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Genetic and taxonomic studies on the genus *Clonopsis*, with the description of a new species (Phasmatodea, Bacillidae)

Introduction

In spite of the vast literature existing on the subject, the taxonomy of Phasmatodea (= Cheleutoptera = Phasmoptera = Phasmida) is still in state of flux, both at the higher and lower taxa level. This is due to various reasons, among which the following: 1. the large use in the taxonomy of this order of characters being of poor phylogenetic value; 2. the existence of a remarkable morphological variability both at the intra- and inter-population level; 3. the relatively few specimens collected and preserved; 4. the widespread occurrence of thelytokous parthenogenesis, either as the only way of reproduction of the species (obligatory parthenogenesis), or associated with bisexual reproduction in the same population (accidental or facultative parthenogenesis), or limited to parts of the species' range (geographic parthenogenesis); 5. the relatively high rate of interspecific hybridization taking place in this group; 6. the existence of hybrid parthenogenetic species, often difficult to recognize from non-hybrid ones.

In the last decades, our knowledge on the taxonomy, biology and evolution of various stick-insect species has greatly improved by the use of different approaches: extensive laboratory breedings (Cappe de Baillon *et al.*, 1934, 1935, 1937, 1938; Cappe de Baillon and de Vichet, 1940; Boisson, 1942; Bergerad, 1958; Bullini, 1964, 1965, 1966, 1967, 1968, 1969; Scali, 1968, 1970, and 1982), hybridization experiments (Favrelle and de Vichet, 1937, 1948; Nascetti *et al.*, 1985; and unpublished), fine investigation, e.g. by scanning electron microscope, of characters such as egg morphology (Clark, 1976 *a, b, c,* 1978, 1979; Mazzini and Scali, 1977, 1980 *a, b,* 1983; Mazzini *et al.*, 1982, 1983; Scali and Mazzini, 1977, 1981, 1982; Hinton, 1981; Scali *et al.*, in press), chromosome studies (Cappe de Baillon *et al.*, 1934, 1935, 1937, 1938; Cappe de Baillon and de Vichet, 1940; Hughes-Schrader, 1947 *a, b,* 1959; de Toledo Piza, 1950; Bergerad, 1958; Pijnacker, 1964, 1966, 1967, 1969; Pijnacker and Harbott, 1980; Virkki, 1970; Craddock, 1970, 1972, 1974; Bullini and Bianchi Bullini, 1971; Koch *et al.*, 1972; Scali, 1972;

White, 1976; Mosti and Scali, 1975; Scali and Mosti, 1975; Goday *et al.*, 1981; Marescalchi *et al.*, 1985), analysis of the genetic structure of natural populations by means of multilocus electrophoresis (Bullini, 1982; Bullini *et al.*, 1977, 1983; Nascetti and Bullini, 1980, 1982 *a, b;* Gasperi *et al.*, 1983; Nascetti *et al.*, 1983, 1985).

The last approach is applied in the present paper, together with chromosomal and morphological analyses, to the study of stick-insects of the genus *Clonopsis* Pantel, 1915 (family Bacillidae). This genus till now included two species: the bisexual *Clonopsis algerica* (Pantel, 1890) and the parthenogenetic *C. gallica* (Charpentier, 1825), with the forms *occidentalis* (Bolivar, 1894) and *affinis* (Salfi, 1925). The taxonomic status of *C. gallica* and *C. algerica* has been for a long time highly controversial: they were considered as geographical races, or as bisexual and parthenogenetic forms of the same species, or as fully distinct species (Pantel, 1890, 1915; Finot, 1895; Chopard, 1943, 1951; etc.). In this paper, the genetic relationships of the taxa so far included in the genus *Clonopsis* are analyzed, and their taxonomic status and evolutionary relationships are discussed. Moreover, a new *Clonopsis* species from Morocco is described, for which the name *Clonopsis maroccana* is proposed.

Materials and methos

The specimens studied were from the following localities (Fig. 1):
Clonopsis gallica (Charpentier, 1825) — Moncique (Portugal), Ronda, Torremolinos, Motril, Benisa, and Gerona (Spain), Mallorca (Balearic Islands), Le Perthus, Banyuls-sur-mer, Frontignan, and Arles (France), Albenga, Genoa, La Spezia, Porretta, Florence, Bolgheri, Tolfa mountains, Neaples, Lamezia Terme, and Bianco (Italy), Alghero, Nuoro, and Iglesias (Sardinia), Messina, Palermo, and Anapo valley (Sicily), Bizerte, Sedjenane, Tabarqa, and Ain Draham (Tunisia), El Kseur, Adekar, Blida, Médéa, Misserghin, and Tlemcen mountains (Algeria), Chechaouen, Tetouan, and Tanger (Morocco).
Clonopsis algerica (Pantel, 1890) - Stidia, Kristel, and Misserghin (Algeria).
Clonopsis maroccana n. sp. - Targuist and Torres de Alcala, Rif (Morocco).

Morphological characters - All the specimens studied were measured by the same person with a precision calliper (mostly using a binocular microscope) for the following characters: total body length, excluding antennae and cerci; antennae length; number of antennae articles; length of pronotum, mesonotum, metanotum, median segment,

fore femora, median femora, and hind femora. Other characters considered were: body colour, meso- and metanotum granulation, development of dorsal tubercles, shape and size of subgenital plate and subanal vomer, femora denticulation, egg morphology.

Electrophoretic techniques - Horizontal starch gel electrophoresis was performed on muscle tissue of single specimens, both larvae and adults, for the following enzymes: α-glycerophosphate dehydrogenase (α-GPDH), lactate dehydrogenase (LDH), malate dehydrogenase

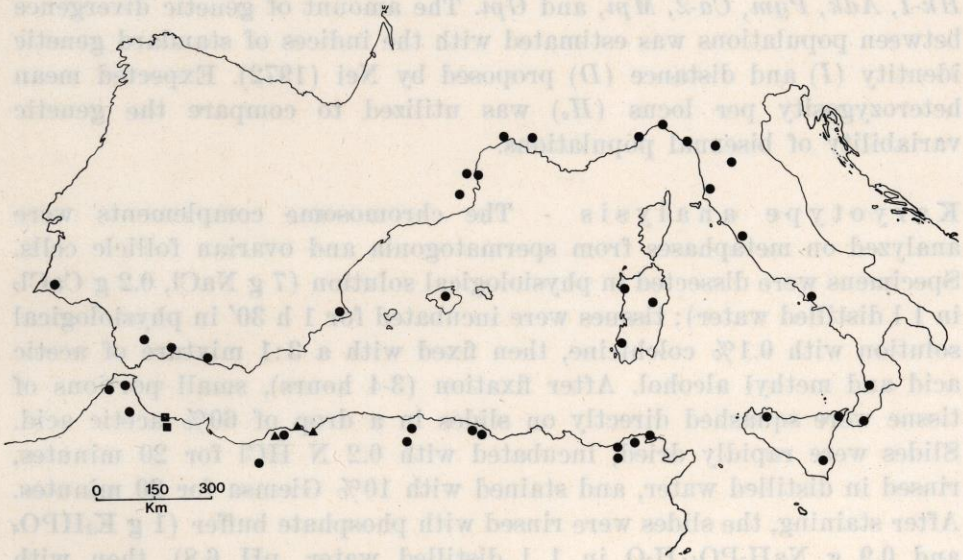

Fig. I

Collecting sites of *Clonopsis gallica* (circles), *C. algerica* (triangles), and *C. maroccana* (squares).

(MDH), isocitrate dehydrogenase (IDH), 6-phosphogluconate dehydrogenase (6PGDH), glyceraldehyde-3-phosphate dehydrogenase (G3PDH), superoxide dismutase (SOD), glutamate oxaloacetate transaminase (GOT), hexokinase (HK), adenylate kinase (ADK), phosphoglucomutase (PGM), carbonic anhydrase (CA), mannose phosphate isomerase (MPI), and glucose phosphate isomerase (GPI). The buffer systems used were: 1) tris-versene-borate (Brewer and Sing, 1970) for SOD, CA, and MPI; 2) tris-versene-maleate (Brewer and Sing, 1970) for LDH and PGM; 3) phosphate-citrate (Harris, 1966) for α-GPDH, MDH, 6PGDH, and GPI; 4) continuous tris-citrate II (Selander *et al.*, 1971) for IDH, G3PDH, GOT, HK, and ADK. The staining techniques were, with minor modifications, those described by Brewer and Sing, 1970

(LDH, PGM), Shaw and Prasad, 1970 (MDH, IDH, 6PGDH), Selander *et al.*, 1971 (SOD, GOT, GPI), Ayala *et al.*, 1972 (α-GPDH, G3PDH, HK, ADK), and Harris and Hopkinson, 1976 (CA, MPI).

The following loci and alleles designation was adopted: isozymes were numbered in order of decreasing mobility from the most anodal; allozymes were named numerically according to their mobility relative to the commonest allele (= *100*) found in a reference clone of *C. gallica* from southern France (Banyuls-sur-mer).

