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The shape of endemics: Notes on male and female genitalia in the genus *Maniola* (Schrank, 1801), (Lepidoptera, Nymphalidae, Satyrinae)

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Abstract

Butterflies of the genus *Maniola* are known for their large morphological variation, at the inter- as well as intraspecific level. Given the overlap in wing-patterns, habitat selection, and geographic distribution of various *Maniola* species, genitalia morphology is sometimes the only possibility to tell specimen apart. In this paper we describe diagnostic characters to distinguish different *Maniola* species by means of their genitalia. Included is also the first detailed description and illustration of the genitalia apparatus of the Sardinian endemic *Maniola nurag*. Further, we describe two Sardinian individuals with intermediate characteristics between *Maniola nurag* and *Maniola jurtina*, and propose that they are hybrids. Further, we shortly discuss the justification of the species status for the island endemics *Maniola chia* and *Maniola cypricola*.

Contents

Introduction

"Made up of concave and convex hills and valleys", was one of the first descriptions of the genital structure of a male Meadow Brown, *Maniola jurtina* (L.), meant to emphasize that this species' genitalia are very irregularly shaped for a Satyrid (Muschamp, 1915). Since then, geographic variation in genital morphology of the Meadow Brown has been extensively discussed (Thomson, 1973, 1976; Goulson, 1993). In recent decades, two new *Maniola* species have been described (Thomson, 1987, 1990). First, the island endemic, *Maniola chia* Thomson, 1987, whose distribution is restricted to the Greek island of Chios. Second, *Maniola halicarnassus* Thomson, 1990, which flies on the Bodrum peninsula (Turkey) and the Aegean island of Nissiros. *Maniola nurag* (Ghiliani, 1852) is endemic to Sardinia, and a third endemic has been described from the island of Cyprus, *Maniola cypricola* (Graves, 1928) (for distribution areas of species see Fig. 1). *Maniola megala* (Oberthür, 1909) occurs on the Greek island of Lesbos, throughout southern Turkey and in Iran.

Although neighbouring islands would be in flight distance for all island endemics, the ranges of the island-*Maniola* species are well confined to the borders of the respective island. In Chios, *M. chia* is said to entirely replace *M. jurtina* and *Maniola telmessia* (Zeller, 1847), species that are commonly found on the neighbouring islands and the Turkish mainland, which is only a few kilometers distant from Chios. In Sardinia, on the other hand, *M. nurag* flies sympatrically with *M. jurtina*. Although, the latter species is usually concentrated on the coast, whereas the Sardinian endemic has its distributional centres in the mountain areas of the island (> 500 m), there is a zone of overlap at intermediate altitudes (500 – 900 m), where both species fly contemporarily at the same sites (Grill, 2003).

Butterflies of the genus *Maniola* are known for their large morphological variation (Fig. 2), at in-

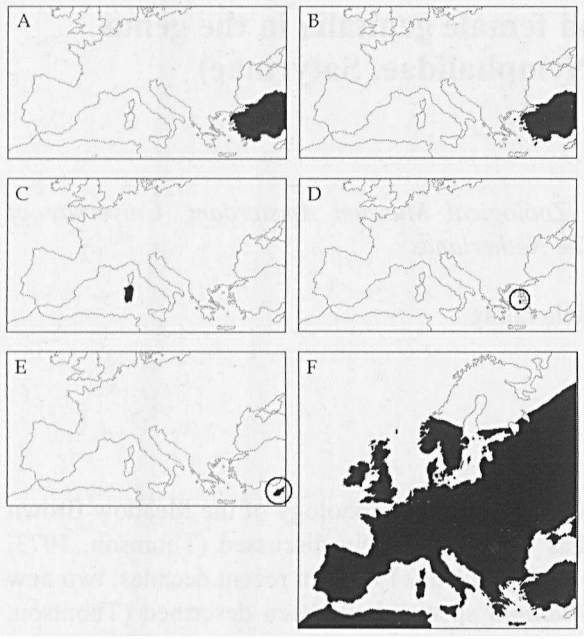

Fig. 1. Distribution areas of the six European species of the genus *Maniola*: (A) *Maniola halicarnassus*, (B) *M. telmessia*, (C) *M. nurag*, (D) *M. chia*, (E) *M. cypricola*, (F) *M. jurtina*.

