spatial model consisted of the quadratic function of longitude, latitude, the interaction between longitude and latitude and the interaction between longitude and the quadratic term of latitude. Only the first four ones with larger independent contribution (Long, Lat*Long, $Long^2$ and Lat^2*Long) were included in the final spatial model (pseudo-$F_{4,31} = 7.01$, $p < 0.001$), which explained 47.5% of the variation. Partitioning of the variation in spe-

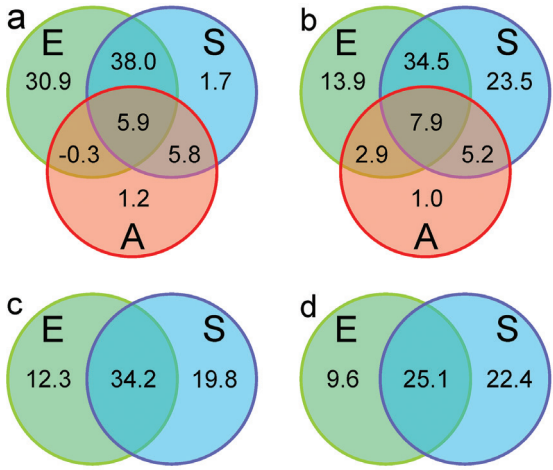

Figure 2. Partitioning of the variation (%) in species richness (**a, b**) and species composition (**c, d**) among groups of explanatory sets (A=area, E=environment and S=spatial variables) for European Cryptocephalinae (left column: **a, c**) and Chrysomelinae (right column: **b, d**).

Table 3. Relationships between variation in cryptocephaline species composition (β_{sim}) and variables, and models for each group of variables. Percentages of variation explained are shown, with their respective Pseudo-*F* parameters, degrees of freedom (d.f.) and p-values. S and E are the spatial and environmental models, respectively.

Variable	Variation (%)	Pseudo-*F*	d.f.	p
log(Area)	3.5	0.77	1, 34	0.288
Long	12.7	4.96	1, 34	0.002
$Long^2$	6.3	2.30	1, 34	0.058
$Long^3$	5.2	1.87	1, 34	0.094
Lat	25.1	11.37	1, 34	<0.001
Lat^2	23.8	1.74	1, 34	0.085
Lat^3	22.4	9.82	1, 34	<0.001
Long*Lat	11.4	4.37	1, 34	0.004
$Long^2$*Lat	6.1	2.22	1, 34	0.067
Long*Lat^2	11.4	4.35	1, 34	0.003
Alt	8.6	3.19	1, 34	0.023
Alt_{ran}	3.6	1.28	1, 34	0.252
T_{ann}	23.2	10.27	1, 34	<0.001
T_{ran}	3.2	1.12	1, 34	0.339
T_{max}	22.1	9.67	1, 34	<0.001
T_{min}	18.6	7.79	1, 34	<0.001
P_{ann}	6.1	2.22	1, 34	0.038
P_{ran}	5.2	1.85	1, 34	0.089
P_{dri}	6.0	2.17	1, 34	0.047
P_{drn}	4.0	1.42	1, 34	0.196
Model for E	46.5	6.73	4, 31	<0.001
Model for S	54.0	9.08	4, 31	<0.001
Model E+S	66.3	6.64	8, 27	<0.001

Table 4. Relationships between variation in chrysomeline species composition (β_{sim}) and variables, and models for each group of variables. Percentages of variation explained are shown, with their respective Pseudo-*F* parameters, degrees of freedom (d.f.) and p-values. S and E are the spatial and environmental models, respectively.