A total of 18 enzyme loci were genetically analyzed: *α-Gpdh, Ldh, Mdh-1, Mdh-2, Idh-1, Idh-2, 6Pgdh, G3pdh, Sod-1, Sod-2, Got-1, Got-2, Hk-1, Adk, Pgm, Ca-2, Mpi,* and *Gpi.* The amount of genetic divergence between populations was estimated with the indices of standard genetic identity (*I*) and distance (*D*) proposed by Nei (1972). Expected mean heterozygosity per locus (H_e) was utilized to compare the genetic variability of bisexual populations.

Karyotype analysis - The chromosome complements were analyzed on metaphases from spermatogonia and ovarian follicle cells. Specimens were dissected in physiological solution (7 g NaCl, 0.2 g $CaCl_2$ in 1 l distilled water); tissues were incubated for 1 h 30′ in physiological solution with 0.1% colchicine, then fixed with a 3:1 mixture of acetic acid and methyl alcohol. After fixation (3-4 hours), small portions of tissue were squashed directly on slides in a drop of 60% acetic acid. Slides were rapidly dried, incubated with 0.2 N HCl for 20 minutes, rinsed in distilled water, and stained with 10% Giemsa for 20 minutes. After staining, the slides were rinsed with phosphate buffer (1 g K_2HPO_4 and 0.9 g $NaH_2PO_4 \cdot H_2O$ in 1 l distilled water, pH 6.8), then with distilled water, and finally air dried.

Clonopsis gallica (Charpentier, 1825)

This species (Fig. 2) is widespread in the Mediterranean region; it inhabits Tunisia, Algeria, Morocco, Portugal (including the Azores), Spain (including the Balearic Islands), France (including Corsica), Italy (including Sicily, Sardinia and most of the minor islands), Yugloslavia, and Greece (Chopard, 1943, 1951; Harz and Kaltenbach, 1976; and unpublished). Two subspecies were described: *occidentalis* from the Azores and Morocco, and *affinis* from Calabria (southern Italy).

Laboratory breedings have confirmed that *C. gallica* reproduces by obligatory thelytokous parthenogenesis. No males have been observed in the present study over thousands of individuals examined both in the field and in the lab. The few males recorded in the literature seem rather to be intersexes; they have an abnormal spermatogenesis and seem to

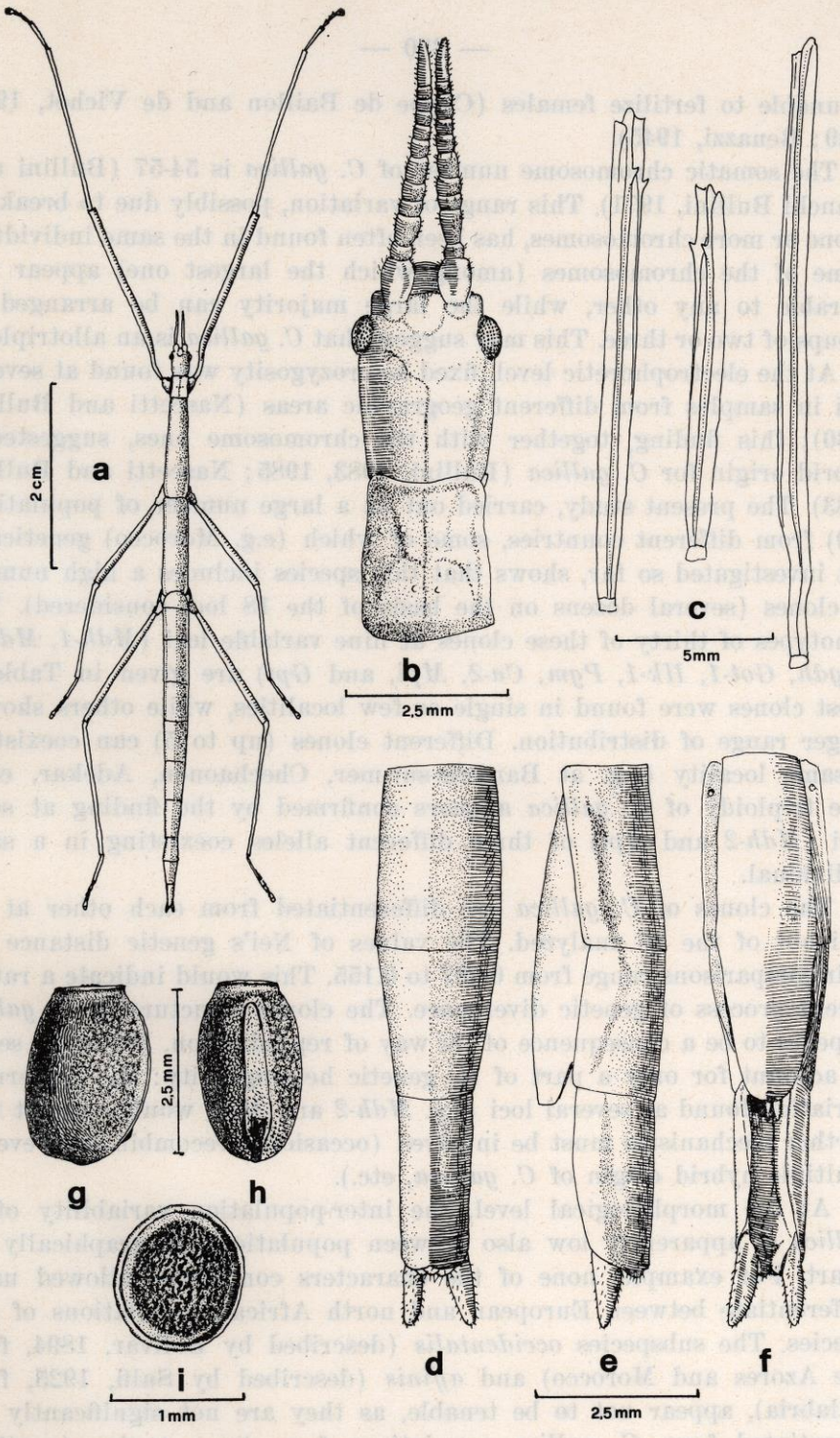

FIG. II

Clonopsis gallica from Tlemcen mountains (Algeria). Adult female, dorsal view (*a*); head and pronotum, dorsal view (*b*); fore, median and hind femora, lateral-ventral view (*c*, respectively from right to left); end of abdomen, dorsal (*d*), lateral (*e*), and ventral (*f*) view; egg, lateral (*g*) and dorsal (*h*) view; egg operculum (*i*).

be unable to fertilize females (Cappe de Baillon and de Vichet, 1935, 1939; Benazzi, 1945).

The somatic chromosome number of *C. gallica* is 54-57 (Bullini and Bianchi Bullini, 1971). This range of variation, possibly due to breaking of one or more chromosomes, has been often found in the same individual. Some of the chromosomes (among which the largest one) appear not pairable to any other, while the large majority can be arranged in groups of two or three. This may suggest that *C. gallica* is an allotriploid.

At the electrophoretic level, fixed heterozygosity was found at several loci in samples from different geographic areas (Nascetti and Bullini, 1980); this finding, together with the chromosome ones, suggested a hybrid origin for *C. gallica* (Bullini, 1983, 1985; Nascetti and Bullini, 1983). The present study, carried out on a large number of populations (40) from different countries, some of which (e.g. Morocco) genetically not investigated so far, shows that this species includes a high number of clones (several dozens on the basis of the 18 loci considered). The genotypes of thirty of these clones at nine variable loci (*Mdh-1, Mdh-2, 6Pgdh, Got-1, Hk-1, Pgm, Ca-2, Mpi,* and *Gpi*) are given in Table 1. Most clones were found in single or few localities, while others show a larger range of distribution. Different clones (up to 5) can coexist in a same locality (e.g. at Banyuls-sur-mer, Chechaouen, Adekar, etc.). The triploidy of *C. gallica* appears confirmed by the finding at some loci (*Mdh-2* and *Gpi*) of three different alleles coexisting in a same individual.

The clones of *C. gallica* are differentiated from each other at 1-8 loci out of the 18 analyzed. The values of Nei's genetic distance for pair comparisons range from 0.007 to 0.155. This would indicate a rather recent process of genetic divergence. The clonal structure of *C. gallica* appears to be a consequence of its way of reproduction. Mutation seems to account for only a part of its genetic heterogeneity: the pattern of variation found at several loci (e.g. *Mdh-2* and *Mpi*) would suggest that further mechanisms must be involved (occasional recombination events, multiple hybrid origin of *C. gallica,* etc.).

At the morphological level, the inter-population variability of *C. gallica* is apparently low also between populations geographically far apart. For example, none of the characters considered allowed us to differentiate between European and north African populations of this species. The subspecies *occidentalis* (described by Bolivar, 1894, from the Azores and Morocco) and *affinis* (described by Salfi, 1925, from Calabria), appear not to be tenable, as they are not significantly differentiated form *C. gallica* populations from terra typica (southern France). Some differences in the egg sculpturing were found by scanning electron microscope between a population from southern Spain (Motril) and others from northern Spain and Italy (Scali and Mazzini, 1982).

However, southern Spain populations appear poorly differentiated both in their external morphology and gene structure (e.g. Nei's *D* is 0.01 between the populations from Motril and Frontignan, southern France, and 0.03 between the former one and those from central and southern Italy).

The genetic, chromosomal, and morphological differences between *C. gallica, C. algerica* and the new species *C. maroccana* will be examined in the next sections.