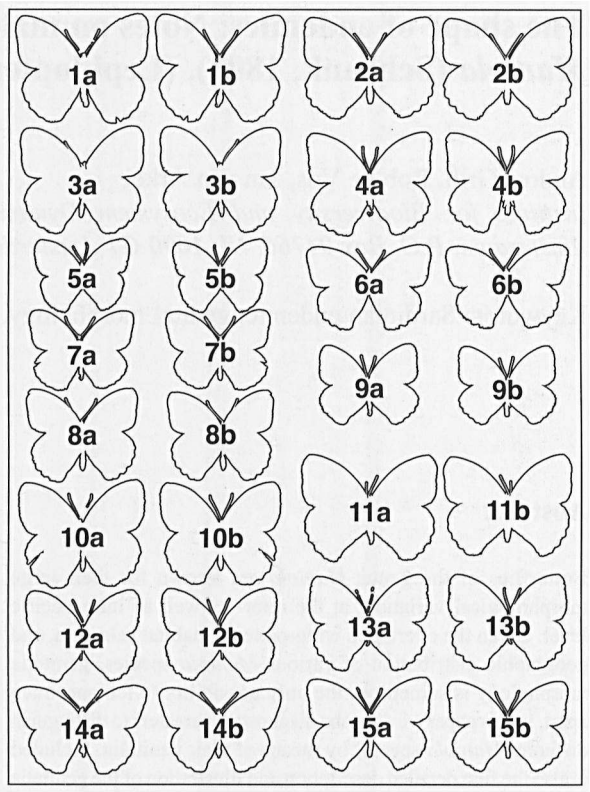

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Fig. 2. Variation in wing pattern in the genus *Maniola*. All the specimens are in the collection of the Zoological Museum Amsterdam. Legend: Column (a) shows the upperside, (b) the underside of the butterflies: 1. *M. jurtina* France (male), 2. *M. jurtina* France (female), 3. *M. jurtina* Sardinia (male), 4. *M. jurtina* Sardinia (female), 5. *M. telmessia* (male), 6. *M. telmessia* (female), 7. *M. nurag* (male), 8. *M. nurag* intermediate form (male), 9. *M. nurag* (female), 10. *M. chia* (male), 11. *M. chia* (female), 12. *M. halicarnassus* (male), 13. *M. halicarnassus* (female), 14. *M. cypricola* (male), and 15. *M. cypricola* (female).

ter- as well as intraspecific level, on both local and continental scale (Ford, 1945; Thomson, 1973). Given the overlap in wing-patterns, habitat selection, and geographic distribution of various *Maniola* species, genitalia morphology is sometimes the only possibility to tell specimens apart. What is more, genitalia shapes can also much vary within a single species (Thomson, 1973). Nevertheless, the species status of *M. chia* and *M. cypricola* has been justified mainly because of differences in the form of the male genitalia; in wing-patterns they resemble *M. jurtina* and *M. telmessia*, respectively. For the third endemic species in this genus, *M. nurag*, genitalia structure and shape has never been described and illustrated in detail as yet.

In this paper, the genital apparatus of *M. nurag* is described and illustrated in detail for the first time. We further describe two Sardinian individuals, whose genitalia seem to be intermediates between *M. nurag* and *M. jurtina*. The genitalia morphology of these Sardinian specimens is compared to the shape and structure of the genital organs in all other *Maniola* species, except *M. megala*, as this species can be unequivocally distinguished from its congenerics by its appreciably larger size, and the wing underside markings.

Ergo, the three main questions we address in this paper are:

1) Are there diagnostic characters in the genitalia of the different *Maniola* species?

2) What is the position of the Sardinian intermediate individuals in the genus *Maniola*?

3) Is species status justified for *M. chia* and *M. cypricola*?

