



The Genetic and Cytogenetic Relationships among Subgenera of *Chrysolina* Motschulsky, 1860 and *Oreina* Chevrolat, 1837 (Coleoptera: Chrysomelidae: Chrysomelinae)

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Abstract

Molecular phylogenetic analyses mainly based on mtDNA and nuDNA sequences and/or, secondarily, on chromosomes and plant trophic affiliations, have been obtained in 84 species of *Chrysolina* and 19 of its closely related genus *Oreina*, belonging to 42 of the 70 described subgenera of the former and to all of the seven ones of the latter. Bayesian and Maximum likelihood phylogenetic trees showed monophyly of *Chrysolina-Oreina*, with four main clades of subgenera with high support values. The first clade includes subgenera *Chrysolinopsis* and *Taeniochrysea*, and the second those of *Chrysomorpha*, *Euchrysolina*, *Melasomoptera* and *Synerga*. The third is much large and composed of three subclades of subgenera, *Chrysocrosita* and *Erythrochrysa*, *Colaphosoma* and *Maenadochrysa*, and *Centoptera*, *Fasta*, the subgenera of *Oreina* and *Timarchoptera*. These three main clades enclose most species with $2n=24(Xy_p)$ male chromosomes and an ancient trophic affiliation with Lamiaceae plants, at the root of the core of *Chrysolina*. However, a host shift from Lamiaceae to Asteraceae was detected in all but one subgenera of *Oreina* and another from Lamiaceae to Apiaceae in *Oreina* s. str. and in *Ch. (Timarchoptera) haemochlora*, a species with $2n(\♂)=23(XO)$ chromosomes. The fourth main clade holds more than a half of subgenera and is ambiguous for ancestral host-plant reconstruction, with nine botanic families. A statistically significant shift for a second association to Apiaceae was found in a subclade with *Crositops*, *Minckia* and *Threnosoma*, and the species *Ch. soiota*, this last should be excluded from *Timarchoptera* and moved to *Paraheliostola* L. Medvedev stat. ressur. thereby these two subgenera could not be synonymous as it was recently suggested. New trophic shifts to *Hypericaceae* were clearly substantiated in *Hypericia* and *Sphaeromela* subclade, and to *Plantaginaceae* in another with *Colaphodes*, *Ovosoma*, *Palaeosticta* and probably *Taeniosticha*. A further great subclade showing a shift to Asteraceae is that of *Allochrysolina*, *Anopachys*, *Apterosoma*, *Chalcoidea*, *Sibiriella* and the species *Ch. (Pezocrosita) convexicollis*. All species in these latter subclades display high chromosome numbers, namely $2n=32(Xy_p)$ in *Sphaeromela*, $2n=38(Xy_p)$ in *Hypericia*, $2n=40(Xy_p)$ in *Anopachys*, *Chalcoidea*, *Colaphodes*, *Minckia* and *Ovosoma*, and $2n(\♂)=47(XO)$ in *Threnosoma*. Possible reversal shifts from *Plantaginaceae* to *Lamiaceae* and to *Asteraceae* were found in the subclades of *Arctolina*, *Colaphoptera* and *Pleurosticha*, whose species have low chromosome numbers, $2n=24(Xy_p)$ or $2n=26(Xyp)$. Near to these previous, the subclade of *Chrysolina* s. str. holds species with a relative polyphagy, on four plant families, and sharing $2n(\♂)=23(XO)$ chromosomes. Furthermore, the subclade of *Stichoptera*, mainly affiliated to *Scrophulariaceae*, is an exception among the subgenera of *Chrysolina* due to its high variation in chromosome numbers from $2n=22(Xy_p)$ to $2n=34(Xy_p)$. Finally, the rising of some subgenera of *Chrysolina* to the genus rank was statistically rejected with highly significant values.

Keywords: *Chrysolina*; *Oreina*; DNA; Chromosomes; Phylogenies

Introduction

A recent insightful and detailed taxonomic review of the genus *Chrysolina* Motschulsky, 1860, based on morphological and male genitalia adult characters [1], has prompted us to set up the possible genetic interrelationships among many of the described subgenera, taking into account mitochondrial gene sequences [2-5], and both mitochondrial and nuclear DNA [6], together with chromosomal studies [7-15], and host-plant affiliations because most species of *Chrysolina* and its near genus *Oreina*, are highly specific feeders on one or less often two botanic families [16-18].