Variable	Variation (%)	Pseudo-*F*	d.f.	p
log(Area)	5.2	1.88	1, 34	0.112
Long	13.6	5.36	1, 34	<0.001
$Long^2$	8.2	3.03	1, 34	0.009
$Long^3$	7.4	2.71	1, 34	0.042
Lat	17.6	7.25	1, 34	<0.001
Lat^2	16.8	6.87	1, 34	<0.001
Lat^3	16.0	6.48	1, 34	<0.001
Long*Lat	12.1	4.66	1, 34	<0.001
$Long^2$*Lat	7.7	2.82	1, 34	0.027
Long*Lat^2	10.9	4.18	1, 34	0.002
Alt	4.5	1.60	1, 34	0.119
Alt_{ran}	3.5	1.24	1, 34	0.268
T_{ann}	16.7	6.81	1, 34	<0.001
T_{ran}	2.4	0.84	1, 34	0.564
T_{max}	15.1	2.82	1, 34	<0.001
T_{min}	15.2	6.08	1, 34	<0.001
P_{ann}	4.9	1.76	1, 34	0.088
P_{ran}	2.5	0.87	1, 34	0.525
P_{dri}	4.9	1.76	1, 34	0.069
P_{drn}	1.8	0.62	1, 34	0.794
Model for E	34.7	4.13	4, 31	<0.001
Model for S	47.5	7.01	4, 31	<0.001
Model E+S	57.1	4.49	8, 27	<0.001

cies composition among sets of variables showed that the largest fraction of variation (25.1%) was again jointly explained by both models, but in Chrysomelinae the unique contribution of the spatial model (22.4%) was almost as large as the joint fraction, and more than two times the unique contribution of the environmental model (9.6%).

Variation in spatial turnover

Assemblage similarity was significantly related to spatial distance in both subfamilies and European regions (Northern and Southern regions, Figure 3a–b), although the fit of exponential decay models was much better in the South ($r^2 = 0.57$, $p < 0.001$ and $r^2 = 0.58$, $p < 0.001$ for Cryptocephalinae and Chrysomelinae, respectively) than in the North ($r^2 = 0.16$, $p < 0.001$ and $r^2 = 0.23$, $p < 0.001$). Likewise, as shown in Figure 3d, the slopes of the distance-decay patterns in Southern Europe (b = -0.00051 and -0.00045 for Cryptocephalinae and Chrysomelinae, respectively) were significantly steeper ($p < 0.001$) than in Northern Europe (b = -0.00010 and -0.00014). Differences