Material and methods

In May 2002 we collected a series of males and females of *M. nurag* (5 males, 3 females) and *M. jurtina* (3 males, 3 females) from Sardinia (Italy), in July 2002 *M. jurtina* (3 males, 3 females) from Amsterdam (The Netherlands), and in September of the same year *M. chia* (1 male, 3 females) from Chios (Greece). These specimens were compared with specimens of *M. telmessia* (2 males, 2 females) and *M. halicarnassus* (2 males, 2 females) collected by H. van Oorschot in Turkey, present in the Zoological Museum Amsterdam. Two of the *M. nurag* we dissected could not unequivocally be attributed to *M. nurag*; according to wing-pattern they could be a light, small *M. jurtina* as well as a dark large *M. nurag*. Small sample sizes are sufficient, as this study aims at a qualitative and not quantitative description of characters. The butterflies were conserved dry or frozen until preparation. Butterflies were identified using characteristics in their wing-patterns following Hesselbarth *et al.* (1995), van Oorschot & van den Brink (1992) and Tolman & Lewington (1997). All individuals studied are in the collection of the Zoological Museum, Amsterdam.

Dissection and photography

Prior to dissection the abdomen of the specimen was separated from the rest of the animal and soaked in Potassium hydroxide (KOH 10%) for approximately 15 hours. To stabilize the samples for photography, they were positioned laterally in a small drop of ethanol (30%), flattened between two glass lids. They were photographed under the microscope (magnification x 25). In order to photograph the form of the signa in the bursa copulatrix, which may show important distinctive characteristics between species, the female genitalia were dyed with chlorazolblack. The dye was fixed in 95% ethanol. For handling, the genitalia were kept in 30% ethanol, as in stronger concentrations of ethanol the chitine hardens and breaks easily. For long term conservation the genitalia will be transferred to a glycerol-tube or Euparol slide.

Results

In males, the main distinctive characters between *Maniola* species are the shape of the valvae, the gnathos, and the strength and size of the Julien organ (Table 1, Fig. 3). There is also some variation in the aedeagus, but it is difficult to use this as a character, as it has a rather soft structure and changes its shape according to the angle from where you look at it. The male genital apparatus also varies in size between species. Among the individuals we studied, *M. jurtina* has the largest and *M. telmessia* the smallest genitalia. In females, we found differences in the shape of the ovipositor lobes and the length of the ductus bursae (Fig. 4). In all species studied in this paper, except *M. jurtina*, the surface of the bursa copulatrix contains two signa that consist of spine-like sclerotised structures (Fig. 5).

Maniola jurtina (Figs. 3A, 4A)

Male: Gnathos markedly swollen at the base, then quickly narrowing. Valvae bigger than all other species except *M. halicarnassus*, in shape most similar to *M. nurag*, but with a characteristic curve towards the distal process; distal and dorsal process round; ventral edge different from the other *Maniola* species, most similar to *M. nurag*. Julien organ always clearly visible, very thick and strong, can be twice the size as in congenerics.

Female: Length of ductus bursae comparable to the other species; notably in none of the dissected females we found signa, although they were clearly visible in all female individuals of the other species. The absence of these marks might be a good distinctive characteristic between *M. jurtina* and the other species in the genus *Maniola*.

Maniola telmessia (Figs. 3B, 4B)

Male: Genital apparatus clearly smaller than in all other *Maniola*. Gnathos similar to *M. chia*, slightly swollen at the base, vesica round. Valvae similar in shape to *M. chia* and *M. halicarnassus*; distal process pointed, similar to *M. chia* and *M. nurag*, dorsal process almost pointed. Ventral edge simi-

Table 1. Comparative listing of characters to differentiate *Maniola* species on the basis of the male genitalia.

character	*M. jurtina*	*M. nurag*	*M. chia*	*M. telmessia*	*M. halicarnassus*	*M. cypricola*
gnathos	markedly swollen at base, then quickly narrowing	swollen at base, gradually narrowing	slightly swollen at base, less so than in *nurag*	slightly swollen at base, smaller than in other *Maniola*	thick at base, but gradually narrowing	swollen at base, gradually narrowing
valvae	larger than all other *Maniola* with characteristic curve towards dorsal process	smaller than in *jurtina*, larger than in *telmessia*	larger than in *telmessia*, comparable to *jurtina* in size	smaller than all other *Maniola*	large, comparable to *jurtina* in size	small, comparable to *telmessia* in size
dorsal process	round	round, flatter than *jurtina*	wide and flatter than *jurtina*	pointed	sharply rounded, not pointed	sharply rounded, considerably longer than in all others
distal process	round	clearly pointed	pointed	pointed	pointed	clearly pointed
ventral edge	begins flat, than curved	begins flat, than curved	begins flat, curves in pointed angle, than continues flat	curved	curved	begins flat, curves in pointed angle, than continues flat
Julien organ	thick and strong	more fragile than in *jurtina* stronger than in *telmessia*	similar to *jurtina*	very fragile, often broken off	fragile, but stronger than in *telmessia*	more fragile than in *jurtina* stronger than in *telmessia*