Clonopsis algerica (Pantel, 1890)

The range of *C. algerica,* the type species of the genus *Clonopsis* Pantel, 1915, seems to be limited to Algeria (Chopard, 1943). Its patria typica is Oran (our population sample from the surroundings of Misserghin is the closest to this town, being 12 km south-west from it). This species reproduces bisexually, showing a sex ratio of about 1:1.

The karyotype of *C. algerica* was not till now described. We have found a chromosome number of 2n = 32 in the female and 2n = 31 in the male, including 3 metacentric and 13 telocentric pairs. This karyotype strongly differs from that of *C. gallica* in being clearly diploid (homologous chromosome pairs, with formation of bivalents), in its chromosome number, and in the morphology of several chromosomes.

At the electrophoretic level, the diploidy of *C. algerica* is confirmed, as well as its bisexual reproduction; in its populations the polymorphic loci are in Hardy-Weinberg equilibrium. The allele frequencies found in the populations of *C. algerica* tested are reported in Table 2. At some loci (*Sod-1, Got-1, Got-2*) the sample from Misserghin appears remarkably differentiated from the other two (Kristel and Stidia), in spite of being all geographically rather close to each other (Nei's $D = 0.16$). Also the level of heterozygosity is quite different, being higher in the Misserghin population ($H_e = 0.11$) than in those from Kristel and Stidia ($H_e = 0.03$). The genetic divergence between *C. algerica* populations respectively west and east of Oran can be related to a geographic barrier, represented by a wide depression: the « grande Sebkra d'Oran », once covered by the sea. Still now this plain, as well as the town of Oran and its surroundings, appears not to be inhabited by *Clonopsis.* The populations of *C. algerica* east of Oran presumably belong to a distinct subspecies, still undescribed. Further research, both morphological and genetic, carried out on a higher number of populations appears necessary to settle this point.

Nei's average genetic distance between *C. algerica* and *C. gallica* is 0.60. The populations of *C. algerica* from Kristel and Stidia are

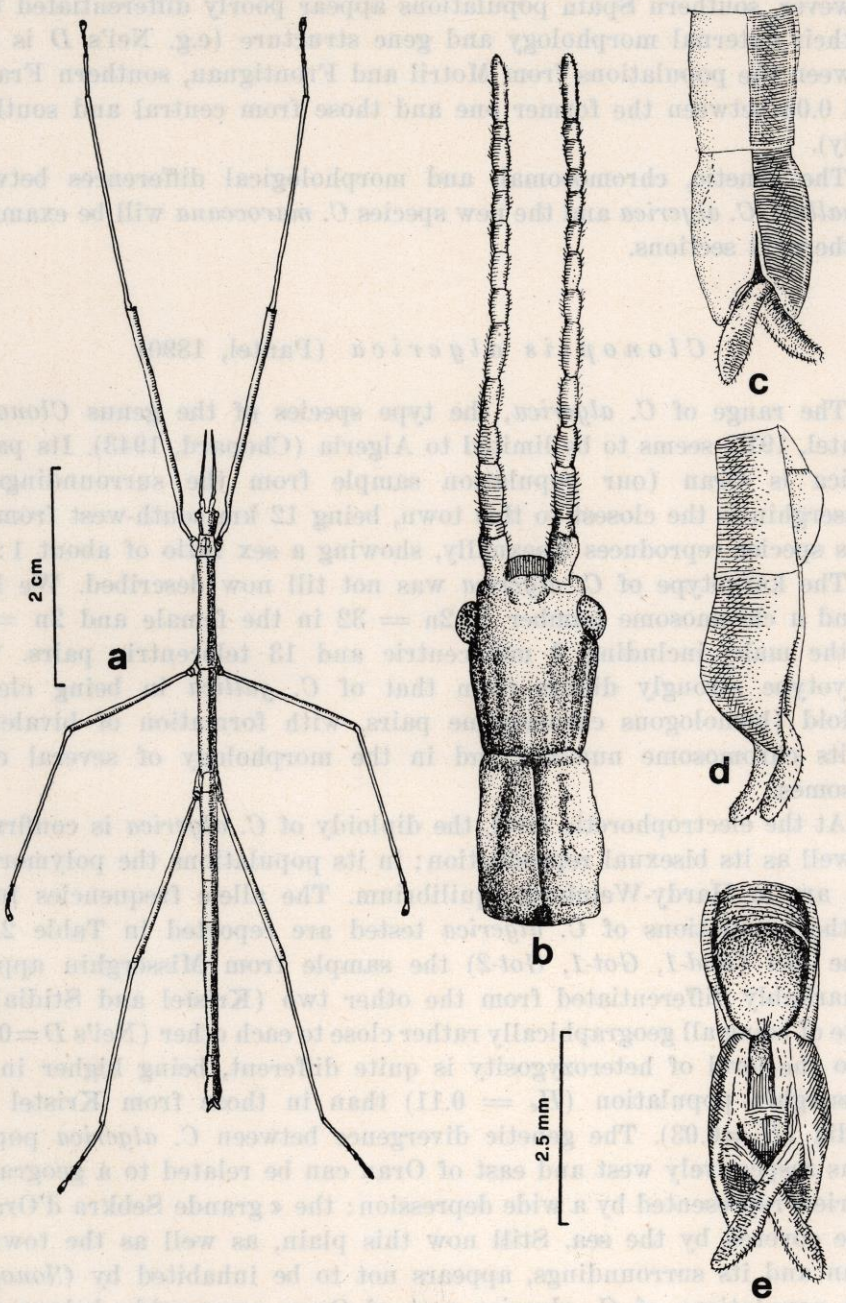

FIG. III

Clonopsis algerica from Misserghin (Algeria). Adult male, dorsal view (*a*); head and pronotum, dorsal view (*b*); end of abdomen, dorsal (*c*), lateral (*d*), and ventral (*e*) view.

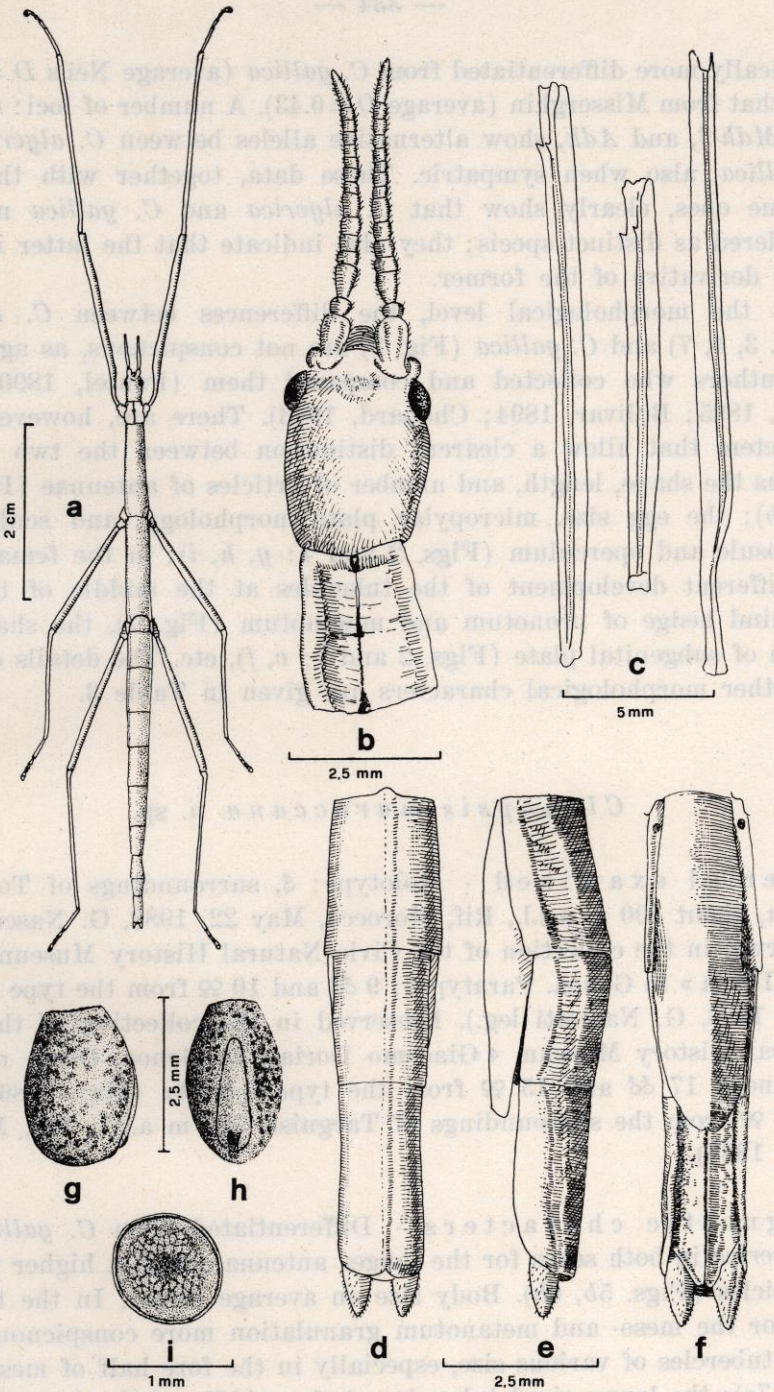

FIG. IV

Clonopsis algerica from Misserghin (Algeria). Adult female, dorsal view (*a*); head and pronotum, dorsal view (*b*); fore, median and hind femora, lateral-ventral view (*c*, respectively from right to left); end of abdomen, dorsal (*d*), lateral (*e*), and ventral (*f*) view; egg, lateral (*g*) and dorsal (*h*) view; egg operculum (*i*).

genetically more differentiated from *C. gallica* (average Nei's $D = 0.68$) than that from Misserghin (average $D = 0.43$). A number of loci: *α-Gpdh, Ldh, Mdh-2,* and *Adk,* show alternative alleles between *C. algerica* and *C. gallica,* also when sympatric. These data, together with the chromosome ones, clearly show that *C. algerica* and *C. gallica* must be considered as distinct specis; they also indicate that the latter is not a direct derivative of the former.