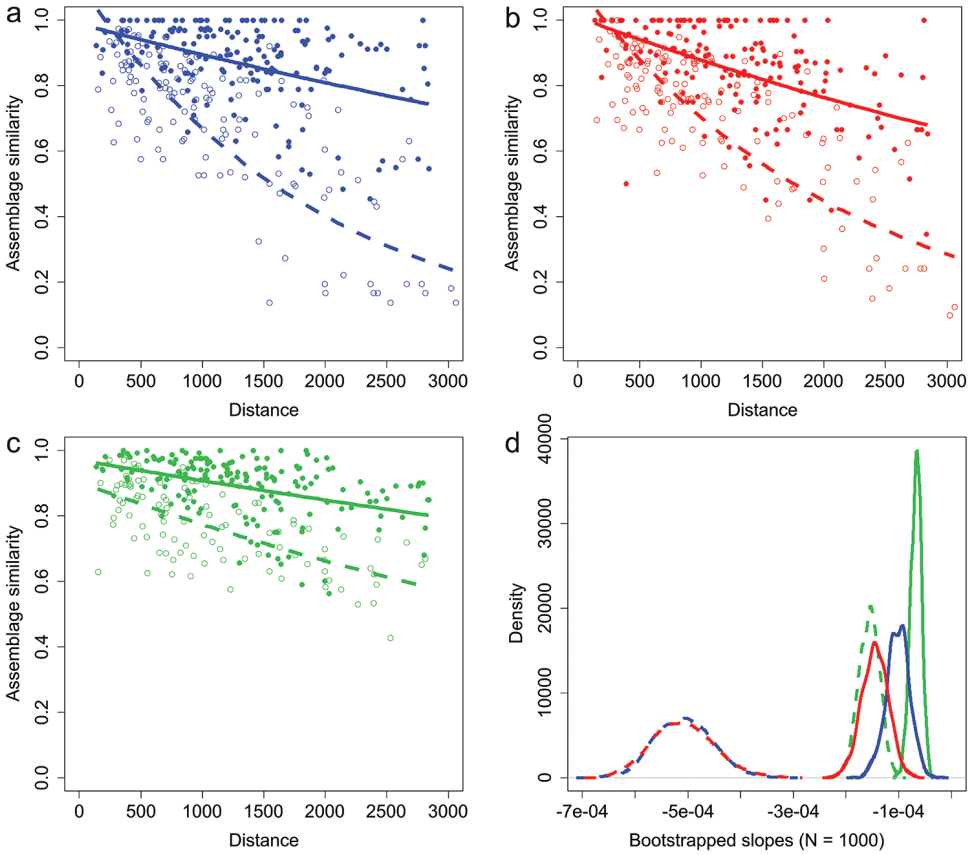

Figure 3. Distance decay of similarity with spatial distance in Northern Europe (solid dots, solid line) and southern Europe (hollow dots, dashed line) for cryptocephalines (**a**, blue), chrysomelines (**b**, red) and longhorn beetles (**c**, green). The density plots in (**d**) show the distribution of 1000 bootstrap replicates of the distance decay slopes (solid lines: northern Europe, dashed lines: southern Europe, colours corresponding to **a, b**, and **c**).

between distance-decay slopes in Cryptocephalinae and Chrysomelinae were neither significant in southern ($p = 0.497$) nor in northern Europe ($p = 0.105$). In contrast, the slopes of both Chrysomelinae and Cryptocephalinae in southern Europe were significantly steeper ($p < 0.001$) than that of the family Cerambycidae (Figure 3c–d).

Discussion

The most prominent pattern in species richness of both cryptocephalines and chrysomelines is the existence of a clear latitudinal gradient. This is an almost universal macroecological pattern (Willig et al. 2003), that in Europe has been thoroughly documented in numerous taxa, especially vertebrates and plants (Araújo et al. 2008;

Montoya et al. 2007; Rodríguez et al. 2005; Svenning and Skov 2007b; Whittaker et al. 2007) and some invertebrates such as butterflies (Hawkins and Porter 2003) and springtails (Ulrich and Fiera 2009), for example. Within Coleoptera, the situation is not very different and most taxa display a clear latitudinal gradient (Baselga 2008; Fattorini and Baselga 2012; Hortal et al. 2011; Schuldt and Assmann 2009). However, the steepness of the latitudinal richness gradient is subject of a great variation among different beetle taxa (Baselga et al. 2012b), with some taxa as families Scolytidae and Silphidae presenting weak (almost flat) gradients, while other taxa as genera *Trechus* (Carabidae) and *Otiorhynchus* (Curculionidae) having steep latitudinal gradients. This variation is partially explained by the differences in dispersal ability among beetle taxa, suggesting that limited post-glacial re-colonization processes are a major determinant of beetle richness gradients in Europe (Baselga et al. 2012b).

Other significant variables of species richness in both cryptocephalines and chrysomelines were country area, temperature, altitude and the range of precipitations. The major difference between both groups was the significant negative relationship between richness and minimum longitude in chrysomelines but not in cryptocephalines. Therefore, species richness models were qualitatively similar in both groups, although a relevant quantitative difference was also observed. While in cryptocephalines the environmental variables were relatively more important than the spatial ones (unique contributions of 31% vs. 2%, respectively), in chrysomelines the situation was reversed, with a largest contribution of spatial rather than environmental factors (unique contributions of 24% vs. 14%, respectively). These figures may be interpreted as cryptocephaline richness being more determined by environmental conditions (mostly temperature), while chrysomeline richness being more subject of undetermined factors causing a spatial structure independent of climatic factors, likely historical factors linked to diversification and limited dispersal. This interpretation is in accordance with the fact that flightless species are common among chrysomelines but not among cryptocephalines. In a wider context, the differences between species richness models for cryptocephalines and chrysomelines suggest that the preponderance of niche-based processes related to energy and water availability dynamics (Currie et al. 2004; Hawkins et al. 2003), niche conservatism and historical climate change (Hortal et al. 2011) or dispersal-based processes (Baselga et al. 2013; Svenning and Skov 2007a) is probably taxon-dependent.

