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PHYLOGENETIC ANALYSES OF DNA AND ALLOZYME DATA SUGGEST THAT *GONIOCTENA* LEAF BEETLES (COLEOPTERA; CHRYSOMELIDAE) EXPERIENCED CONVERGENT EVOLUTION IN THEIR HISTORY OF HOST-PLANT FAMILY SHIFTS

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Abstract.—A phylogenetic analysis of the genus *Gonioctena* (Coleoptera, Chrysomelidae) based on allozyme data (17 loci) and mitochondrial DNA sequence data (three gene fragments, 1,391 sites) was performed to study the evolutionary history of host-plant shifts among these leaf beetles. This chrysomelid genus is characteristically associated with a high number of different plant families. The diverse molecular data gathered in this study are to a large extent congruent, and the analyses provide a well-supported phylogenetic hypothesis to address questions about the evolution of host-plant shifts in the genus *Gonioctena*. The most-parsimonious reconstruction of the ancestral host-plant associations, based on the estimated phylogeny, suggests that the Fabaceae was the ancestral host-plant family of the genus. Although most of the host-plant shifts (between different host species) in *Gonioctena* have occurred within the same plant family or within the same plant genus, at least eight shifts have occurred between hosts belonging to distantly related and chemically dissimilar plant families. In these cases, host shifts may have been simply directed toward plant species available in the environment. Yet, given that two *Gonioctena* lineages have independently colonized the same three new plant families (Salicaceae, Betulaceae, Rosaceae), including four of the same new genera (*Salix, Alnus, Prunus, Sorbus*), some constraints are likely to have limited the different possibilities of interfamilial host-plant shifts. [Allozymes; Chrysomelidae; coevolution; *Gonioctena*; host-plant shifts; insect–plant interactions; mitochondrial DNA; molecular phylogenetics.]

A majority of herbivorous insects are oligophagous (Jermy, 1984; Bernays and Chapman, 1994), which means their diet is restricted to a few host-plant species. This fact has led many biologists to investigate the possible evolutionary pathways responsible for the close association between phytophagous insects and their host plants. In this respect, chrysomelid beetles represent a particularly interesting example of phytophagous insects. In some genera, all species show a high fidelity to the same plant family, and in other genera, different species can feed on different, sometimes distantly related, plant families.

A few workers have inferred the phylogenetic relationships within chrysomelid beetle genera from morphological and/or molecular characters and have discussed the evolutionary history of host-plant shifts in these genera. Farrell and Mitter (1990) suggested that *Phyllobrotica* leaf beetles have evolved in parallel with their lamialean host plants, based on the almost complete correspondence between the phylogenies of the insects and their hosts. Futuyma and McCafferty (1990) and Funk et al. (1995) studied the pattern of host-plant shifts in the genus *Ophraella*, the species of which are associated exclusively with Asteraceae. They observed few host shifts of these beetles among tribes of Asteraceae but several independent host shifts toward the same plant genera within the tribe Helianthae and therefore postulated that similarity among plants (e.g., in secondary chemistry) facilitates host shifts in those phytophagous insects. These phylogenetic studies, along with studies on the genetic variation of the host-plant choice by *Ophraella* leaf beetles (Futuyma et al.,

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1993, 1995), have led their authors to suggest that genetic constraints (lack of genetic variation) were responsible for the conserved history of host-plant shifts in *Ophraella*. These conclusions contrast with those of Dobler et al. (1996), who studied the leaf beetle genus *Oreina* associated with Asteraceae and Apiaceae. They observed no conserved pattern of host-plant shifts below the plant family level and concluded that *Oreina* provides an example of shifts to distantly related and chemically dissimilar plants. Becerra (1997) conducted molecular phylogenetic studies of the leaf beetle genus *Blepharida* and of their host plants belonging mainly to the genus *Bursera* (Burseraceae). This author compared the importance of host cladogenesis and host-plant chemistry in host shifts and concluded that chemistry had a greater influence on the evolution of host use in *Blepharida*.

All of the above studies are based on leaf beetle genera with a diet restricted to one or two different host-plant families. The goal of the present study was to extend the available data on the evolution of host-plant shifts in leaf beetles to a genus utilizing a much more diverse array of host-plant families: the genus *Gonioctena*, associated with at least six distantly related plant families and distributed over a wide geographical range (Asia, Europe, North Africa, and North America; see Table 1).

This genus contains 70 reported species classified into nine subgenera (Table 1). On the basis of morphological characters, it has been suggested that *Gonioctena* forms a well-differentiated monophyletic group (Cantonnet, 1968). The classification into subgenera, with one subgenus containing almost half of the species, is also based on morphological characters (Kimoto, 1962; Gressit and Kimoto, 1963; Takizawa, 1976, 1989b). Each species feeds on one or a small number of host-plant species. Larvae and adult beetles feed on the leaves of their host plant, and pupation occurs in the soil. Because they have a very short active life cycle and return afterwards to the soil, these beetles are visible for only 2–3

TABLE 1. *Gonioctena* subgenera with their reported number of species, distribution range, and the plant genera to which their hosts have been reported to belong. These data were compiled from Brown (1942, 1952), Bechyné (1947), Palmén (1948), Franz and Palmén (1950), Mohr (1966), Cantonnet (1968), Axelsson et al. (1974), Takizawa (1976, 1989a, 1989b), Bourdonné and Doguet (1979), Seeno and Wilcox (1982), Kippenberg (1994), Daccordi (1995, pers. comm.), and Kudo et al. (1995).

Subgenus	No. species	Range (no. species)	Known host plants
Gonioctena	32	Asia (Siberia, China, Korea, Japan) (21), Europe (7), N. America (4)	mainly Salicaceae (*Salix, Populus*),[a] but also Betulaceae (*Alnus, Carpinus*), Fagaceae (*Fagus*), Rosaceae (*Prunus, Sorbus*)
Asiphytodecta	9	Asia	Fabaceae (*Pueraria*)
Brachyphytodecta	12	Asia	Fabaceae (*Robinia, Wisteria, Lespedseza*)
Spartoxena	7	N. Africa (5), SW Europe (Spain, Portugal, France, Italy) (2)	Fabaceae (*Lygos, Genista, Spartium, Adenocarpus, Calycotome, Sarothamnus, Cytisanthus*)
Goniomena	4	N and M Europe	Betulaceae (*Alnus, Corylus*), Rosaceae (*Sorbus, Prunus*), Salicaceae (*Salix*)
Sinomela	3	Asia (China, Japan)	Ulmaceae (*Celtis*)
Spartophila	1	Europe, N Africa	Fabaceae (*Sarothamnus, Genista*)
Spartomena	1	SE Europe	Fabaceae (*Medicago*)
Platyphytodecta	1	Asia (China)	unknown
Total	70		

[a] For 19 species for which the host plant is reported, 15 feed exclusively on Salicaceae.

months (Waloff and Richards, 1958; Axelsson et al., 1974; Takizawa, 1976; Mason and Lawson, 1982).