At the morphological level, the differences between *C. algerica* (Figs. 3, 4, 7) and *C. gallica* (Fig. 2) are not conspicuous, as agreed by the authors who collected and compared them (Pantel, 1890, 1915; Finot, 1895; Bolivar, 1894; Chopard, 1943). There are, however, some characters that allow a clearcut distinction between the two species, such as the shape, length, and number of articles of antennae (Figs. 2*b*, 3*b*, 4*b*); the egg size, micropylar plate morphology, and sculpturing of capsule and operculum (Figs. 2 and 4: *g, h, i*); in the female, also the different development of the tubercles at the middle of the fore and hind hedge of pronotum and mesonotum (Fig. 7), the shape and length of subgenital plate (Figs. 2 and 4: *e, f*), etc. The details of these and other morphological characters are given in Table 3.

Clonopsis maroccana n. sp.

Material examined - Holotype: ♂, surroundings of Torres de Alcala, about 100 m a.s.l., Rif, Morocco, May 22, 1986, G. Nascetti leg. Preserved in the collection of the Civic Natural History Museum « Giacomo Doria » in Genoa. Paratypes: 9 ♂♂ and 10 ♀♀ from the type locality (May 1986, G. Nascetti leg.). Preserved in the collection of the Civic Natural History Museum « Giacomo Doria » in Genoa. Other material examined: 17 ♂♂ and 15 ♀♀ from the type locality (May 1986); 9 ♂♂ and 7 ♀♀ from the surroundings of Targuist, 550 m a.s.l., Rif, Morocco (May 1986).

Diagnostic characters - Differentiated from *C. gallica* and *C. algerica* in both sexes for the longer antennae, with a higher number of articles (Figs. 5*b*, 6*b*). Body size on average larger. In the females, also for the meso- and metanotum granulation more conspicuous, with conic tubercles of various size, especially in the fore half of mesonotum (Fig. 7*c*); the larger-sized tubercles at the middle of the fore and hind hedge of pronotum and mesonotum (Fig. 7*c*); the denticulation of median and hind femora, not limited to the apical part (Fig. 6*c*). Egg smaller, glossy, with a peculiar sculpturing (Fig. 6*g, h, i*). Karyotype: $2n = 22$ in the female, $2n = 21$ in the male.

Holotype description - Adult male (Fig. 5). Body and legs colour: brown. Body length, excluding antennae and cerci: 59.5 mm.

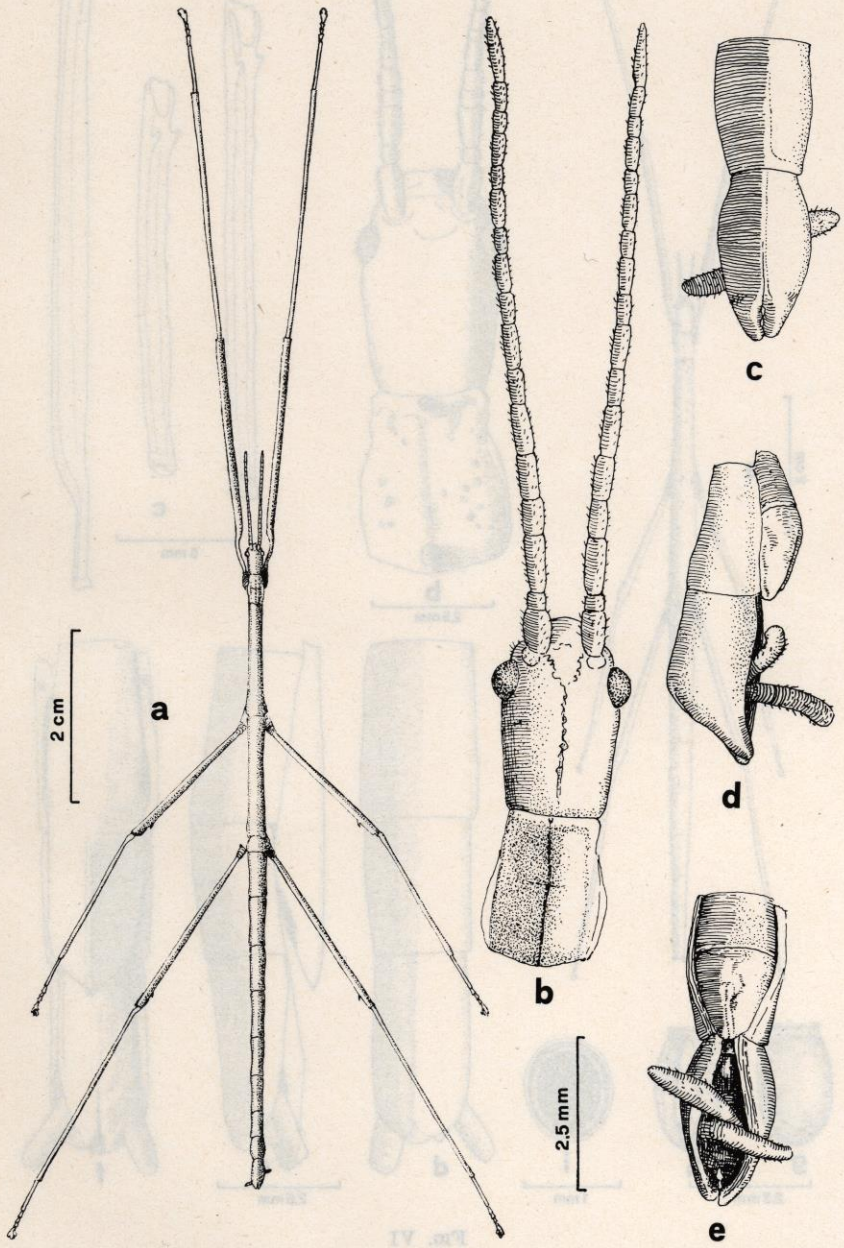

FIG. V

Clonopsis maroccana from Torres de Alcala, Rif (Morocco). Adult male (holotype), dorsal view (*a*); head and pronotum, dorsal view (*b*); end of abdomen, dorsal (*c*), lateral (*d*), and ventral (*e*) view.