The patterns of variation in species composition were, in contrast, very similar in both groups: several European southern regions, namely Iberian and Greek peninsulas and southern Russia, presented singular species composition differing from the rest of the continent, which harboured a relatively homogeneous fauna. Therefore, the environmental correlates of variation in species composition were very similar in both groups, including altitude and several temperature-related variables. The only difference was the significant relationship between species composition and precipitation of the driest quarter in cryptocephalines but not in chrysomelines, suggesting that cryptocephaline species are filtered by water availability but chrysomeline species are not. Likewise, the spatial determinants are similar in both groups, including both latitudinal and longitudinal components. The partition of variation in species composition

between environmental and spatial variables revealed that a strong spatial structure (independent of environmental factors) exist in both groups, as also found in other beetle taxa (Baselga 2008; Fattorini and Baselga 2012). This suggests that, besides the effect of environmental factors causing species turnover as a result of differential physiological requirements, other spatially-structured processes have deeply influenced the replacement of cryptocephaline and chrysomeline faunas across Europe. Speciation processes and limited dispersal are likely behind such spatial patterns, and the effect of Pleistocene glaciations appears as one of the most plausible explanations. Indeed, while northern European faunas were obliterated during Pleistocene glaciations, southern European regions have acted as faunal refugia during glacial cycles. This has impacted southern European faunas in two ways. In first place, pre-Pleistocene assemblages were not obliterated, providing more time for in-situ diversification (Hewitt 2004), compared to northern regions where beetle assemblages are necessarily recent (i.e. arrived there since last glaciation). Indeed, several studies have reported the pre-Pleistocene origin of Mediterranean endemics (Andújar et al. 2012; Condamine et al. 2013; Hidalgo-Galiana and Ribera 2011; Ribera et al. 2010). In second place, glacial refugia have also acted as speciation centres (Gomez-Zurita et al. 2012; Ribera and Vogler 2004), increasing the singularity of southern faunas. Among the different glacial refugia that could serve as sources of re-colonization of northern regions, the Italian peninsula is the only one that does not present a markedly singular fauna (despite its high species richness), a fact also observed on longhorn and darkling beetles (Baselga 2008; Fattorini and Baselga 2012). This might suggest that the Italian peninsula was one of the major re-colonization sources, making most of the European faunas similar to the Italian one, but less so to the Iberian and Greek assemblages, in which re-colonization events seem to have been much more limited. Finally, the higher relevance of spatial factors for species composition compared to species richness suggests that species composition is more linked to dispersal and historical contingencies than species richness, which would be more controlled by environmental limitations.

The effects of Pleistocene glaciations seem also to be behind the differences in the patterns of distance decay of similarity between northern and southern regions. As it could be predicted from the visual inspection of maps illustrating the distribution of major clusters, the spatial turnover of cryptocephaline and chrysomeline assemblages with spatial distance is much steeper in southern Europe compared to northern Europe. As previously suggested elsewhere (Baselga 2010; Baselga et al. 2012a), this difference could be interpreted as a consequence of Pleistocene glaciations. Southern regions have retained beetle assemblages during larger periods of time, allowing speciation processes to accumulate and thus increasing the differences between assemblages. In contrast, beetle assemblages in northern regions were obliterated during glaciations, and the species currently present there are all generalist ones with high dispersal ability (which have allowed them to colonize the continent from southern refugia). Interestingly, differences between northern and southern slopes of the distance decay pattern are much larger in both leaf beetle taxa than in longhorn beetles. The reason for this larger difference is that, in southern Europe, leaf beetles present a much steeper distance decay of similar-

ity than longhorn beetles. In contrast, the decay of similarity with spatial distance in northern Europe is more similar for the three taxa. In other words, differences in diversity patterns between the two leaf beetle taxa and the longhorn beetles are more marked in southern than in northern Europe, suggesting again that the relative homogeneity of northern faunas is a phenomenon resulting from a common process in all taxa, likely post-glacial re-colonization by only those species with high dispersal ability, in line with previous evidence (Baselga et al. 2012b). In contrast, southern faunas would be composed by older assemblages and differences in diversity patterns of taxa had more time to accumulate, reflecting dispersal, ecological and evolutionary differences.

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