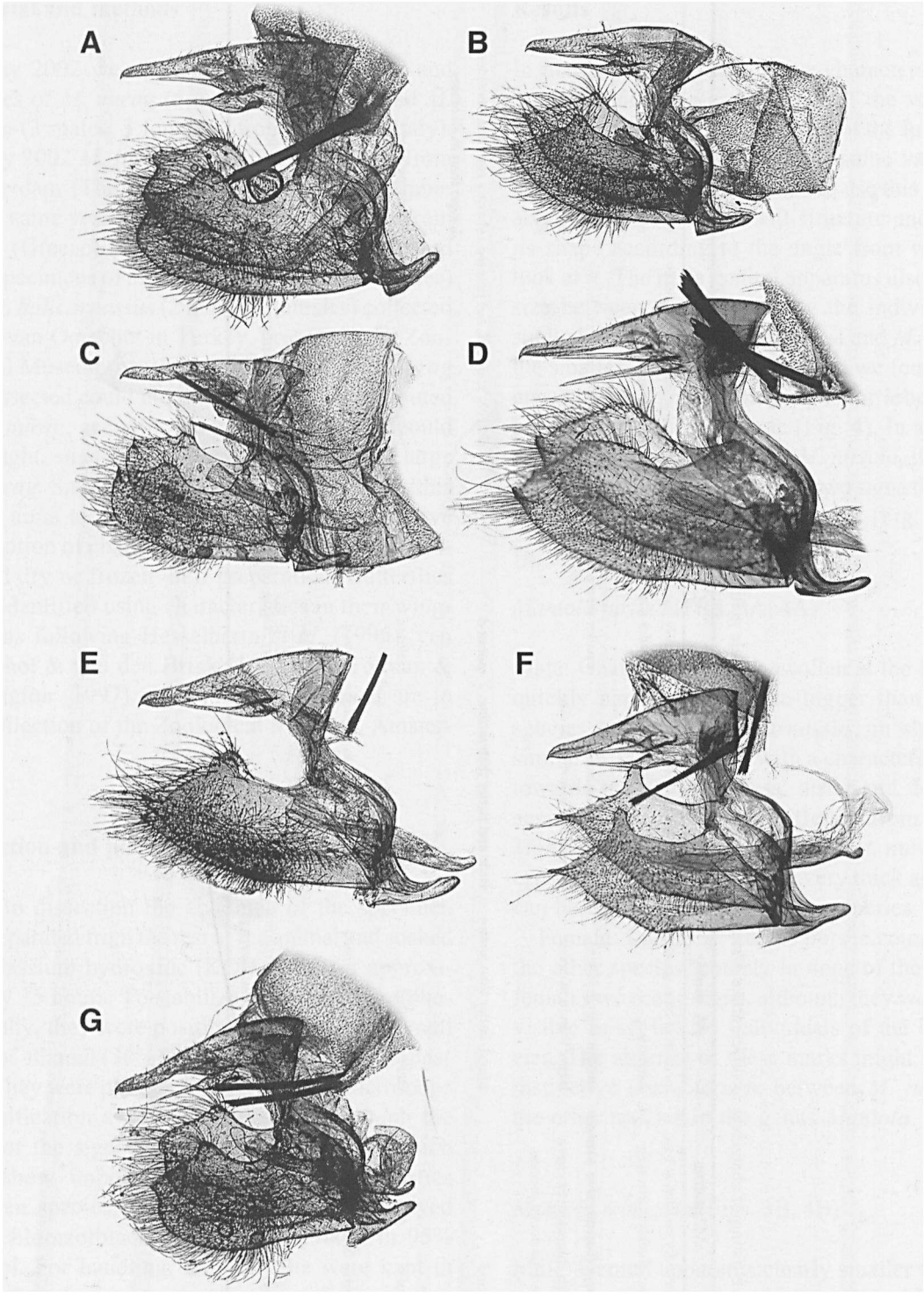

Fig. 3. Male genital apparatus of different *Maniola* species. (A) *M. jurtina,* (B) *M. telmessia,* (C) *M. nurag*, (D) *M. chia*, (E) *M. halicarnassus*, (F) *M. cypricola,* and (G) intermediate form (from Sardinia).

lar to *M. chia* and *M. halicarnassus*, clearly different from *M. jurtina*. Julien organ present but very fragile. In *M. telmessia* the Julien organ often brakes off, and in earlier literature it was considered to be lacking (see references in Thomson, 1973).