Although some subgenera display a partly conserved history of host-plant affiliation (e.g., the subgenus *Gonioctena* is almost entirely associated with Salicaceae), the genus as a whole utilizes very diverse plant families (Table 1). These plant families are morphologically very different and were placed by Cronquist (1988) in three different subclasses (Rosidae, Hamamelidae, Dilleniidae) of the six defined dicotyledon subclasses. Moreover, recent phylogenetic studies of the major groups of angiosperms seems to show that these families are phylogenetically distantly related (e.g., Chase et al., 1993; Soltis et al., 1997). The pattern of host affiliation of these insects raises two main questions about the host-plant shifts that occurred in *Gonioctena*: (1) Do the *Gonioctena* subgenera that feed on the same host-plant families form a monophyletic group, or did the host-plant shifts toward these plant families occur several times independently during evolution? (2) What is the ancestral host-plant family for *Gonioctena*? To address these questions, a robust hypothesis of the phylogenetic relationships among *Gonioctena* subgenera is needed. Therefore, in this study, we collected allozyme data (17 loci) and mitochondrial DNA (mtDNA) sequences (1,391 sites) of *Gonioctena* and inferred a molecular phylogenetic hypothesis, which we used to estimate the evolution of host associations.

Materials and Methods

Insect Collection

Table 2 shows the leaf beetle populations collected for the present study. Two outgroup species were used, *Oreina cacaliae* and *Chrysomela tremula*, both belonging (as does *Gonioctena*) to the subfamily Chrysomelinae, tribe Chrysomelini. We sampled representatives of all *Gonioctena* subgenera, except for one monotypic subgenus (*Platyphytodecta*). All four extant species of the subgenus *Goniomena* were collected, allowing the inference of the history of host-plant shifts within this subgenus. Although the study of the evolutionary history of host-plant shifts in the other subgenera would also have been informative, a more complete sampling was difficult to achieve given the transcontinental geographical range of this genus. All samples from European populations were brought alive to the laboratory, where they were identified and frozen in liquid nitrogen. We obtained specimens of Asian and North American species that had been preserved in 100% ethanol; hence, these samples could be used for DNA sequencing but not for the allozyme study.

Host-Plant Affiliation

The food plants of each species were determined during field collection and by reference to the literature (see Table 1). In general, more confidence was placed in records from reports specifically oriented toward the study of *Gonioctena* species than from general reviews providing an overview of the classification of the Chrysomelidae. In addition, we confirmed the diet of the collected European species in the laboratory, testing whether adults and larvae of each species fed on all the plants cited in the literature. For each test, groups of approximately 20 individuals of the same sampled population were placed in a feeding arena with leaves of a single plant species. If feeding was not observed after 3 days, we concluded that the insects did not accept that host plant. Plants that were mentioned in general reviews but that were not accepted by the insects in our feeding tests were not considered as part of that species' diet.

Allozyme Electrophoresis

Horizontal starch gel electrophoresis was performed using the standard procedures and modified protocols of Werth (1985) and Murphy et al. (1990). We screened all the collected European populations for 17 allozyme loci, which were resolved with the following buffer systems: glucose-6-phosphate isomerase (*GPI*, EC 5.3.1.9) and dihydrolipoamide dehydrogenase (*DDH*, EC 1.8.1.4) on Tris-citrate

TABLE 2. Collection information on the populations of *Gonioctena* used in this study. *Chrysomela tremula* and *Oreina cacaliae* are the two outgroup species. The host plants cited are those on which the insects were found.

Collected species	Subgenus	Locality[a]	Date	Host plant
Gonioctena viminalis	*Gonioctena*	(1) Tshiertshen (Alps, Switzerland),	Jul 91	*Salix caprea*
		(2) Le Breitfirst (Vosges, France)	May 92	*Salix caprea*
G. linnaeana	*Gonioctena*	Les Rousses (Lozère, France)	Jun 91	*Salix purpurea*
G. rufipes	*Gonioctena*	Oignies (Belgium)	May 92	*Populus tremula*
G. holdausi	*Gonioctena*	Gramais (Alps, Austria)	Jun 93	*Salix waldsteiniana*
G. occidentalis	*Gonioctena*	Jasper National Park (Alberta, Canada)	Jun 92	*Salix* sp.
G. kamikawai	*Gonioctena*	Liukuei (Kaohsiung, Taiwan)	Mar 95	unknown
G. tredecimmaculata	*Asiphytodecta*	Liukuei (Kaohsiung, Taiwan)	Mar 95	*Pueraria lobata*
G. rubripennis	*Brachyphytodecta*	Komagawa (Saitama Pref., Japan)	May 95	*Wisteria floribunda*
G. variabilis	*Spartoxena*	(1) Malaga (Spain),	May 91	*Spartium* sp.
		(2) Sines (Portugal)	May 93	*Lygos* sp.
G. quinquepunctata	*Goniomena*	(1) Le Breitfirst (Vosges, France),	May 92	*Sorbus aucuparia*
		(2) Zastler (Black Forest, Germany),	May 92	*Sorbus aucuparia*
		(3) Hald (Denmark)	Jul 92	*Sorbus aucuparia*
G. pallida	*Goniomena*	(1) Mittlach (Vosges, France),	May 92	*Corylus avellana, Salix caprea*
		(2) Tshiertshen (Alps, Switzerland),	Jun 92	*Salix caprea*
		(3) Gramais (Alps, Austria)	Jun 93	*Salix caprea*
G. intermedia	*Goniomena*	Matshertal (Alps, Italy)	Jun 93	*Prunus padus*
G. interposita	*Goniomena*	(1) Rosslach (Alps, Austria),	Jun 92	*Alnus viridis*
		(2) Matshertal (Alps, Italy)	Jun 93	*Alnus viridis*
G. nigroplagiata	*Sinomela*	Komagawa (Saitama Pref., Japan)	May 95	*Celtis chinensis*
G. olivacea	*Spartophila*	(1) Wibrin (Belgium),	May 92	*Sarothamnus scoparius*
		(2) Hald (Denmark),	Jul 92	*Sarothamnus scoparius*
		(3) Sines (Portugal)	May 93	*Sarothamnus scoparius*
G. fornicata	*Spartomena*	Petric (Bulgaria)	Jun 94	*Medicago sativa*
Chrysomela tremula		Oignies (Belgium)	May 92	*Populus tremula*
Oreina cacaliae		Le Howald (Vosges, France)	Jun 90	*Adenostyles alliariae*

[a] Numbers in parentheses indicate different populations.