Material and Methods

A total of 84 species of *Chrysolina* of 43 subgenera and 19 species of *Oreina* of the seven described subgenera have been genetically analyzed, either by DNA sequences and/or by karyotypes. Three different molecular markers were selected for building a phylogenetic tree: a partial sequence of the mitochondrial 16S rDNA (*rrnL*), a partial sequence of the mitochondrial cytochrome c oxidase subunit 1 gene (*cox1*), and a fragment from the nuclear histone 3 gene (*nuH3*) [6]. We have also used the published mitochondrial phylogenetic trees based on the small subunit ribosomal RNA (12S) and the large subunit ribosomal RNA (16S) [5], that on the large subunit ribosomal RNA (16S) and the cytochrome c oxidase subunit 1 gene (*cox1*) [3], and that on the large subunit ribosomal RNA (16S) only [4]. The chromosomal analyses in meiotic and mitotic testis cells, by classical methods, have given chromosome numbers, male sex-chromosome systems, and in some cases detailed karyotypes of *Chrysolina* and *Oreina* species, reported in multiple publications [6,7,10]. In addition, host-plant affiliations to different botanic families are also considered as worth data for phylogenetic trees. Bayesian, Neighbour-joining (NJ), Maximum Likelihood (ML) and/or Maximum Parsimony (PAUP) trees have been implemented in the different published cladograms [2-6].

Results

The genetic findings, DNA sequences and/or karyotypes, on 84 species of *Chrysolina* among a total number of almost 500, belonging to 43 subgenera, 61% of the whole 70 described ones [1], have been used for our review. The DNA data of *Chrysolina* are compiled in 63 species and the chromosomes in 69 species, both corresponding to 35 subgenera, and in the closely related genus *Oreina*, 19 species of the seven described subgenera, have been analyzed by DNA sequences [3-6] and/or chromosomes [7-25]. Moreover, the findings on plant affiliations in these species of *Chrysolina* and *Oreina* are also taken from several authors [26-31]. Therefore, we believe that this sample is enough representative for getting reliable genetic interrelationships among these subgenera of

Chrysolina and *Oreina*.

Discussion and Conclusions

The Bayesian and Maximum Likelihood phylogenetic trees obtained from the combined analysis of mtDNA *cox1* and *rrnL* genes and nuDNA *H3* gene, have shown three distinctive most plesiomorphous clades of species feeding on Lamiaceae plants (P=0.98 [6]: 1) that including *Ch. (Taeniochrysa) americana* and *Ch. (Chrysolinopsis) gemina*, 2) another with three species of the subgenus *Synergia* Weise, 1900, *Ch. coeruleans*, according with Hsiao and Pasteels [5], *Ch. herbacea* (Duftschmid, 1825) and *Ch. viridana* (Küster, 1844), two species of the subgenus *Melasomoptera* Bechyné, et al. [32], *Ch. grossa* (Fabricius, 1792), according with Hsiao and Pasteels [5], and *Ch. lucida* (Olivier, 1807), one of the subgenus *Chrysomorpha* Motschulsky, et al. [33], *Ch. cerealis cyaneoaurata* (Motschulsky, 1860) and another of the subgenus *Euchrysolina* Bechyné [32], *Ch. graminis* (Linnaeus, 1758), this latter species according with Hsiao and Pasteels [5], 3) a third large clade made of three taxa of the subgenus *Maenadochrysa* Bechyné [32] *Ch. affinis baetica* (Suffrian, 1851), *Ch. affinis mesatlantica* (Kocher, 1958) and *Ch. femoralis* (Olivier, 1790), close to them the monotypic subgenus *Erythrochrysa* Bechyné [32], with *Ch. polita* (Linnaeus, 1758), the Asian subgenus *Chrysocrosita* Bechyné [32], with *Ch. jakowlewi* (Weise, 1894), and also in the same clade but in an intermediate position between the subgenus *Maenadochrysa* and those of these two latter taxa, *Ch. (Colaphosoma) sturmi* (Westhoff, 1882), the unique species of this last subgenera, plus a doubtful and misleading subclade composed of *Ch. (Centoptera) bicolor* (Fabricius, 1775), *Ch. (Fasta) fastuosa* (Scopoli, 1763). All species of these three clades, except *Ch. sturmi* which has not been cytogenetically studied yet, display $2n=24(Xy_p)$ chromosomes and similar karyotypes [11,19-20,23], and the nine karyotypes which have been carefully analyzed, show meta- or submetacentric chromosomes and among them a satellited first autosome [7,23], that could be a marker chromosome for all these group of species, and bearing the ribosomal DNA locus, very likely. Two further species feeding also on Lamiaceae, that were only chromosomally analyzed but not by DNAs, and sharing also $2n=24(Xy_p)$, *Ch. (Bittotaenia) salviae* (Germar, 1824) and *Ch. (Rhyssoloma) fragariae* (Wollaston, 1824) (7,43,44), should probably join to any one of these three previous clades of *Chrysolina*.