Female: Ductus bursae similar to *M. nurag* and *M. chia;* signa clearly visible, short, pointed at the posterior end, broadening at the anterior end (Fig. 5A).

Maniola nurag (Figs. 3C, 4C)

Male: Gnathos substantially swollen at the base, vesica round at its extremity. Valvae considerably smaller than in *M. jurtina* but larger than in *M. telmessia.* Dorsal process clearly visible, round, flatter than in *M. jurtina*; distal process clearly pointed, sharper than in *M. jurtina*; ventral edge curved, similar to *M. jurtina*. Julien organ present, more fragile than in *M. jurtina*, but stronger than in *M. telmessia.*

Female: Ductus bursae similar to other *Maniola* species; Bursa with two elongated signa, which vary considerably in length and visibility (Fig. 5B).

Maniola chia (Figs. 3D, 4D)

Male: Gnathos slightly swollen at the base, but less than in *M. nurag*, shape and size of aedeagus similar to *M. nurag*. Valvae larger than in *M. telmessia*, comparable to those of *jurtina* in size, but not in shape; distal process pointed similar to *M. telmessia* and *M. nurag*, dorsal process wider and flatter than in *M. jurtina* and *M. nurag*, slightly pointed. Ventral edge differently curved than in *M. jurtina*, similar to *M. telmessia*. Julien organ like in *M. jurtina*.

Female: Ductus bursae relatively short, notably shorter than in *M. halicarnassus;* Bursa in all individuals with two crescent-formed signa (Fig. 5C).

Maniola halicarnassus (Figs. 3E, 4E)

Male: Gnathos thicker at the base but gradually narrowing towards the end. Valvae of similar size as in *M. jurtina*, but different in shape; distal process pointed, dorsal process sharply rounded but not pointed, connection between distal and dorsal process straighter than in other species. Ventral edge similar to *M. telmessia*. Julien organ thinner than in *M. jurtina, M. nurag* and *M. chia*, but stronger than in *M. telmessia*.

Female: Ductus bursae slightly longer than in the other species; signa variable, but clearly visible, pointed towards the posterior end, broadening towards the anterior end (Fig. 4D).

Maniola cypricola (Fig. 3F)

Male: Gnathos swollen at base, gradually narrowing. Valvae small, comparable to *M. telmessia* in size and shape, line towards distal process straight, as opposed to all other species, where it is slightly curved; distal process clearly pointed, dorsal process sharply rounded, basis considerably longer than in other *Maniola*.

Female: Ductus bursae relatively short; signa clearly visible, elongated, round at the ends (Fig. 4E).

Intermediate form (Fig. 3G)

The two individuals with wing-patterns that seemed intermediates between *M. nurag* and *M. jurtina*, were also intermediate in genitalia structure.

Male: Gnathos markedly swollen at base, then quickly narrowing. Valvae larger than in *M. nurag;* distal process pointed like in *M. nurag*, but position like in *M. jurtina*, dorsal process slightly pointed as opposed to the other two species; ventral edge similar to *M. jurtina*. Julien organ thicker, but not as solid as in *M. jurtina.*

We were not able to identify any females of this type.

Discussion and conclusions

(1) Are there diagnostic characters in the genitalia of the different *Maniola* species?