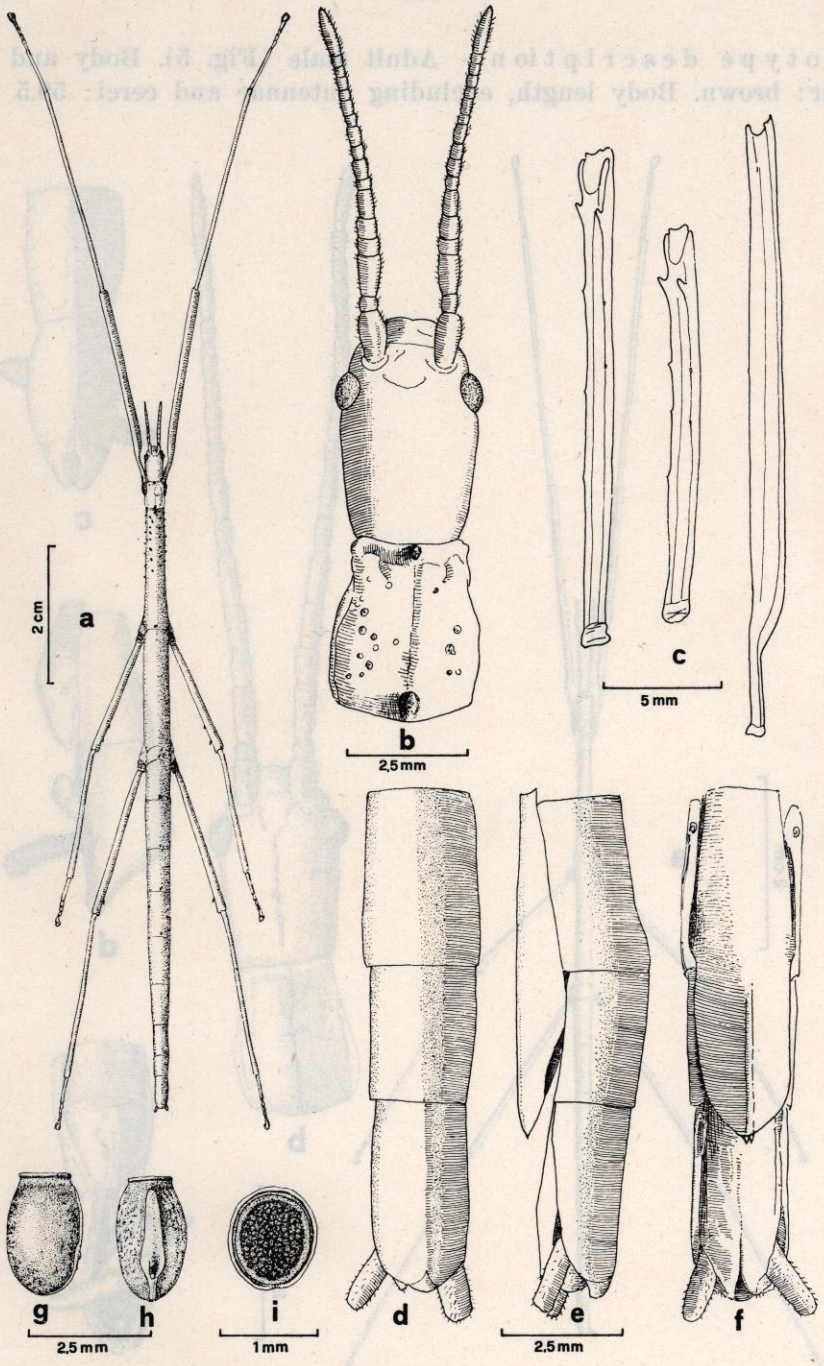

FIG. VI

Clonopsis maroccana from Torres de Alcala, Rif (Morocco). Adult female (paratype), dorsal view (*a*); head and pronotum, dorsal view (*b*); fore, median and hind femora, lateral-ventral view (*c*, respectively, from right to left); end of abdomen, dorsal (*d*), lateral (*e*), and ventral (*f*) view; egg, lateral (*g*) and dorsal (*h*) view; egg operculum (*i*).

Antennae elongated and thin, 10.4 mm long, with 17 articles. Pronotum 2.5 mm, mesonotum 11.8 mm, metanotum + median segment 13 mm long. Meso- and metanotum without evident granulation. Tubercles at the middle of the fore and hind hedge of pronotum and mesonotum slightly developed. 10th tergum deeply excised and denticulated at the apex; subgenital plate as in Fig. 5*d, e*; vomer very small. Cerci 1.8 mm long. Fore femora not denticulated, 22.8 mm long; median and hind

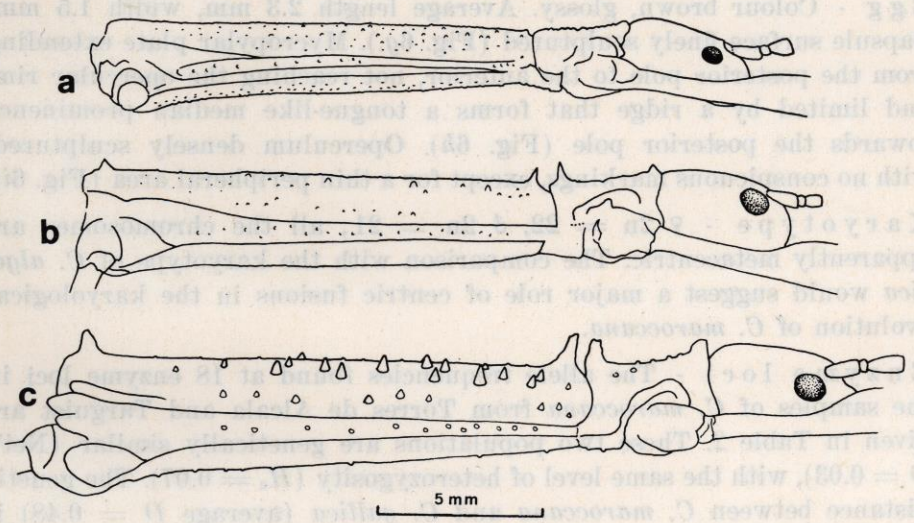

FIG. VII

Head, pronotum and mesonotum of *Clonopsis gallica* (*a*), *C. algerica* (*b*), and *C. maroccana* (*c*) females, lateral view, showing the different development of dorsal tubercles and granulation in the three species.

femora subapically denticulated, respectively 14.2 mm and 18.2 mm long. Tibiae length: fore 25.3 mm, median 14.1 mm, hind 19.0 mm.

Paratypes description - All adults. Body and legs colour: males brown to dark brown; females from light to dark brown, often darkly mottled on the body and on the femora. Total length (excluding antennae and cerci): ♂♂ 55-63.5 mm, ♀♀ 68.5-88.5 mm. Antennae with 17-18 articles, length ♂♂ 9.2-10.7 mm, ♀♀ 6.5-8 mm. Pronotum ♂♂ 2.1-2.6 mm, ♀♀ 3-3.8 mm; mesonotum ♂♂ 10-11.8 mm, ♀♀ 13.8-15 mm; metanotum + median segment ♂♂ 11.5-13.3 mm, ♀♀ 14.5-17.2 mm. ♂♂ without evident granulation, ♀♀ with the meso- and metanotum granulation conspicuous, and conic tubercles of various size, especially in the fore half of mesonotum. Tubercles at the middle of the fore and hind hedge of pronotum and mesonotum slightly developed in the males, large and conspicuous in the females. Subgenital plate broad, in the male distally tapered,

reaching the end of the 9th segment; in the female obtuse-ended, distinctly exceeding the 9th segment and covering the ovipositor. Fore femora not denticulated, ♂♂ 22-24 mm, ♀♀ 22.2-28 mm long; median and hind femora ♂♂ subapically denticulated, respectively 13.8-15.2 mm and 16.9-19.5 mm long; ♀♀ denticulated not only subapically, respectively 14.8-18 mm and 18.2-22 mm long. Tibiae length: fore ♂♂ 23.2-27 mm, ♀♀ 23.5-30.5 mm; median ♂♂ 13.5-15.8 mm, ♀♀ 13.2-17.8 mm; hind ♂♂ 18.4-21 mm, ♀♀ 18-23 mm.

Egg - Colour brown, glossy. Average length 2.3 mm, width 1.5 mm. Capsule surface finely sculptured (Fig. 6*g*.). Mycropylar plate extending from the posterior pole to the anterior, not reaching the opercular rim, and limited by a ridge that forms a tongue-like median prominence towards the posterior pole (Fig. 6*h*). Operculum densely sculptured, with no conspicuous markings, except for a thin peripheral area (Fig. 6*i*).

Karyotype - ♀ 2n = 22, ♂ 2n = 21; all the chromosomes are apparently metacentric. The comparison with the karyotype of *C. algerica* would suggest a major role of centric fusions in the karyological evolution of *C. maroccana*.

Enzyme loci - The allele frequencies found at 18 enzyme loci in the samples of *C. maroccana* from Torres de Alcala and Targuist are given in Table 2. These two populations are genetically similar (Nei's $D = 0.03$), with the same level of heterozygosity ($H_e = 0.07$). The genetic distance between *C. maroccana* and *C. gallica* (average $D = 0.48$) is lower than between *C. maroccana* and *C. algerica* (average $D = 0.63$). In the latter comparison, the genetic divergence is lower with the Misserghin population of *C. algerica* ($D = 0.44$) than with those from Kristel and Stidia ($D = 0.72$). These two populations of *C. algerica* are therefore the most differentiated ones from both *C. maroccana* and *C. gallica*.

Four out of the 18 loci studied show alternative alleles between *C. maroccana* and *C. algerica* (i.e. *α-Gpdh*, *Ldh*, *Adk*, and *Gpi*); the same loci, plus a fifth: *Mdh-2*, are diagnostic between *C. maroccana* and *C. gallica*. These loci allow a reliable identification of the three species at the electrophoretic level, as shown in Table 4. This is particularly useful in the early life stages, when the specimens cannot be classified morphologically.

Reproduction - *C. maroccana* reproduces bisexually, with a sex-ratio of about 1:1 (males apparently slightly outnumber females).

Distribution - Collected up to now only in the Rif region, Morocco, from sea level to about 600 m a.s.l.

Derivatio nominis - Latinization of the adjective « Moroccan », from the country where the new species was discovered.

Discussion and conclusions

The present study on the genus *Clonopsis,* carried out at the gene, chromosome and morphological level, changes considerably our picture of this group. Among the forms previously described, two: *gallica* and *algerica,* often considered as geographical races or as reproductive forms of the same species, have been shown to be fully distinct species. The other two forms, *affinis* and *occidentalis,* generally treated as subspecies of *C. gallica,* appear not to be tenable, due to the lack of consistent morphological and genetic differentiation. The peculiar clonal structure of *C. gallica,* together with the fact that different clones can live sympatrically, make anyway difficult its splitting in subspecific taxa. In the bisexual *C. algerica,* on the contrary, we have found evidence suggesting that at least two subspecies are present: *C. algerica algerica* west of Oran and a second one, still undescribed, east of this town. The former is genetically the closest both to *C. gallica* ($D = 0.43$) and to *C. maroccana* ($D = 0.44$), while the latter is the most differentiated from these two species ($D = 0.68$ from *C. gallica* and $D = 0.72$ from *C. maroccana*).