In the Maximum Likelihood phylogenetic tree obtained by a combined analysis of the three sequenced genes [6], and close to the latter subclade of *Ch. bicolor* and *Ch. fastuosa*, there is another one (P=0.527 [6], made of five species of *Oreina*, of two different subgenera, *Chrysochloa* Hope, 1840 and *Oreina s.str.*, all of them but one sharing karyotypes with $2n=24(Xy_p)$ chromosomes [8,21], but with an additional

species of *Chrysolina*, *Ch. (Timarchoptera) haemochlora* (Gebler, 1823). This last species displays a highly distinct karyotype of $2n(\♂)=27(XO)$ chromosomes, strikingly diverse in size, with two autosome pairs threefold larger than any of the remainders and lacking the Y-chromosome [11]. The feeding affiliations of the species in this subclade are very heterogeneous, *Ch. haemochlora* and two species of *Oreina* on Apiaceae, and the other three species of *Oreina* on Asteraceae, contrary to all the above mentioned species on Lamiaceae plants [5,6,29]. Therefore, the presence in this subclade of at least one species of *Chrysolina* with five ones of *Oreina* has induced us to apply an Approximately Unbiased test [34] to see whether *Oreina* is a different genus from *Chrysolina*, and the AU test has given a statistically significant value of 0.016, $P < 0.05$, rejecting this hypothesis [6], so they might be joined, although most recent authors still keep them separated [1,18,35,36].

Another new and very heterogeneous main clade, the fourth one, includes subgenera affiliated to all the nine plant families so far recorded in *Chrysolina*. Those on Apiaceae plants encloses the three analyzed species in the subgenus *Threnosoma* Motschulsky, 1860, *Ch. helopioides* (Suffrian, 1851), *Ch. obscurella* (Suffrian, 1851) and *Ch. timarchoides*, one of the subgenus *Crositops* Marseul, 1883, *Ch. pedestris*, one also in the subgenus *Timarchoptera* Motschulsky, 1860, *Ch. soiota* [6], and three in the subgenus *Minckia* Strand, 1835 (= *Sulcicollis* J. Sahlberg, 1913), *Ch. oricalcia* (O.F. Müller, 1776), *Ch. peregrina* (Herrich-Schäffer, 1831) and *Ch. rufoaenea* (Suffrian, 1851) [5,6], all of them, out of maybe *Ch. soiota*, feeding on this plant family [6,16,17,29]. Their chromosome numbers and sex-chromosome systems are different but all showing high values, thereby the three quoted species of *Threnosoma* share $2n(\♂)=47(XO)$, those of *Minckia*, *Ch. oricalcia* $2n=40(Xy_p)$, *Ch. peregrina* $2n=46(Xy_p)$ and *Ch. rufoaenea* $2n=50(Xy_p)$, the highest updated number in the *Chrysolina* and the subfamily Chrysomelinae, plus the unique species of the subgenus *Cyrtochrysolina* Kippenberg (2012), *Ch. marcasitica* (Germar, 1824) $2n=42(Xy_p)$ [8,11,21,23], and *Ch. pedestris* with $2n(\♂)=47(XO)$, as those of the subgenus *Threnosoma*, though having two large ring-shaped autosome bivalents, very remarkable among the remaining medium or small ones, and a tiny X-chromosome. Mikhailov [38], and recently also Bienkowski [1], synonymized the subgenera *Timarchoptera* Motschulsky (1860) and *Paraheliostola* L. Medvedev, et al. [38], but the molecular phylogeny of their respective typical species, *Ch. haemochlora* and *Ch. soiota*, does not support this view, because when we applied the AU Shimodaira test, the hypothesis of monophyly for both species was statistically rejected [6]. Therefore, *Paraheliostola* L. Medvedev (1992) should be *stat. ressur.* as a good new subgenus.