pH 6.3/6.7 buffer; malate dehydrogenase (*MDH*, EC 1.1.1.37), a peptidase (*pep-D*, EC 3.4.13.9), phosphoglucomutase (*PGM*, EC 5.4.2.2), and glycerol-3-phosphate dehydrogenase (*G3PDH*, EC 3.5.4.3) on Tris-citrate-EDTA buffer; fumarate hydratase (*FUMH*, EC 4.2.1.2), two additional peptidases (*PEP-B*, EC 3.4.11.4 and one dipeptidase using L-leucyl-DL-alanine as substrate *PEP-LA*, EC 3.4.13.11), and formaldehyde dehydrogenase (*FDH*, EC 1.2.1.1) on Tris-HCl buffer; adenylate kinase (*AK*, EC 2.7.4.3), arginine kinase (*ARK*, EC 2.7.3.3), and aspartate aminotransferase (*AAT-1*, EC 2.6.1.1) on amine-citrate pH 6.1 buffer; and triose phosphate isomerase (*TPI*, EC 5.3.1.1), isocitrate dehydrogenase (*IDH*, EC 1.1.1.42), and a second aspartate aminotransferase (*AAT-2*, EC 2.6.1.1) on Tris-citrate buffer (electrode buffer: 0.343 M Tris, 0.078 M citric acid, pH 8.0; gel buffer: 0.076 M Tris, 0.005 M citric acid, pH 8.7). Before loading the gel, the whole insect was ground in a homogenization buffer (Tris-HCl buffer, pH 7, EDTA, mercaptoethanol). We screened 30 individuals per population when there was more than one population per species and 50 individuals when only one population was available. The insects were distributed on the gels by groups of five individuals from the same population in such a way that each combination of species was observed side by side at least once for each locus. Sharing of electromorphs was established by comparison on the same gel. When there were doubts about the similarity of two electromorphs (especially with rare alleles), samples were rerun.

DNA Sequencing

We sequenced 353 base pairs (bp) from the small subunit (12S) ribosomal RNA (rRNA), 485 bp from the large subunit (16S) rRNA, and 553 bp from the cytochrome oxidase II (COII), for a total of 1,391 bp. These mtDNA fragments were sequenced for one individual per species, except for *G. intermedia*, *G. interposita*, and *G. fornicata* (two individuals per species). Whole insects were ground in an SDS homogenization buffer and incubated overnight with proteinase K (2 mg/ml) at 40°C. Several phenol/chloroform extractions were done, followed by ethanol precipitation and resuspension in TE buffer (10 mM Tris, 1 mM EDTA). The target portions of mtDNA were amplified by asymmetric PCR (after an initial denaturation step of 30 sec at 94°C, 38 cycles of 25 sec at 93°C, 60 sec at 53–55°C, and 70 sec at 72°C). The primers used were modified from those of Simon et al. (1994): modSR-J-14233 (5'AAGAG(CT)GACGGGCGATGTGT3') and SR-N-14588 (5'AAACTAGGATTAGATACCCTATTAT3') for the 12S; modLR-J-12887 (5'CCGGT(CT)TGAACTCAGATCA(CT)GT3') and modLR-N-13398 (5'CGCCTGTTTA(CT)CAAAAACAT3') for the 16S; mod TL2-J-3037 (5'ATGGCAGATTAGTGCA(AT)T(AG)G3') and modC2-N-3661 (5'CCACAAATTTC(AT)GAACATTGACCA3') for COII. Sequencing reactions were performed with the PRISM™ Cycle Sequencing Ready Reaction Kit CS+ (ABI) following the manufacturer's protocol. Sequencing products were separated by vertical electrophoresis on a 4.75% acrylamide–urea gel in an ABI Stretch 373 DNA Sequencer. Both strands, amplified from two different asymmetric PCR reactions, were sequenced to ensure accurate results.

Phylogenetic Analyses of Allozyme Data

Allozyme data were transformed into discrete characters for parsimony analysis. Loci were treated as characters, the combination of alleles present in each taxon was considered a different character state, and step matrices assigning a cost for each possible character state transformation were constructed using the method proposed by Mabee and Humphries (1993). This method was implemented using the additional procedure of Mardulyn and Pasteels (1994), defining new character states not present in the studied taxa but that may need to be assigned to ancestral nodes during reconstruction of the most-parsimonious solution. Because this procedure is difficult to apply manually when dealing with enzymatic loci displaying many alleles, we used the program ASAP

to code the data (Thumfort and Sampson, 1996).

We agree with Swofford and Berlocher (1987) that the coding of allele frequencies into discrete states is subject to sampling error. However, our insect samples were large enough (50–90 individuals per taxon) to allow detection of rare alleles with a high probability. For the present data set (mostly fixed differences between species), the origin of new alleles supplies more phylogenetic information than the change of allele frequencies.

A heuristic search (100 random addition sequences, TBR swapping) and a bootstrap analysis (400 replicates; heuristic search: stepwise addition, swap on minimal trees only, simple addition sequence, TBR swapping) were performed with PAUP 3.1.1 (Swofford, 1993). Bremer support values (Bremer, 1994) were calculated for each node using the computer programs TreeRot (Sorenson, 1996) and PAUP 3.1.1.

Phylogenetic Analyses of DNA Sequence Data

Sequences were aligned with Clustal W 1.5 (Thompson et al., 1994) and compared with alignments based on the criterion of maximum parsimony using the program Malign 2.5 (command "build," randorderns 10, align swap) (Wheeler and Gladstein, 1995). Gaps (present in the 12S and 16S sequences) were coded as single characters irrespective of their length and added to the nucleotide data set. When gaps of different lengths overlapped, each size class was considered a different character state. To check for possible saturation of nucleotide substitution types, we plotted the number of transitions (Ti) against the number of transversions (Tv) for each pair of taxa for each gene, 12S, 16S, and COII, and for each of the three positions of COII separately.

We inferred the most-parsimonious trees using PAUP 3.1.1 (heuristic search, 100 random addition sequences, TBR swapping) for each of the three genes separately and for the combined DNA data set. All characters were weighted equally for the above analyses. However, different classes of sites (e.g., the three codon positions of a protein-coding gene) or different types of changes (e.g., Ti vs. Tv) are subject to different evolutionary rates, which may, in specific cases, justify differential weighting (Milinkovitch et al., 1996; Swofford et al., 1996). We therefore also compared our unweighted analyses with a weighted-parsimony analysis on the combined DNA data set. The Ti/Tv plots were used as a guide to develop our weighting strategy. For each of the above DNA parsimony analyses, we estimated the reliability of the various inferred clades by performing a bootstrap analysis (400 replicates, heuristic search, simple addition sequence), although bootstrap values may be misleading estimates of accuracy under specific conditions (e.g., Milinkovitch et al., 1996). We also calculated Bremer support values for the unweighted parsimony analyses.

An unweighted parsimony analysis of the combined data sets (DNA + allozymes) was also performed. Because some species available for the DNA sequencing were not available for the allozyme study, and only some populations used in the allozyme study were included in the DNA sequencing, this combined data set contained many missing characters, coded as such for the PAUP analysis.