C. maroccana, the new species described in this paper, is differentiated from *C. algerica* and *C. gallica* at the gene, chromosome, and morphological level. Centric fusions appear to have played an important role in its karyotypic evolution. *C. maroccana* and *C. algerica* presumably diverged in geographic isolation, with an allopatric mode of speciation. A different mechanism was apparently involved in the speciation of *C. gallica.* Three hypotheses can be made on its origin: 1) from a single bisexual species, by the shift to parthenogenetic reproduction; 2) from the cross between two bisexual species, giving rise to a parthenogenetic hybridogen; 3) from the backcross between a diploid parthenogenetic hybridogen and the male of one of its bisexual ancestors, giving rise to a triploid hybrid species. According to the first hypothesis, the relatively high heterozygosity found in *C. gallica* at both gene and chromosome level would be the consequence of a stochastic building up of mutations after the shift of this species from bisexual to parthenogenetic reproduction. This does not agree with the pattern of genetic variation found in *C. gallica,* that generally involves relatively few loci, with a limited number of alleles, the same ones recurring in different clones. Moreover, in the karyotype of this species most chromosomes can be arranged in groups of two or three, while only few remain unpairable; a high number of the latter would be expected in the case of a random building up of chromosomal mutations.

The second and especially the third hypotheses appear in better agreement with the gene and chromosome data found in *C. gallica.* The pattern of speciation suggested by the latter hypothesis was recently

demonstrated in another stick insect (belonging, as the genus *Clonopsis*, to the family Bacillidae): *Bacillus lynceorum* Bullini, Nascetti and Bianchi Bullini, 1983. This triploid parthenogenetic hybridogen arose from the backcross between the hybrid parthenogenetic *B. whitei* Nascetti and Bullini, 1982, and one of its bisexual parental species: *B. grandii* Nascetti and Bullini, 1982 (Bullini, 1985; Bullini and Nascetti, 1986, 1987). In the case of *C. gallica* neither its hypothetical bisexual parental species, nor the diploid parthenogenetic hybridogen have been detected so far (gene and chromosome data rule out *C. algerica* and *C. maroccana* as possible parental species of *C. gallica*). This is not per se a proof against a hybrid origin of *C. gallica*. The genus *Clonopsis* has been poorly investigated so far and new species can well be discovered, as shown by the present study. Moreover, competition between a parthenogenetic hybridogen and its bisexual parental species must be taken into account. This phenomenon often causes the extinction of the bisexual relatives, or a strong reduction of their original ranges (Bullini, 1985). A hybrid origin of *C. gallica* could account for its wide distribution all over the Mediterranean region, compared to the restricted ranges of the bisexual *Clonopsis*. It was shown (Bullini, 1985) that hybrid species generally gain a strong selective advantage over their bisexual relatives, due both to their mode of reproduction (often by thelytokous parthenogenesis), that doubles the species' intrinsic rate of increase (demographic advantage), and to their enhanced level of heterozygosity (heterotic advantage).

Further research on *Clonopsis* populations, both bisexual and parthenogenetic, especially from areas not investigated so far, appear necessary to get a deeper insight into speciation mechanisms, systematics, and evolution of this group.

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SUMMARY

Gene, chromosome and morphological data are presented on *Clonopsis* populations from different countries (Portugal, Spain, Balearic Islands, France, Italy, Tunisia, Algeria, and Morocco). The following 18 enzyme loci have been genetically analyzed: *α-Gpdh*, *Ldh*, *Mdh-1*, *Mdh-2*, *Idh-1*, *Idh-2*, *6Pgdh*, *G3pdh*, *Sod-1*, *Sod-2*, *Got-1*, *Got-2*, *Hk-1*, *Adk*, *Pgm*, *Ca-2*, *Mpi*, and *Gpi*, on a total of 45 samples. The populations studied cluster in three main groups, corresponding to distinct species: *C. gallica*, *C. algerica*, and a third one, *C. maroccana*, described in the present paper. The taxa *occidentalis* and *affinis*, generally treated as subspecies of *C. gallica*, appear not to be tenable, due to the lack of consistent morphological and genetic differentiation.

C. gallica has been confirmed to reproduce by obligatory thelytokous parthenogenesis; no males have been observed both in the field and in laboratory breedings, over thousands of individuals examined. The karyotype of this species is presumably allotriploid, with a somatic chromosome number ranging from 54 to 57, even in the same individual; the higher figures are possibly due to chromosome breaking. Most chromosomes can be arranged in groups of two or three, while only few remain unpairable. At the gene level, *C. gallica* populations show fixed heterozygosity at several loci; its triploidy appears confirmed by the finding at some loci (i.e. *Mdh-2* and *Gpi*) of three different alleles coexisting in the same individual. A clonal structure has been found in this species, related to its way of reproduction. The interclonal variation generally involves relatively few loci, with a limited number of alleles, the same ones recurring in different clones. Several dozens of clones have been found, on the basis of the 18 loci analyzed; most of them are present in single or few localities, while others show a larger range of distribution; different clones can coexist in the same area. The genetic distance between clones is relatively low (Nei's D from 0.007 to 0.155), indicating a rather recent process of divergence. Also at the morphological level, *C. gallica* appears remarkably homogeneous.

C. algerica, often considered as a geographical race or as the bisexual form of *C. gallica*, has been found to be differentiated from the latter both at the gene and chromosome level. The karyotype of *C. algerica*, 2n = 32 in the female and 2n = 31 in the male, includes 3 metacentric and 13 telocentric chromosome pairs. At the gene level, the population from the surroundings of Misserghin (near Oran, patria typica of *C. algerica*) appears rather differentiated from those east of Oran (D = 0.16), the latter presumably belonging to a distinct subspecies, still undescribed. Their genetic variability is also different, H_e being respectively 0.11 at Misserghin and 0.03 in the samples east of Oran. A number of loci (*α-Gpdh*, *Ldh*, *Mdh-2*, and *Adk*) show alternative alleles between *C. algerica* and *C. gallica*, also when sympatric. Their average genetic distance is 0.60. Both chromosome and gene data indicate that *C. gallica* is not a direct derivative of *C. algerica*. The main morphological characters allowing their identification are reviewed.

The new species *C. maroccana* has been discovered in the Rif region (Morocco); it reproduces bisexually and shows a sex ratio of about 1:1 (males apparently slightly outnumber females). The main morphological character that differentiates this species from *C. gallica* and *C. algerica* is the longer antennae, with a higher number of articles. Body size is on average larger. *C. maroccana* females show further diagnostic characters: meso- and metanotum granulation more conspicuous, with conic tubercles of various size, especially in the fore half of mesonotum; larger-sized tubercles at the middle of the fore and hind hedge of pronotum and mesonotum; denticulation of median and hind femora not limited to the apical part, etc. The eggs of *C. marocccana* are smaller, glossy, with a peculiar sculpturing of the capsule and operculum.

C. maroccana has a chromosome number of 2n = 22 in the female, 2n = 21 in

the male, all pairs being apparently metacentric; centric fusions appear to have played an important role in its karyotypic evolution. At the gene level, 4 out of the 18 loci studied show alternative alleles between *C. maroccana* and *C. algerica* (i.e. *α-Gpdh*, *Ldh*, *Adk*, and *Gpi*); the same loci, plus a fifth: *Mdh-2*, are diagnostic between the former and *C. gallica*. In spite of this finding, the average genetic distance is lower between *C. maroccana* and *C. gallica* ($D = 0.48$) than between *C. maroccana* and *C. algerica* ($D = 0.63$). This is due to the high genetic divergence of *C. algerica* populations east of Oran, that are the most differentiated not only from *C. maroccana* ($D = 0.72$), but also from *C. gallica* ($D = 0.68$). On the basis of the loci found diagnostic between the three species, biochemical keys are given allowing their electrophoretic identification in both sexes and at any life stage.

The possible mechanisms involved in the speciation of *C. gallica*, *C. algerica*, and *C. maroccana* are considered. The last two species presumably diverged in geographic isolation, with an allopatric mode of speciation. As to *C. gallica*, different hypotheses are discussed, among which the most consistent with the genetic data involves a hybrid origin of this species (a mode of speciation demonstrated in other parthenogenetic stick insects of the family Bacillidae). The selective advantages gained by hybridogens are discussed. They could account for the wide distribution of *C. gallica* all over the Mediterranean region, compared to the restricted ranges of the bisexual *Clonopsis*. Unlike other stick insect hybridogens (e.g. *Bacillus whitei* and *B. lynceorum*), the hypothetical parental species of *C. gallica* have not been detected so far (*C. algerica* and *C. maroccana* appear ruled out on the basis of gene and chromosome data). The possible causes are discussed, among which the reduction of the parental species' range, or even their extinction, due to competition with their parthenogenetic hybrid derivative.