In females, the most unequivocal characteristic to distinguish *M. jurtina* from the other five spe-

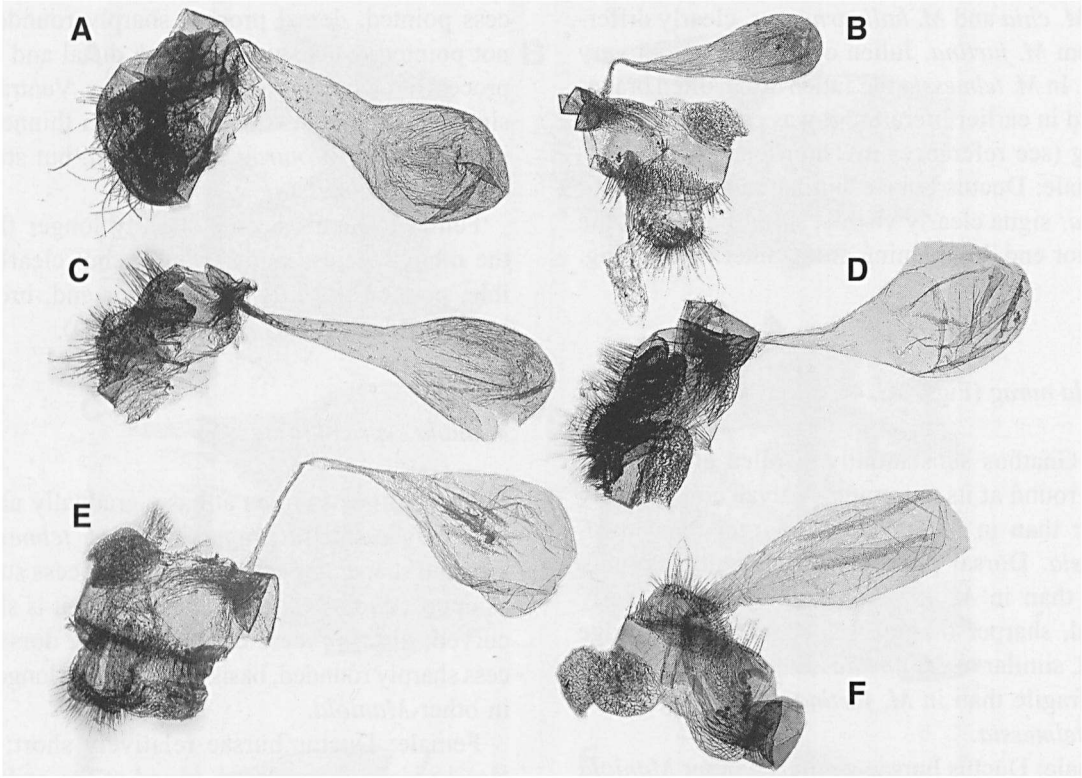

Fig. 4. Female genital apparatus of different *Maniola* species in comparison. (A) *M. jurtina,* (B) *M. telmessia,* (C) *M. nurag,* (D) *M. chia,* (E) *M. halicarnassus,* and (F) *M. cypricola.*

cies we studied, seems the absence of signa on the female bursa. Signa were present in all studied individuals *M. nurag, M. chia, M. cypricola, M. telmessia,* and *M. halicarnassus*, but absent in *M. jurtina*. However, it might be, that this characteristic is just much rarer in *M. jurtina* than in the other species, but still occasionally present (Thomson, pers. comm.). The female genitalia of the other *Maniola* species do not show diagnostic characters. As usual, the main characters to differentiate between species are in the male genital apparatus.

Maniola jurtina can be clearly distinguished by shape and size of the valvae and the Julien organ. *Maniola nurag* is generally well recognisable by the form of its valvae. *Maniola telmessia* is distinctive by its smaller size, the outline of the valvae and the shape of distal and dorsal processes. In *M. halicarnassus* the form of the dorsal process as well as the connection between dorsal and distal process are distinctive. Diagnosis is further facilitated through the wing-patterns, which additionally to genitalia structure, differentiate this species from the other *Maniola*. More of a problem poses *M. chia*, which is very similar to *M. telmessia* in the shape of its genital apparatus, and almost indistinguishable from *M. jurtina* by its wings. Maybe it is this intermediate position between *M. telmessia* and *M. jurtina*, that can serve as a distinctive characteristic: *chia* = genitalia like *telmessia* plus wing-pattern of *jurtina*. But obviously, for this species more samples are necessary to obtain a better picture.