As increasingly complex models of nucleotide evolution are developed, the maximum likelihood method (Felsenstein, 1981) is becoming more reliable and widely used to infer phylogenetic trees from DNA sequences (Swofford et al., 1996). Maximum likelihood may outperform unweighted parsimony methods under some models of evolution (Swofford et al., 1996). Consequently, two maximum likelihood estimates of the *Gonioctena* phylogeny from our combined sequence data set were obtained (with empirically determined base frequencies) using fastDNAml (Olsen et al., 1994): one applied a Ti/Tv ratio of 1 and a second applied a Ti/Tv ratio of 3. Bootstrap replicates by the maximum likelihood method were not performed because of the high computational burden.

Reconstruction of Ancestral Host-Plant Association

The ancestral host-plant association was reconstructed using the criterion of maxi-

TABLE 3. Host-plant associations known for the genus *Gonioctena*, inferred from the literature (see Table 1) and from our observations. If an insect species did not feed in the laboratory on a plant genus that was mentioned in the literature, that plant genus is not shown in this table. Such discordances were observed only for plant genera mentioned in general reviews. Some plant genera cited in Table 1 are not shown here because, although they were assigned in the literature to a particular *Gonioctena* subgenus, no details were given on which insect species they are associated with.

Species	Subgenus	Host plants
G. americana	*Gonioctena*	*Populus*
G. arctica	*Gonioctena*	*Salix*
G. flavicornis	*Gonioctena*	*Salix*
G. hiranoi	*Gonioctena*	*Alnus, Fagus*
G. holdausi	*Gonioctena*	*Salix*[a]
G. honchuensis chujoi	*Gonioctena*	*Salix*
G. japonica	*Gonioctena*	*Alnus*
G. kaufmanni	*Gonioctena*	*Salix*
G. linnaeana	*Gonioctena*	*Salix*[a]
G. moritomoi	*Gonioctena*	*Prunus, Sorbus*
G. nivosa	*Gonioctena*	*Salix*
G. notmani	*Gonioctena*	*Salix*
G. occidentalis	*Gonioctena*	*Salix*
G. rufipes	*Gonioctena*	*Salix, Populus*[a]
G. sibirica	*Gonioctena*	*Salix*
G. sorbina	*Gonioctena*	*Salix*
G. springlovae	*Gonioctena*	*Salix*
G. takahashii	*Gonioctena*	*Fagus*
G. viminalis	*Gonioctena*	*Salix, Populus*[a]
G. tredecimmaculata	*Asiphytodecta*	*Pueraria*
G. rubripennis	*Brachyphytodecta*	*Wisteria*
G. secsaouia	*Spartoxena*	*Adenocarpus, Calycotome*
G. sexnotatus	*Spartoxena*	*Genista, Lygos*
G. variabilis	*Spartoxena*	*Spartium, Sarothamnus, Lygos*[a]
G. gobanzi	*Spartoxena*	*Genista, Cytisanthus*
G. intermedia	*Goniomena*	*Prunus*[a]
G. interposita	*Goniomena*	*Alnus*[a]
G. pallida	*Goniomena*	*Corylus, Salix*[a]
G. quinquepunctata	*Goniomena*	*Sorbus*[a]
G. nigroplagiata	*Sinomela*	*Celtis*
G. olivacea	*Spartophila*	*Sarothamnus, Genista*[a]
G. fornicata	*Spartomena*	*Medicago*[a]

[a] Plant diet was tested in the laboratory.

mum parsimony with McClade 3.01 (Maddison and Maddison, 1992). Each insect taxon was assigned a "host plant" state corresponding to the family of its host plant(s). If a species was associated with more than one plant family, a state was defined containing all plant families associated with this species. A step matrix was used to assign a cost to each transformation from one state to another by adding one step for each gain or each loss of a plant family.

RESULTS

Host-Plant Affiliation

The host plants of *Gonioctena* species inferred from the literature and from our observations are shown in Table 3.

Allozyme Data

Allele frequency data are shown in Appendix 1 (and can be downloaded from the *Systematic Biology* server at www.utexas.edu/depts/systbiol/). One species, *G. (Spartomena) fornicata*, exhibits a single fixed allele

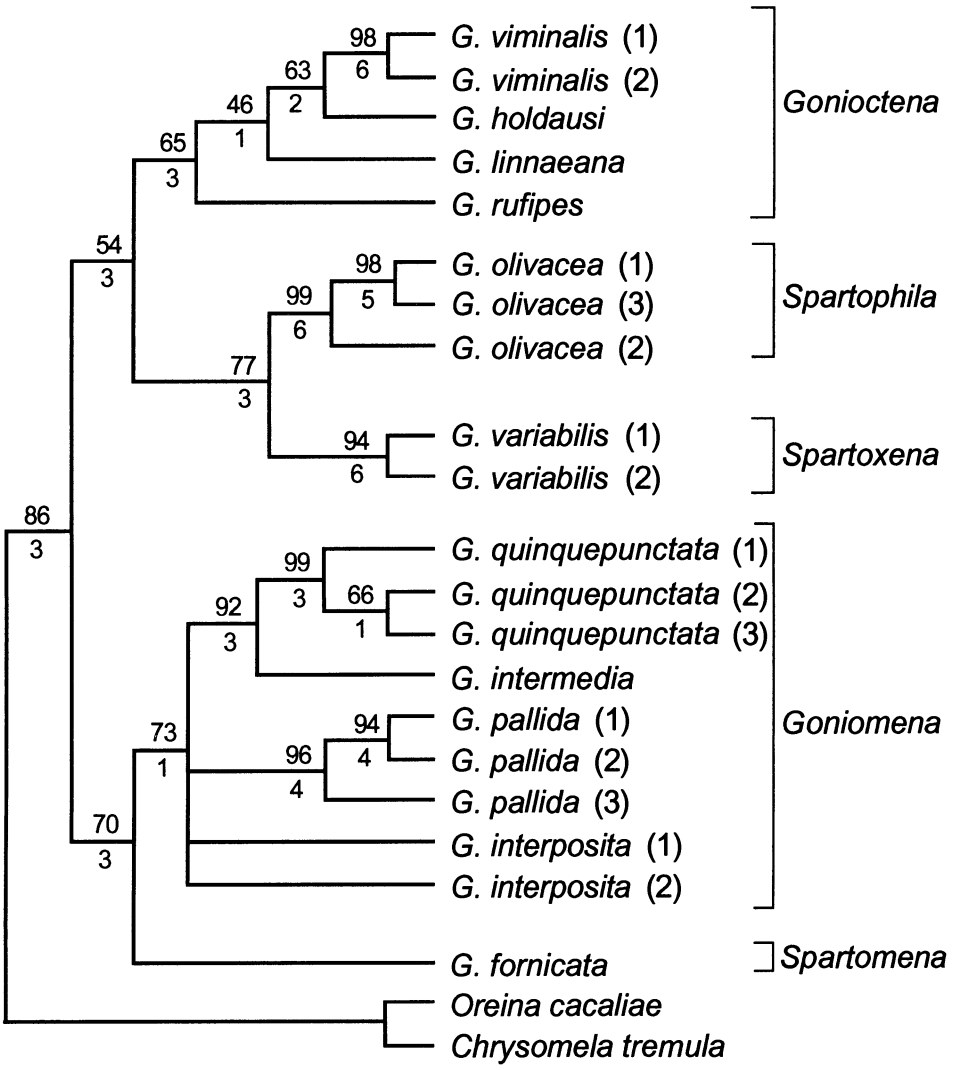

FIGURE 1. Strict consensus of the three most-parsimonious trees (length = 351) inferred from *Gonioctena* allozyme data. Bootstrap proportions (above branches) and Bremer support values (below branches) are given. Numbers in parentheses after the species name refer to the populations (see Table 2).