Two further subgenera, *Hypericia* Bedel, 1899 and the

monotypic *Sphaeromela* Bedel, 1899, the first with four DNA sampled species, *Ch. brunsvicensis* (Gravenhorst, 1807), *Ch. geminata* (Paykull, 1799), *Ch. hyperici* (Forster, 1771) and *Ch. quadrigemina* (Suffrian, 1851), and the second with *Ch. varians* (Schaller, 1783) [3-6,16,17], constitute a well-defined subclade. All these species are feeding on Hypericaceae plants, a botanic affiliation which has been found in no other species of the remaining subgenera [16,17], out of the Moroccan *Ch. seriatopora*, of the monotypic subgenus *Atlasiana* Bourdonné et Doguet, 1991 [17], which has not been genetically studied yet. The chromosomes of the three checked species of *Hypericia* share $2n=38(Xy_p)$ elements, mostly metacentrics of medium and small size [8,21], while *Ch. varians* displays $2n=32(Xy_p)$ [8,24].

The five updated DNA and/or cytogenetically sampled species of the subgenus *Chalcoidea* Motschulsky, 1860, *Ch. analis* (Linnaeus, 1767), *Ch. carnifex* (Fabricius, 1792), *Ch. interstincta* (Suffrian, 1851), *Ch. janbechynei* Cobos, 1953 (= *Ch. curvilinea* Weise, 1884) and *Ch. marginata* (Linnaeus, 1758) belong to a clearly supported subclade [3,5,6], with three more species of two subgenera, *Ch. (Pezocrosita) convexicollis* (Jacobson, 1901), very close to *Ch. carnifex*, and two a bit more distant to the *Chalcoidea* species, *Ch. (Anopachys) aurichalcea* (Gebler, 1825) and *Ch. (Anopachys) eurina* (Fridvaldszky, 1883). All these species feed on Asteraceae plants [6,16,17], and at least the five species of *Chalcoidea* share $2n=40(Xy_p)$ chromosomes [0,12,14,19,21], whereas the cytogenetic findings on *Ch. aurichalcea*, $2n(\♂)=31(XO)$ to $2n(\♂)=45(XO)$ chromosomes, have proved the existence of several cryptic species [21,40]. Although none of the three chromosomally studied species of the subgenus *Apterosoma* Motschulsky, 1860 (= *Caudatochrysa* Bechyné, et al. [32] have been surveyed at the DNA level, their similar cytogenetic results of $2n(\♂)=39(XO)$ and $2n=42(XY)$ chromosomes [13], and their trophic affiliation on Asteraceae (Jolivet and Petitpierre, 1976), point out to a probable close genetic resemblance, in agreement with the proposal by Bourdonné and Doguet [17] who joined the species of *Chalcoidea*, *Anopachys*, *Apterosoma* (sub *Caudatochrysa*) and *Allochrysolina* Bechyné, 1950, in his group 9. In relation with this last subgenus *Allochrysolina*, the molecular phylogeny of the two analyzed species, *Ch. fuliginosa* (Olivier, 1807) and *Ch. lepida* (Olivier, 1807) join them in a minor subclade within the large one of *Anopachys* and *Chalcoidea* subgenera [6], and also in accordance with an identical chromosome number of $2n=42(Xy_p)$, although *Ch. fuliginosa* and *Ch. lepida* have distinguishable karyotypes [8]. In relation to the subgenus *Pezocrosita*, the DNA analysis of only one species, *Ch. convexicollis*, may not assume that the subgenus should be included in this latter clade, because it is a highly complex taxon made of 34 species in seven different groups [1], and three or four more species of *Pezocrosita* at least, should be genetically analyzed before coming to this

conclusion.