Ricerche genetiche e tassonomiche sul genere *Clonopsis* e descrizione di una nuova specie (Phasmatodea, Bacillidae)

RIASSUNTO

Vengono esposti i dati di ricerche elettroforetiche, cariologiche e morfologiche su 45 popolazioni del genere *Clonopsis* provenienti da vari paesi (Portogallo, Spagna, Isole Baleari, Francia, Italia, Tunisia, Algeria e Marocco). Sono stati analizzati 18 loci enzimatici: *α-Gpdh*, *Ldh*, *Mdh-1*, *Mdh-2*, *Idh-1*, *Idh-2*, *6Pgdh*, *G3pdh*, *Sod-1*, *Sod-2*, *Got-1*, *Got-2*, *Hk-1*, *Adk*, *Pgm*, *Ca-2*, *Mpi* e *Gpi*. Le popolazioni studiate formano tre gruppi principali, che corrispondono a specie diverse: *C. gallica*, *C. algerica* e una terza, *C. maroccana*, descritta nel presente lavoro. I taxa *occidentalis* e *affinis*, generalmente considerati sottospecie di *C. gallica*, non risultano validi poiché non presentano differenze significative a livello morfologico e genetico.

Risulta confermato che *C. gallica* si riproduce per partenogenesi telitoca obbligatoria; non sono stati osservati maschi né sul campo né negli allevamenti di laboratorio, su migliaia di individui esaminati. Il cariotipo di questa specie è presumibilmente allotriploide, con un numero cromosomico che varia nelle cellule somatiche da 54 a 57, anche nello stesso individuo; i numeri cromosomici più alti sembrano dovuti a rottura di uno o più cromosomi. La maggior parte dei cromosomi forma gruppi di due o tre elementi, mentre solo pochi risultano non appaiabili. A livello genico le popolazioni di *C. gallica* mostrano eterozigosi fissata ad alcuni loci; la triploidia di questa specie risulta confermata dall'aver trovato tre alleli diversi coesistere ad alcuni loci (*Mdh-2* e *Gpi*) in uno stesso individuo. *C. gallica* risulta costituita da un numero molto elevato

di cloni (alcune decine sulla base dei 18 loci studiati); questo fenomeno va correlato con le modalità riproduttive della specie. Il differenziamento genetico tra cloni riguarda di solito un numero relativamente basso di loci, ciascuno con pochi alleli che ricorrono in cloni diversi. La maggior parte dei cloni è stata rinvenuta in una singola o in poche località, mentre altri mostrano una distribuzione geografica più ampia; cloni diversi possono coesistere in una medesima località. La distanza genetica tra i vari cloni è relativamente bassa; *D* secondo Nei varia da 0,007 a 0,155 nei diversi confronti a coppie. Ciò indica che in questa specie il processo di divergenza genetica è relativamente recente. Anche a livello morfologico *C. gallica* risulta notevolmente omogenea.

C. algerica, spesso considerata una razza geografica di *C. gallica* o la sua forma bisessuata, risulta differire da quest'ultima sia a livello genico che cromosomico. Il cariotipo di *C. algerica* è infatti 2n = 32 nelle femmine e 2n = 31 nei maschi; esso comprende 3 coppie metacentriche e 13 telocentriche. A livello genico la popolazione dei dintorni di Misserghin (vicino a Orano, patria typica di *C. algerica*), risulta notevolmente differenziata da quelle ad est di Orano (*D* = 0,16); queste ultime appartengono presumibilmente a una sottospecie distinta, non ancora descritta. Anche la variabilità genetica risulta differire tra le due presunte sottospecie; l'eterozigosi media per locus è infatti 0,11 nella popolazione dei dintorni di Misserghin e 0,03 in quelle raccolte ad est di Orano. Vari loci (*α-Gpdh*, *Ldh*, *Mdh-2* e *Adk*) mostrano alleli alternativi fra *C. algerica* e *C. gallica* anche in condizioni di simpatria tra le due specie. La loro distanza genetica media è 0,60. Sia i dati cromosomici che quelli elettroforetici indicano che *C. gallica* non è derivata direttamente da *C. algerica*. Vengono riassunti i principali caratteri morfologici che consentono l'identificazione di queste due specie.

La nuova specie di *Clonopsis* descritta nel presente lavoro: *C. maroccana* è stata scoperta nella regione del Rif (Marocco). Si riproduce per anfigonia e presenta un rapporto sessi di circa 1:1 (i maschi sembrano un po' più numerosi delle femmine). Il principale carattere morfologico che differenzia questa specie da *C. gallica* e *C. algerica* è rappresentato dalle antenne più lunghe, con più alto numero di articoli. Le dimensioni corporee sono in media maggiori. Le femmine di *C. maroccana* presentano ulteriori caratteri diagnostici: la granulazione del meso- e del metanoto è più sviluppata e vi sono tubercoli conici di varia dimensione specialmente nella metà anteriore del mesonoto; i tubercoli situati al centro del margine anteriore e posteriore del pronoto e del mesonoto sono nettamente più grandi; la denticolazione dei femori intermedi e posteriori non risulta limitata alla loro porzione apicale, ecc. Le uova di *C. maroccana* sono più piccole, translucide, con una peculiare microscultura sulla superficie esterna della capsula e dell'operolo.

Il numero cromosomico di *C. maroccana* è 2n = 22 nelle femmine e 2n = 21 nei maschi. Tutte le coppie sono apparentemente metacentriche; la fusione centrica sembra aver giocato un ruolo importante nell'evoluzione cariologica di questa specie. A livello genico 4 dei 18 loci studiati presentano alleli alternativi tra *C. maroccana* e *C. algerica* (*α-Gpdh*, *Ldh*, *Adk* e *Gpi*); gli stessi loci, più un quinto: *Mdh-2*, sono diagnostici tra *C. maroccana* e *C. gallica*. Nonostante questo, la distanza genetica media di *C. maroccana* è più bassa da *C. gallica* (*D* = 0,48) che da *C. algerica* (*D* = 0,63). Ciò è dovuto all'elevato differenziamento genetico delle popolazioni di *C. algerica* ad est di Orano; esse risultano le più differenziate non solo da *C. maroccana* (D = 0,72), ma anche da *C. gallica* (*D* = 0,68). Sulla base dei loci trovati diagnostici tra le tre specie, vengono date chiavi biochimiche che consentono la loro identificazione elettroforetica in entrambi i sessi ed anche negli individui molto giovani, non classificabili morfologicamente.

Vengono presi in considerazione i possibili meccanismi di speciazione di *C. gallica*, *C. algerica* e *C. maroccana*. Le ultime due specie si sono verosimilmente differenziate in isolamento geografico, con una speciazione di tipo allopatrico. Quanto a *C. gallica* vengono discusse diverse ipotesi, tra cui quella di una sua origine ibrida. Questo mec-

canismo di speciazione, recentemente dimostrato in altri fasmidi partenogenetici della famiglia Bacillidae, sembra meglio degli altri in accordo con i dati elettroforetici e cromosomici ottenuti in questa specie. Vengono discussi i vantaggi selettivi propri delle specie di origine ibrida. Essi potrebbero spiegare perché *C. gallica* presenti un areale così vasto (comprendente tutta la regione mediterranea), rispetto a quelli molto ristretti delle specie bisessuate di *Clonopsis*. A differenza di altri fasmidi di origine ibrida (come *Bacillus whitei* e *B. lynceorum*), le ipotetiche specie parentali di *C. gallica* non sono state fino ad ora scoperte (i dati elettroforetici e cromosomici portano ad escludere che *C. algerica* e *C. maroccana* abbiano avuto questo ruolo). Ciò potrebbe essere dovuto a fenomeni di competizione tra le specie bisessuate parentali e la specie partenogenetica ibrida da esse derivata, con conseguente forte riduzione dell'areale delle prime od anche loro estinzione.

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TABLE 1 - Genotypes at 9 variable loci for thirty of the clones observed in *Clonopsis gallica*.