at each locus studied. This species is a pest (the only known case in *Gonioctena*) of lucerne crops (*Medicago sativa*), and all individuals used in this study were collected in the same field, which might explain the absence of allele diversity observed for this species. Parsimony analysis of the allozyme data resulted in three most-parsimonious (MP) trees. The strict consensus tree is presented in Figure 1. The branch grouping *Spartoxena* + *Spartophila* to subgenus *Gonioctena* is supported by a low bootstrap value (BV).

Sequence Data

Using Malign, two best (MP) alignments were found for the 12S (gap costs of 6, 8, and 10 vs. change cost of 1), and eight alignments were found for the 16S (four, eight, and eight alignments, respectively, for gap costs of 6, 8, and 10 vs. change cost of 1). The Clustal W (same gap:change

cost ratios) alignment of each gene fragment was identical to one of the alignments inferred with Malign. The parsimony analysis of both alignments for the 12S gene resulted in the same two MP trees. Two regions of ambiguity were identified in the 16S alignments. The first region showed two equally parsimonious patterns of alignment that yielded the same MP tree. The second region showed four equally parsimonious alignments that produced slightly different MP trees. Consequently, all phylogenetic analyses involving the 16S fragment were performed four times, once for each alignment of the second region. In the alignments, six gaps were present in the 12S, eight gaps in the 16S, and no gap in the COII sequences. All gaps had a length of a single nucleotide, except for one gap in the 16S fragment that had a length of two nucleotides. Although the base compositions of the 12S (A: 42%, C: 14%, G: 7%, T: 37%) and 16S (A: 41%, C: 16%, G: 8%, T: 35%) fragments were very similar, the COII fragment exhibited a slightly higher frequency of G and lower frequency of A (A: 35%, C: 16%, G: 11%, T: 38%). Uncorrected pairwise sequence divergences were 0.8–15% for the 16S, 3–21% for the COII, and 3–17% for the 12S fragments. All sequences are available from GenBank, under accession numbers AF014580–AF014642. Aligned sequences are shown in Appendix 2 (and can be downloaded from the *Systematic Biology* server).

Results of the unweighted parsimony analyses conducted on the three mitochondrial gene fragments separately and on the combined DNA data set are presented in Figure 2. Each tree shown is the strict consensus of the MP trees. If we take into account only the clades supported by a BV >50% (thick branches), the four phylogenetic trees are very similar (two differences within the subgenus *Gonioctena*: presence of the clades *G. rufipes* + *G. linnaeana* and *G. occidentalis* + *G. viminalis* on the 16S tree). The separate analyses of each of the three genes resolve different parts of the tree.

Figure 3 shows the different Ti/Tv plots. There seems to be a saturation of Ti in the COII fragment after 10–20 Tv (Fig. 3c). Moreover, when the three codon positions of this fragment are considered separately (Figs. 3d–f), it appears that the suggested saturation of Ti occurs mainly at the third-codon positions. The pattern of saturation of substitution types in the 12S and 16S fragments is much more difficult to appraise. It could be interpreted from Figure 2 that significant saturation of Ti has occurred in the 16S gene, which is consistent with the observation that the 16S Ti/Tv ratio is unusually low for mtDNA. Ti/Tv ratios were estimated for each gene fragment from a regression slope calculated from the far left portion of each graph (below a limit value of 10 Tv), where Ti are unlikely to be saturated (Figs. 3a–c). A ratio of 3.1 was deduced from the COII fragment, 2.7 from the 12S fragment, and 1.2 from the 16S fragment. Based on these observations, we performed a weighted parsimony analysis of the combined DNA data set in which Ti in COII third positions and in all 16S positions were ignored (weight of 0). Results of this weighted parsimony analysis are shown in Figure 4. If we consider only clades supported by a BV >50%, the resulting tree is almost identical to the tree of Figure 2d; *G. pallida* is paraphyletic in Figure 2d but is monophyletic in Figure 4.

The parsimony analysis of the unweighted combined data sets (DNA + allozymes) resulted in four MP trees, whose consensus is presented in Figure 5. This tree is congruent with the trees of Figures 2d and 4. Bootstrap values are similar to those of Figure 2d, with two differences: (1) the support for the clade *Spartoxena* + *Spartophila* has increased from 58–59% to 80–83%, which is not surprising because the clade is supported also by the allozymes with a reasonably high BV (77%); (2) the support for the clade *Spartophila* + *Spartoxena* + *Goniomena* + *Sinomela* + *Spartomena* has decreased from 69–72% to 55–58%, which reflects the fact that the allozymes support an alternative clade.

Both maximum likelihood analyses of the combined DNA data set, employing two different Ti/Tv ratios, yielded the same single tree, which is very similar to