Pasteels et al. [42] suggested a possible relationship between the subgenera *Chalcoidea* and *Hypericia*, based on their defensive toxins, and Hsiao and Pasteels [5] another between the subgenera *Allochrysolina* and *Anopachys* based on mtDNA phylogeny, host-plant affiliation and chemical defense, and both proposals have not been rejected by our AU tests ($P=0.066$ and $P=0.215$, respectively [6]. Likewise, Bourdonné [46] proposed a monophyly for the subgenera *Allochrysolina*, *Chalcoidea* and *Pezocrosita*, based on adult morphology and host-plant affiliations, an hypothesis that could not be statistically rejected by our AU test too ($P=0.205$) [6]. Therefore, these three phyletic assertions can be possible.

The five checked species of the subgenus *Chrysolina* s. str. Motschulsky, 1860, *Ch. bankii* (Fabricius, 1775), *Ch. costalis* (Olivier, 1807, = *Ch. obsoleta* Brullé, 1838), *Ch. rufa* (Duftschmid, 1825), *Ch. staphylaea* (Linnaeus, 1758) and *Ch. wollastoni* Bechyné, 1957 (= *Ch. rutilans* Wollaston, 1864 nec Gravenhorst, 1807), constitute a highly supported subclade either by DNA molecular systematics [3,4,6] or by their $2n(\text{♂})=23(XO)$ chromosomes (19,21,23; Petitpierre et al., 1988; Petitpierre et al., 2004). Nevertheless, in another species, *Ch. (Lithopteroides) exanthematica*, which shares also $2n(\text{♂})=23(XO)$ chromosomes, we do not know whether it is related to this previous clade or not, because we should await to DNA analyses. Moreover, Bienkowski [1] placed the Canarian endemic species *Ch. costalis* and *Ch. wollastoni* within the subgenus *Rhyssoloma* Wollaston, 1854, whose typical species is the Madeira island endemic *Ch. fragariae* a unique species for this subgenus, according with Bechyné [32], Bienkowski [33] and Kippenberg [35], a viewpoint that we follow too because *Ch. fragariae*, apart from being a Madeiran endemic, has a very unusual longitudinal relief in elytra and it shows $2n=24(Xy_p)$ chromosomes [7] instead of $2n(\text{♂})=23(XO)$ chromosomes of the above mentioned species, despite of the absence of DNA findings in *Ch. fragariae* that may give an additional stronger support to this view.

The subgenera *Arctolina* Kontkanen, 1959, with two genetically sampled species, *Ch. oirota* Lopatin, 1990, and *Ch. poretzky*, and *Pleurosticha* Motschulsky, [33], with three other ones, *Ch. gebleri* L. Medvedev, et al. [38] *Ch. lagunovi* Mikhailov, et al. [31] and *Ch. sylvatica* (Gebler, 1823), display a remarkable DNA resemblance, with $2n=26(Xy_p)$ or $2n=26(XY_p)$ chromosomes, that may allow to a possible synonymisation of these two subgenera. However, *Ch. oirota* and *Ch. poretzky* seem to have a double feeding affiliation on Lamiaceae and Scrophulariaceae, whereas *Ch. lagunovi* Mikhailov, 2006 and *Ch. sylvatica* have only one on Lamiaceae and Ranunculaceae, respectively.