Thomson (1973) divides the species *M. jurtina* into three main types, the eastern, the western, and the primitive type, where in the *primitive type* the dorsal process has an irregular 'fringe', which usually extends to the distal process, in the *western type* the dorsal process is long with a pointed or very sharply rounded extremity or a short flat top, and in the *eastern* type the dorsal process is fairly short with a flat or almost flat top. Also the gnathos and the ventral edge vary among the three types.

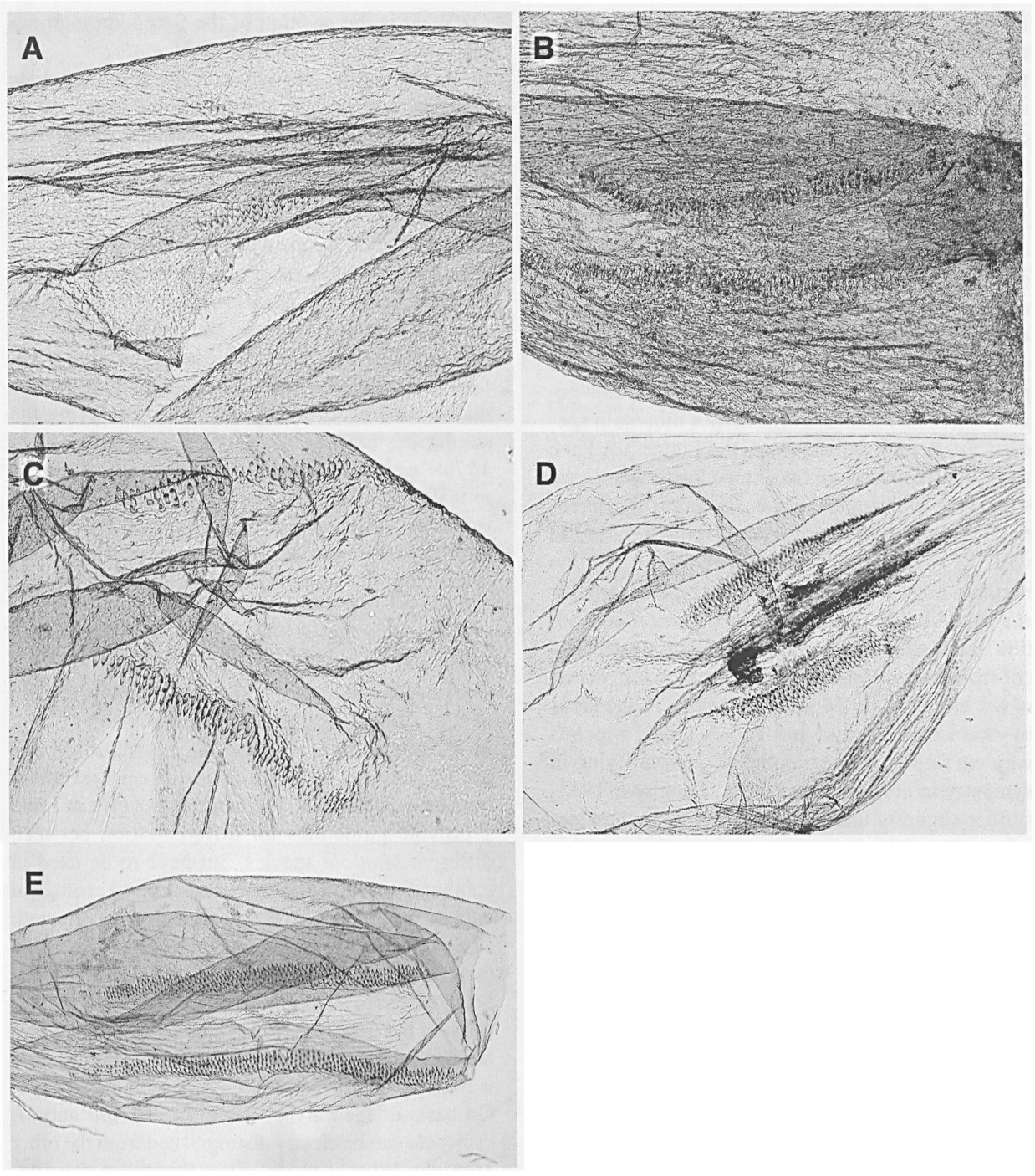

Fig. 5. Signa in the female bursa copulatrix of (A) *M. telmessia*, (B) *M. nurag*, (C) *M. chia*, (D) *M. halicarnassos*, and (E) *M. cypricola*.