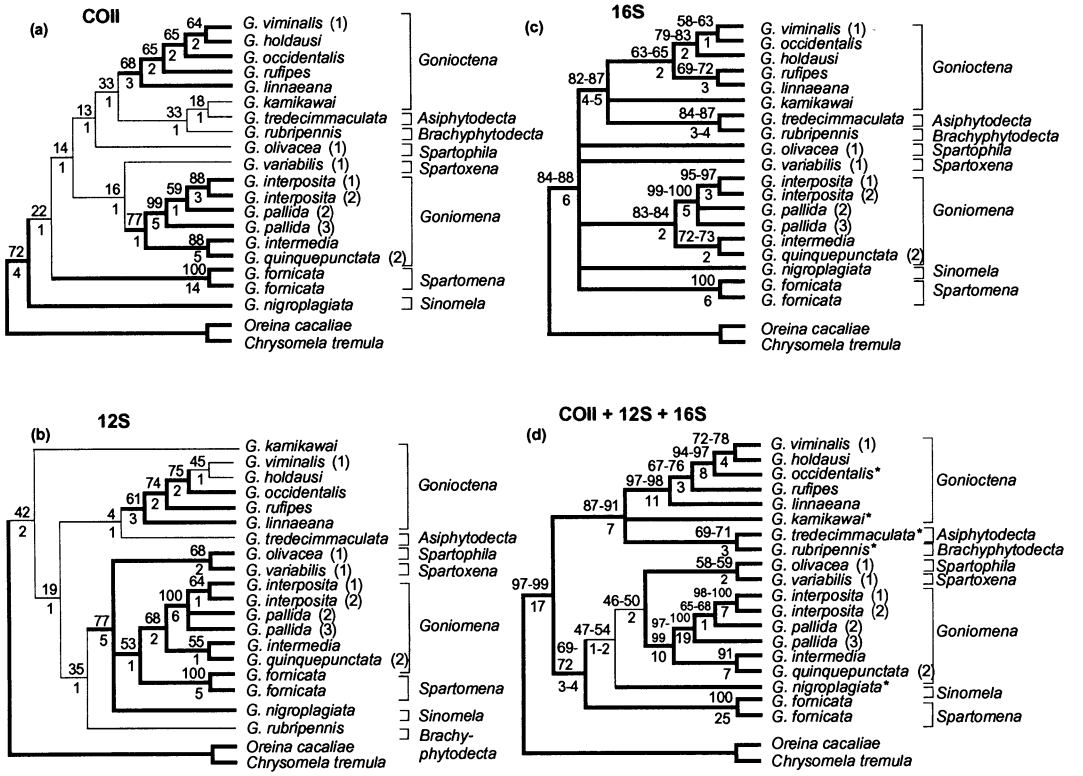

FIGURE 2. Strict consensus trees of the most-parsimonious trees inferred by unweighted parsimony analysis of *Gonioctena* DNA data. Bootstrap proportions (BV, above branches) and Bremer support values (BS, below branches) are given. Thick branches represent clades supported by a BV >50%. Each analysis that included the 16S fragment was conducted four times, once for each of the four best alignments, hence the differences in tree length and consistency index (CI). When different alignments yielded different BV or BS, the range is shown. Numbers in parentheses after the species name refer to the populations to which this individual belongs (see Table 2). (a) COII: one most-parsimonious tree (length = 639, CI = 0.487). (b) 12S: two most-parsimonious trees (length = 214, CI = 0.642). (c) 16S: eight most-parsimonious trees (length = 276–277, CI = 0.623–0.628). (d) Combined DNA data set: two most-parsimonious trees (length = 1150, CI = 0.541–0.543). Species not included in the allozyme study are indicated by an asterisk.

the consensus shown in Figure 2d (one difference: *Sinomela* and *Spartomena* are sister groups on the maximum likelihood trees).

The diverse molecular data (allozymes and three different mitochondrial gene fragments) gathered in this study are, to a large extent, congruent. Except for one node, the allozyme tree is similar to the DNA trees (Figs. 2d, 4), as far as the taxa represented in both data sets are concerned. Five taxa included in the DNA data set were not available for the allozyme analysis. The two kinds of data disagree on one point: the clade formed by *Spartophila* + *Spartoxena* is placed as a sister group to subgenus *Gonioctena* on the allozyme tree but is placed with *Goniomena* + *Spartomena* + *Sinomela* on the DNA trees. Given that the phylogenetic pattern of the DNA trees in this case is supported by a higher bootstrap proportion (69–75% vs. 54% on the allozyme tree) and that the species sampling is more complete for the DNA data set (three more subgenera, especially important in resolving relationships at this higher phylogenetic level), we have more confidence in the clade supported by the DNA data, which is also supported by the results of the combined (DNA + allozymes) analysis (Fig. 5).

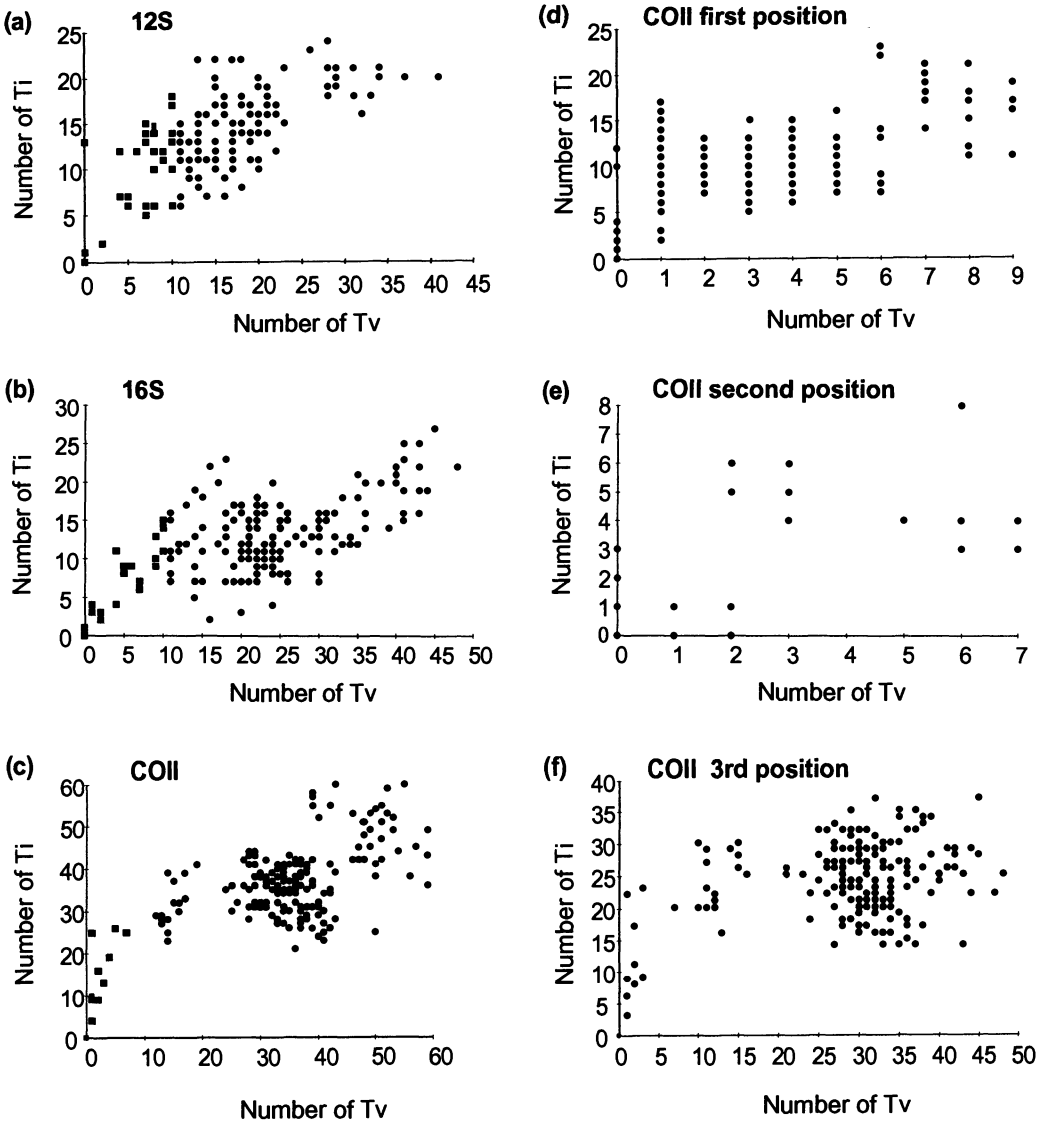

FIGURE 3. Plotted number of transitions (Ti) against number of transversions (Tv) for the *Gonioctena* 12S (a), 16S (b), and COII (c) fragments and separately for each of the three codon positions of COII (d–f). ■ = areas near the origin (where Ti are not saturated) used to infer the Ti/Tv ratios for each gene fragment.