Two species belonging to the subgenus *Allohypericia* Bechyné, et al. [32], *Ch. aeruginosa* (Faldermann, 1835) and *Ch. perforata* (Gebler, 1830), plus probably three more of the subgenus *Colaphoptera* Motschulsky, 1860, *Ch. bigorrensis* (Fairmaire, 1865), *Ch. globosa* (Panzer, 1805), and *Ch. purpurascens* (Germar, 1822), join to a sister subclade of the previous one of *Arctolina* and *Pleurosticha* subgenera. The chromosome number of *Ch. bigorrensis* (Fairmaire, 1865) and *Ch. globosa* (Panzer, 1805), $2n=26(Xy_p)$, agrees with those of *Ch. (Arctolina) poretzkyi* (Jacobson, 1897), *Ch. (Pleurosticha) gebleri* L. Medvedev, 1979, *Ch. (Pleurosticha) lagunovi* and *Ch. (Pleurosticha) sylvatica* whereas that of *Ch. purpurascens crassimargo* (Germar, 1824), $2n=24(Xy_p)$, is quite similar. Moreover, the host-plants of *Ch. bigorrensis* and *Ch. globosa* are Lamiaceae, but those of *Ch. aeruginosa* and *Ch. perforata* are Lamiaceae and Asteraceae, as well as those of *Ch. oirota* and *Ch. Poretzky*.

Another well-supported subclade ($P=0.91$, [6]), include sister minor subclades of species mostly feeding on Plantaginaceae plants, such as those of the subgenera *Taeniosticha* Motschulsky, 1860, *Ch. petitpierrei* Kippenberg, 2004 and *Ch. reitteri* (Weise, 1884), whose feedings are not yet known, *Palaeosticta* Bechyné, 1952, *Ch. diluta* (Germar, 1824) and *Ch. kocheri* (Codina Padilla, 1961), *Colaphodes* Motschulsky, 1860, *Ch. haemoptera* (Linnaeus, 1758), and *Ovosoma* Motschulsky, 1860, *Ch. vernalis pyrenaica* (Dufour, 1843) and probably *Ch. susterai* Bechyné, 1950, though this last species has only been studied chromosomally but not by DNA. The chromosomes of the species included in this large subclade are known for *Ch. diluta*, $2n=36(Xy_p)$ [21] (Petitpierre, 1981), *Ch. haemoptera*, *Ch. vernalis pyrenaica* and *Ch. susterai*, all of them with $2n=40(Xy_p)$ [11,20,21,44]. Another species that would be possibly included in this subclade, when the DNA sequences were become available, is *Ch. (Zeugotaenia) limbata* (Fabricius, 1775), because it feeds on Plantaginaceae [16,17], it shows a similar chromosome number of $2n=42(Xy_p)$ [8], and shares some morphological features with the subgenus *Taeniosticha* [37], sub genus *Craspeda* Motschulsky, 1860), and the subgenus *Palaeosticta* [37].

An additional highly evolved subclade is that of the subgenus *Stichoptera* Motschulsky, 1860, where eight species have been analyzed, either by their DNA sequences and/or by their chromosomes [8,9,12,21,23,39], with a highly variable numbers from $2n=22(Xy_p)$ of *Ch. kuesteri* (Helliessen, 1912) to $2n=34(Xy_p)$ of *Ch. sanguinolenta* (Linnaeus, 1758), a modal value of $2n=24(Xy_p)$ of *Ch. colasi* (Cobos, 1952), *Ch. latecincta* (Demaison, 1896) and *Ch. oceanoripensis* Bourdonné, Doguet and Petitpierre, [39] (= *Ch. ripocanensis* auct.), and three species with high chromosome numbers, *Ch. lucidicollis grossepunctata* (Lindberg, 1950) with $2n=30(Xy_p)$, and *Ch. gypsophilae* (Küster, 1845) and *Ch. rossia* (Illiger, 1802)

with $2n=32(Xy_p)$. A distinctive cytogenetic trait of these eight karyotypes is their asymmetry of autosome sizes, with a few large autosome pairs and the remaining small pairs, being the number of these large autosome pairs inversely correlated with the chromosome numbers [9]. The trophic affiliation of these species of *Stichoptera* is quite eclectic, mainly on Scrophulariaceae, but at least two species only on Lamiaceae, and another one with a secondary choice on Plantaginaceae in adults [6,39].