Loci and Genotypes									
Mdh-1	*Mdh-2*	*6Pgdh*	*Got-1*	*Hk-1*	*Pgm*	*Ca-2*	*Mpi*	*Gpi*	Geographic origin
100/100/100	78/ 88/100	100/108/108	100/100/100	96/100/100	100/100/100	90/100/100	100/100/100	100/100/100	France
100/100/100	88/100/100	100/108/108	100/100/100	96/100/100	95/100/100	90/100/100	100/100/100	100/100/100	France
100/100/100	78/ 88/100	100/108/108	100/100/100	96/100/100	95/100/100	90/100/100	100/100/100	100/100/100	France
100/100/100	78/ 88/100	100/100/108	100/100/105	100/100/100	100/100/100	90/100/100	100/100/100	100/100/100	France and Italy
100/100/100	78/ 88/100	100/100/108	100/100/105	100/100/100	100/100/100	90/100/100	100/100/100	100/100/104	Spain
93/100/100	78/ 88/100	100/100/108	100/100/105	100/100/100	100/100/100	90/100/100	100/100/100	100/100/100	France
100/100/100	78/ 88/100	100/100/108	100/100/105	100/100/100	100/100/100	90/100/100	100/112/112	100/100/100	Portugal
100/100/100	78/ 88/100	100/100/100	100/100/105	100/100/100	95/100/100	90/100/100	100/112/112	100/100/100	Italy, Tunisia, Algeria
100/100/100	78/ 88/100	100/100/100	100/100/105	100/100/100	95/100/100	90/100/100	112/112/112	100/100/100	Algeria
100/100/100	78/ 88/100	100/100/100	100/100/105	100/100/100	95/100/100	90/100/100	100/112/112	100/100/104	Algeria
100/100/100	78/ 88/100	100/100/100	100/100/100	100/100/100	95/100/100	90/100/100	100/112/112	100/100/100	Spain
100/100/100	78/ 88/100	100/100/100	100/100/100	96/100/100	95/100/100	90/100/100	112/112/112	100/100/100	Balearic Islands
100/100/100	78/ 88/100	100/100/108	100/100/100	100/100/100	95/100/100	90/100/100	112/112/112	100/100/100	Spain
100/100/100	88/100/100	100/100/100	100/100/105	100/100/100	95/100/100	90/100/100	112/112/124	90/100/100	Morocco
100/100/100	88/100/100	100/100/100	100/100/100	100/100/100	100/100/100	100/100/100	100/112/112	100/100/100	Morocco
100/100/100	78/ 88/ 88	100/100/100	100/100/100	100/100/100	100/100/100	100/100/100	100/112/112	100/100/100	Morocco
100/100/100	78/ 88/ 88	100/100/100	100/100/105	100/100/100	100/100/100	90/100/100	100/112/112	100/104/104	Algeria
100/100/100	88/100/100	100/100/108	100/100/105	100/100/100	100/100/100	90/100/100	112/112/124	100/100/100	Morocco
100/100/100	78/ 88/100	100/100/108	100/100/105	100/100/104	100/100/100	90/100/100	112/112/124	100/100/104	Spain
100/100/100	78/ 88/100	100/100/108	100/100/105	100/100/100	100/100/100	90/100/100	112/112/124	100/100/104	Spain
100/100/100	78/ 88/100	100/100/108	100/100/105	100/100/100	100/100/100	90/100/100	112/112/124	100/100/100	France
100/100/100	78/ 88/ 88	100/100/108	100/100/105	100/100/100	100/100/100	90/100/100	112/112/124	100/100/100	Algeria
100/100/100	78/ 88/ 88	100/100/108	100/100/105	100/100/100	100/100/100	90/100/100	112/112/112	100/100/100	Algeria
100/100/100	78/ 88/100	100/100/100	100/100/105	100/100/100	100/100/100	90/100/100	112/112/112	100/100/104	Morocco
100/100/100	78/ 88/100	100/100/100	100/100/100	100/100/100	100/100/100	90/100/100	112/112/112	100/100/104	Morocco
100/100/100	88/100/100	100/100/100	100/100/105	100/100/100	100/100/100	90/100/100	112/112/112	100/100/104	Morocco
100/100/100	88/100/100	100/100/108	100/100/105	100/100/100	100/100/100	85/ 90/100	112/112/112	100/100/104	Spain
100/100/100	88/100/100	100/100/108	100/100/100	100/100/100	100/100/100	100/100/112	112/112/124	100/100/104	Morocco
100/100/100	78/ 88/100	100/100/108	100/100/100	100/100/100	100/100/100	100/100/112	112/112/124	100/100/104	Algeria
100/100/100	78/ 88/ 88	100/100/100	100/100/105	100/100/100	100/100/105	90/100/100	112/112/118	100/104/108	Algeria

TAB. 2. - Allele frequencies in populations of *Clonopsis algerica* (STI, KRI, MIS) and *C. maroccana* (TOR, TAR). STI = Stidia, Algeria; KRI = Kristel, Algeria; MIS = Misserghin, Algeria; TOR = Torres de Alcala, Morocco; TAR = Targuist, Morocco. H_e = expected mean heterozygosity per locus.

Loci	Alleles	STI	KRI	MIS	TOR	TAR
α-Gpdh	*70*	0.00	0.00	0.00	0.63	0.50
	78	0.00	0.00	0.00	0.00	0.50
	82	1.00	1.00	1.00	0.00	0.00
	85	0.00	0.00	0.00	0.37	0.00
Ldh	*107*	1.00	1.00	1.00	0.00	0.00
	109	0.00	0.00	0.00	1.00	1.00
Mdh-1	*90*	0.00	0.05	0.00	1.00	0.64
	100	1.00	0.95	1.00	0.00	0.36
Mdh-2	*105*	1.00	1.00	1.00	1.00	1.00
Idh-1	*97*	0.00	0.10	0.09	0.00	0.00
	100	1.00	0.90	0.90	1.00	1.00
	110	0.00	0.00	0.01	0.00	0.00
Idh-2	*100*	0.02	0.00	0.10	1.00	1.00
	110	0.98	1.00	0.90	0.00	0.00
6Pgdh	*100*	1.00	1.00	1.00	1.00	1.00
G3pdh	*100*	1.00	1.00	1.00	1.00	1.00
Sod-1	*90*	1.00	1.00	0.00	0.00	0.00
	100	0.00	0.00	1.00	1.00	1.00
Sod-2	*100*	1.00	1.00	1.00	1.00	1.00
Got-1	*92*	0.00	0.00	0.28	0.00	0.00
	100	0.00	0.12	0.42	0.88	0.79
	105	0.98	0.88	0.29	0.00	0.14
	110	0.02	0.00	0.01	0.12	0.07
Got-2	*100*	0.02	0.04	1.00	1.00	1.00
	106	0.98	0.96	0.00	0.00	0.00
Hk-1	*96*	0.00	0.00	0.00	0.43	0.00
	100	1.00	1.00	1.00	0.57	1.00
Adk	*93*	1.00	1.00	1.00	0.00	0.00
	96	0.00	0.00	0.00	1.00	1.00
Pgm	*95*	0.00	0.00	0.22	0.00	0.00
	100	1.00	1.00	0.73	1.00	1.00
	105	0.00	0.00	0.05	0.00	0.00
Ca-2	*88*	0.00	0.00	0.05	0.00	0.00
	100	1.00	1.00	0.93	1.00	1.00
	110	0.00	0.00	0.02	0.00	0.00
Mpi	*118*	0.50	1.00	0.48	0.00	0.00
	124	0.50	0.00	0.52	1.00	1.00
Gpi	*100*	1.00	1.00	1.00	0.00	0.00
	102	0.00	0.00	0.00	1.00	1.00
H_e		*0.034*	*0.031*	*0.115*	*0.065*	*0.073*

TAB. 3 - Comparison of morphological characters between *Clonopsis gallica*, *C. algerica*, and *C. maroccana*. Measures are given in mm.

Character	Sex	*C. gallica*	*C. algerica*	*C. maroccana*
body length	♀♀	62-73	62-80	68-90
	♂♂	—	48-58	55-64
antennae length	♀♀	3.5-4.5	4.1-5.8	5.5-8
	♂♂	—	6-8.1	9-11.1
antennae articles	♀♀	12-13	14-16	17-18
	♂♂	—	13-14	17
pronotum	♀♀	2.5-3.1	2.5-3.3	3-3.8
	♂♂	—	2-2.4	2-2.6
mesonotum	♀♀	11-13.9	11-14	13.7-15.2
	♂♂	—	9-10.5	10-11.8
metanotum + median segment	♀♀	11.3-14.5	11.9-15.5	14.5-17.3
	♂♂	—	10.6-11.5	11.2-13.6
fore femora	♀♀	16-23.2	19.5-25.8	22-28.3
	♂♂	—	17.9-22.5	21.8-24.2
median femora	♀♀	10-14.3	12.5-17.2	14.4-19.5
	♂♂	—	11.8-14.7	13.5-16.1
hind femora	♀♀	14-18.3	15.4-20.5	16.7-22.2
	♂♂	—	14.9-19	16.5-20.6

TAB. 3 (*continues*)

Character	Sex	*C. gallica*	*C. algerica*	*C. maroccana*
meso- and metanotum granulation	♀♀	slightly granulated	more granulated than in *C. gallica*	more granulated than in *C. algerica*, with conic tubercles of various size
	♂♂	—	without evident granulation	without evident granulation
tubercles at the middle of the fore and hind hedge of pronotum and mesonotum	♀♀	slightly developed, one or more often lacking	generally well developed, bigger than in *C. gallica*	conspicuous, always more developed than in *C. algerica*
	♂♂	—	slightly developed	slightly developed
femora denticulation	♀♀	fore without, median and hind denticulated subapically	fore without, median and hind denticulated subapically	fore without, median and hind denticulated not only near the apex
	♂♂	—	fore without, median and hind denticulated subapically	fore without, median and hind denticulated subapically
subgenital plate	♀♀	broad, reaching the end of the 9th segment	broader than in *C. gallica*, exceeding the 9th segment	broad as or more than *C. algerica*, exceeding the 9th segment
	♂♂	—	broad, distally tapered, reaching the end of the 9th segment	broad, distally tapered, reaching the end of the 9th segment
egg (length x width)		2.6 x 1.8	2.4 x 1.6	2.3 x 1.5
karyotype	♀♀	3n = 54-57	2n = 32	2n = 22
	♂♂	—	2n = 31	2n = 21

TAB. 4. - Biochemical keys to the identification of *Clonopsis maroccana*, *C. algerica*, and *C. gallica*, based on the allozymes at the loci *α-Gpdh*, *Ldh*, and *Adk*.

Loci	Alleles		Species
α-Gpdh	*100*	⟶	*C. gallica*
	82	⟶	*C. algerica*
	70, 78, 85	⟶	*C. maroccana*
Ldh	*100*	⟶	*C. gallica*
	107	⟶	*C. algerica*
	109	⟶	*C. maroccana*
Adk	*100*	⟶	*C. gallica*
	93	⟶	*C. algerica*
	96	⟶	*C. maroccana*

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