Reconstruction of Ancestral Host-Plant Associations

The phylogenetic relationships shown in Figure 5 were used to estimate the ancestral host-plant associations in the genus *Gonioctena*. In Figure 6, phylogenetic relationships within subgenera are shown only for *Goniomena*, the only subgenus for which all species were available. In this MP reconstruction of the ancestral host-plant affiliation, the ancestor of the genus *Gonioctena* is associated with plants of the Fabaceae. This hypothesis is based on the assumption that the association of *Gonioctena* with any species of a particular plant family corresponds to a single character state, i.e., the different genera of Fabaceae, for example, are much more similar to each oth-

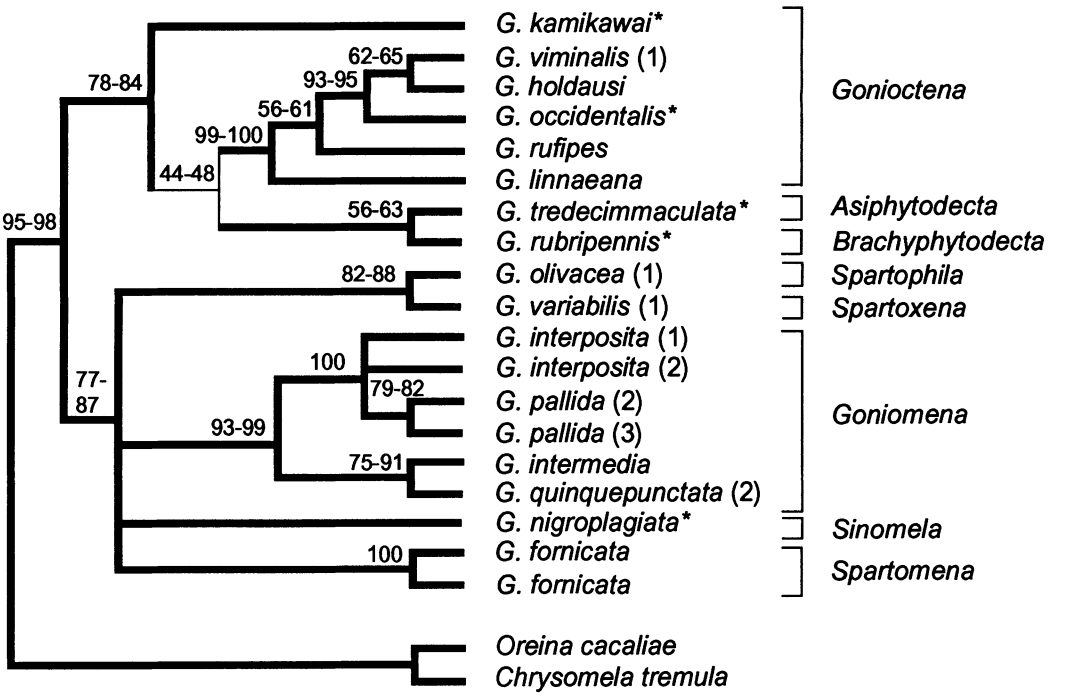

FIGURE 4. Strict consensus of the three most-parsimonious trees (length = 889) inferred by weighted parsimony analyses of the total *Gonioctena* DNA data set, ignoring transitions in the 16S region and in third positions of COII. Bootstrap proportions are given. Thick branches represent clades supported by a bootstrap value >50%. This analysis was conducted four times, once for each of the MP 16S alignments. Numbers in parentheses after the species name refer to the populations to which this individual belongs (see Table 2). Species not included in the allozyme study are indicated by an asterisk.

er (from the insect's point of view) than any is to the Salicaceae, Betulaceae, and Rosaceae genera associated with these insects.

DISCUSSION

Our evaluation of the evolution of host-plant shifts in the genus *Gonioctena* is based on the following assumptions. First, all *Gonioctena* subgenera defined in the traditional classification are true monophyletic groups. This assumption is supported by adult and larval morphological characters (Brown, 1942; Bechyné, 1947; Kimoto, 1962; Cantonnet, 1968; Takizawa, 1976, 1989b) and is compatible with the molecular phylogenetic results for the species included in these analyses. Second, the overall picture of the host-plant affiliation of *Gonioctena* shown in Figure 6 is correct, although we do not know the host plants of all *Gonioctena* species and some additional host-plant genera or families could still be discovered. Third, the cladogram inferred from this study, depicted in Figure 5, represents the true phylogenetic relationships.

Most of the host shifts between different plant species in *Gonioctena* occurred within the same plant family or within the same plant genus. For example, 15 of the 19 leaf beetle species of subgenus *Gonioctena* are associated with different species of *Salix* and *Populus*, and all species of subgenus *Spartoxena* are associated with Fabaceae plants. Nonetheless, the pattern of host-plant affiliation in this genus clearly suggests that at least eight major shifts between distantly related host-plant families occurred during the evolutionary history of these insects.

A striking observation that can be made from Figure 6 is that some *Gonioctena* sub-