Finally, the subgenus *Sibiriella* L. Medvedev, 1999, has been cytogenetically studied by two species, *Ch. montana* (Gebler, 1848) and *Ch. schewyrewi* (Jacobson, 1895), with $2n=40(XY_p)$ and $2n=40(Xy_p)$ chromosomes, respectively [8,11], This number of $2n=40$ chromosomes and their feeding on Asteraceae plants are also found in species of the subgenus *Chalcoidea* which might place *Sibiriella* phyletically close to them. Likewise, the monotypic subgenus *Sphaerochrysolina* Kippenberg [35], with *Ch. umbratilis* (Weise, 1887), having $2n=30(XY)$ chromosomes [14], but without DNA findings, as in the previous subgenus, we could not place it in a molecular phylogenetic tree. However, *Sphaerochrysolina* resembles morphologically, among others, to the subgenera *Chrysolina s.str.*, *Colaphoptera* and *Cyrtochrysolina* [1], but it is strikingly different by its karyotype of $2n=30$ chromosomes and the chiasmatic XY sex-chromosome system, whereas *Chrysolina s.str.* Displays $2n(\text{♂})=23(XO)$, *Colaphoptera* $2n=26(Xy_p)$ and $2n=24(Xy_p)$ y *Cyrtochrysolina* $2n=42(Xy_p)$ chromosomes. Furthermore, the trophic affiliation of *Sphaerochrysolina* on Asteraceae plants [29], is clearly different from those of the above subgenera on Lamiaceae, Plantaginaceae, Ranunculaceae and Apiaceae [6].

Although Lamiaceae are the first plant-affiliation for the genus ($P=0.98$) [6] the species of *Chrysolina-Oreina* have extended their trophism to eight other botanic families [6,15,16,17]. The different shifts from Lamiaceae to new host-plants families have not been unique events for each of them, except for Hypericaceae selected by the subgenera *Hypericia* and *Sphaeromela* [3,6] namely, that from Lamiaceae to Apiaceae, has taken place in a clade grouping *Oreina s.str.* and *Ch. (Timarchoptera) haemochlora* ($P=0.37$) [6], in a subclade with the subgenera *Minckia* and *Threnosoma*, and *Ch. (Timarchoptera) soiota* ($P=0.62$) [6]. Also, that from Lamiaceae to Asteraceae in a subclade of *Oreina* subgenus *Chrysochloa* ($P=0.54$) [6] and in another with the subgenera *Allochrysolina*, *Anopachys*, *Aptosoma*, *Chalcoidea* and *Pezocrosita* (*Ch. convexicollis*) ($P=0.94$) [46]. In agreement with this last subclade, Bourdonné [46] has put within his "genus" *Chalcoidea* Motschulsky, the subgenera *Allochrysolina*, *Chalcoidea s.str.* and *Pezocrosita*. It is interesting to note that some subgenera subclades, such as that of *Stichoptera*, shows a double trophic shift from possibly Plantaginaceae to Scrophulariaceae and Lamiaceae

($P=0.66$) [6], while others such as *Allohypericia*, *Arctolina* and *Pleurosticha* display feedings in three plant families, on Asteraceae, Lamiaceae and Ranunculaceae [15,30,31], probably also from a Plantaginaceae ancestor, in reversal shifts ($P=0.5$ in these two cases) [6]. The species of subgenus *Chrysolina s. str.* are paradigmatic in this sense because some of them, as *Ch. bankii*, can feed on three plant families at least, Asteraceae, Lamiaceae and Plantaginaceae, and *Ch. staphylaea* on two families, Lamiaceae and Ranunculaceae [16]. In addition, *Ch. (Euchrysolina) graminis* has a double feeding too, on Asteraceae and Lamiaceae [16,17], as well as the two sampled species of the subgenus *Allohypericia*, *Ch. aeruginosa* and *Ch. perforata*, quoted before. In all these examples of a relative polyphagy, there are no new plant-affiliations, because these selected botanic families are always chosen by other congeneric species. Thus, most species of *Chrysolina-Oreina* have possibly maintained a common potential genetic basis in their genomes for plant affiliations in these botanic families.

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