He postulated that the genital apparatus of *M. nurag* and *M. telmessia* were close to the western type he found in *M. jurtina*. At the time his publication appeared, *M. halicarnassus* and *M. chia* were not described yet, and consequently he could not discuss them at the time. According to his ideas, the primitive type individuals in the eastern range of *M. jurtina* are relict elements of the original type of *Maniola*, which were the ancestors of all *Maniola* species we know in Europe today. He found the eastern forms with the primitive valvae in mountain localities from 1500 – 2000 metres a.s.l., and concluded that the Iranian forms of *M. jurtina* are probably the oldest surviving ancestors of this genus, which he considers originally a mountain species. From Iran, he suggests, the butterflies have travelled westwards in two flows, one towards the south, the other one towards the north. Differentiation of the southern migrant groups resulted in what he calls the western type of valvae, and the northern migrants led to the eastern type. *M. telmessia* would be the result of an extreme differentiation of the western type, that had become so different from the ancestors that it resisted a reinvasion of the eastern type *M. jurtina* later on in the areas of what we call Greece and Turkey today, which is why we find *M. telmessia* and *M. jurtina* flying in sympatry in most of their ranges. Thomson (1973) further suggests that *M. nurag* is the furthest development of the *M. jurtina* ancestor. He bases this on the fact that the valvae are purely of western form, and the fulvous of the butterfly is very extensive. Although all this reasoning is very intriguing, and indeed partly convincing, it remains in the realm of conjecture. To answer questions like that, large scale phylogenetic and phylogeographic analysis based on molecular data are indispensable.

The illustrations presented in figure 3 show that based on male genitalia shape, the six species we investigated would fall into two groups: *M. telmessia, M. halicarnassus, M. chia, M. cypricola* on the one hand, and *M. nurag* and *M. jurtina* on the other. This pattern corresponds well to the geographic distribution of these species: the first four are flying in the eastern Mediterranean, the latter two in the western Mediterranean. It also confirms our genetic data on the close genetic relationship of *M. nurag* and *M. jurtina* (Grill, 2001; Grill, 2003).

(2) What is the position of the Sardinian intermediate individuals in the genus *Maniola*?
In the two Sardinian individuals with intermediate wing-patterns also the genitalia are of intermediate form; in their contours they resemble *M. jurtina*, but they are smaller and the distal process is pointed like in *M. nurag* and *M. telmessia*. The explanations for this are twofold: (I) These two individuals are hybrids between *M. jurtina* and *M. nurag*, and therefore have intermediate wings as well as genital structure. (II) There is a third form of *Maniola* flying in Sardinia. This intermediate type, however, is clearly more similar to *M. nurag* and *M. jurtina*, than to any of the other *Maniola* species, which makes the hybrid-idea plausible. Considering the similarities in size and structure of the genitalia in *M. jurtina* and *M. nurag*, hybridisation seems theoretically possible. The new 'intermediate' form we found in Sardinia is another example, for the potential of the genus *Maniola* as an interesting model system to study adaptation and speciation processes.

(3) Is species status justified for *M. chia* and *M. cypricola*?
Considering the intraspecific variation in the male genital apparatus, illustrated by Thomson (1973), in *M. jurtina* from different areas in Europe, the use of genitalia structure to justify species status remains problematic. The characters we give in Table 1 provide guidelines for differentiation between different *Maniola* species, but have to be used in combination with wing characteristics and ecological data of the site where the specimen was collected. The wing-patterns of *M. chia*, for example, resemble those of *M. jurtina* (Fig. 2) so closely, that these two species are indistinguishable without taking into account the geographic provenance and male genitalia structure of the specimen. Also *M. cypricola* is phenotypically extremely similar to *M. telmessia*. On basis of the valvae, however, *M. chia* and *M. cypricola* can be clearly distinguished from the other *Maniola*. What is more, given that these two species are island endemics and therefore completely isolated from other congeneric populations, they are a genetically distinct entity. In a nature conservation context they would therefore be considered as 'evolutionary significant units', regardless if they are 'real' endemic species or not (Gärdenfors et al., 1999).

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