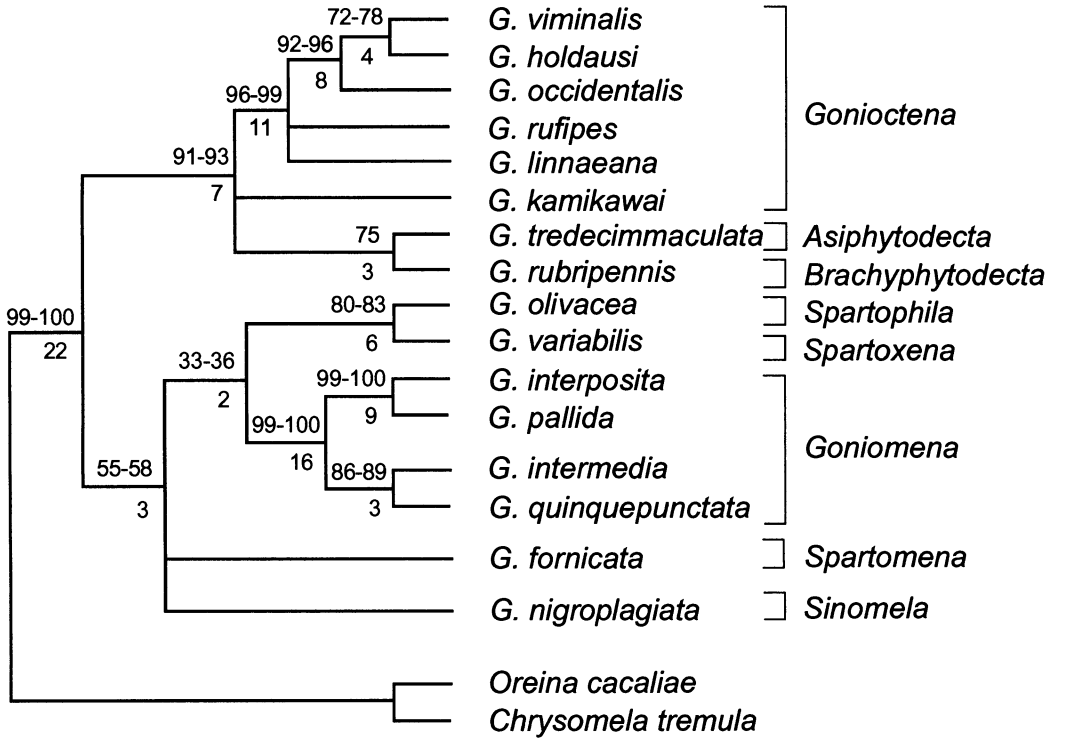

FIGURE 5. Strict consensus of four most-parsimonious trees (length = 1,512, CI = 0.539–0.542) obtained by the unweighted parsimony analysis on the combined (DNA + allozymes) *Gonioctena* data set. Bootstrap values (above branches) and Bremer support values (below branches) are given. This analysis was conducted four times, once for each of the MP 16S alignments. Each species is represented by a single branch (in the resulting most-parsimonious trees, each species is monophyletic).

genera feeding on closely related plant species are not closely related. Clearly, the *Gonioctena* species associated with a particular host-plant genus or family do not always form a monophyletic group. This lack of correspondence between host affiliation and insect phylogeny appears to have two very different causes. *Spartophila, Spartoxena,* and *Spartomena* belong to a clade different from that of *Asiphytodecta* and *Brachyphytodecta,* and yet all are associated with Fabaceae. However, our phylogenetic analyses identified this host association as ancestral. It is therefore not surprising that Fabaceae-associated leaf beetles do not form a clade. The subgenera *Goniomena* and *Gonioctena* share three nonancestral host-plant families even though they are separated by several nodes on the tree, which can only be explained by independent convergences in host-plant shifts. These convergences are particularly striking because both taxa have shifted independently toward the same host-plant genera. Specifically, subgenera *Goniomena* and *Gonioctena* each contain species feeding on *Prunus* (Rosaceae), *Sorbus* (Rosaceae), *Alnus* (Betulaceae), or *Salix* (Salicaceae).

This favored evolutionary scenario is two steps shorter than two alternative hypotheses in which the Rosaceae or the Betulaceae is the ancestral host-plant family. In both cases, feeding on Fabaceae plants would have arisen a minimum of three times independently and feeding on the Betulaceae, the Rosaceae, or the Salicaceae would have appeared at least twice independently.

Several possible causes, not mutually exclusive, may have played a role in the oc-

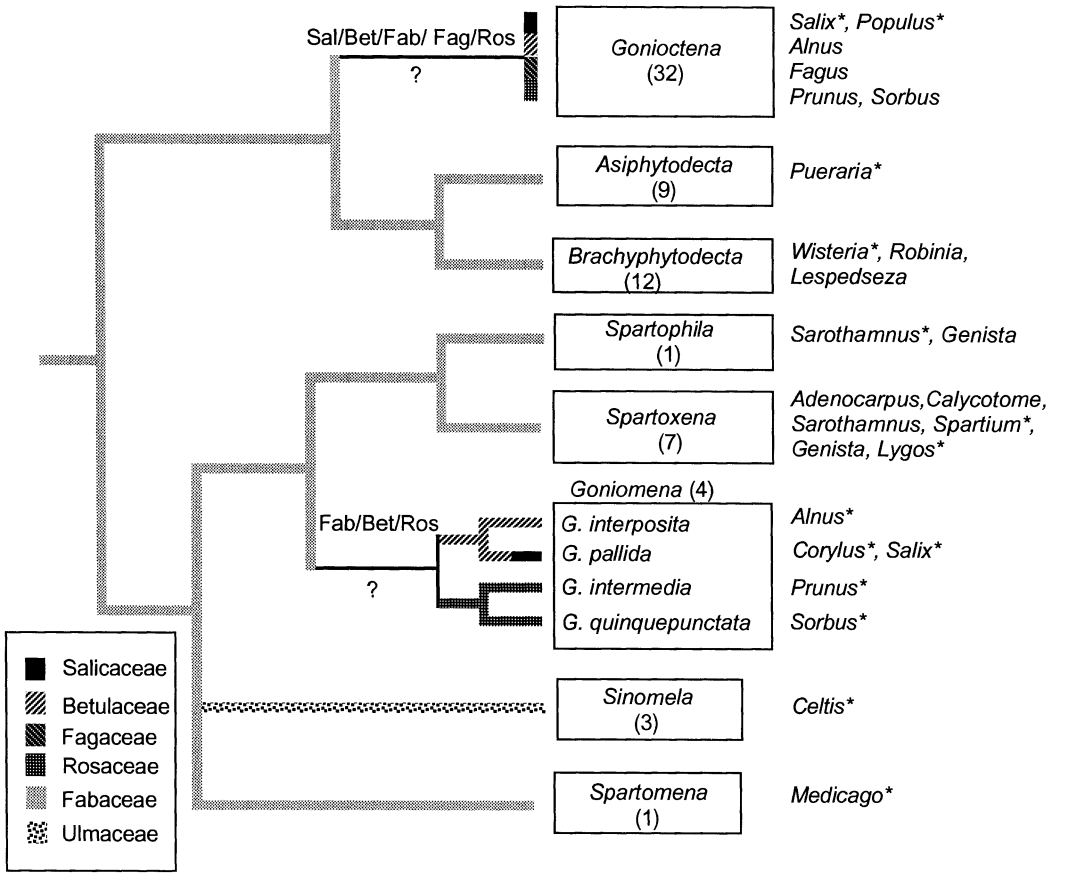

FIGURE 6. Most-parsimonious reconstruction of ancestral host-plant associations in the genus *Gonioctena* based on the phylogenetic relationships shown in Figure 5. The known plant genera associated with each *Gonioctena* subgenus are displayed and the number of species included in each subgenus is given in parentheses. An asterisk indicates that a species feeding on this plant genus was included in our phylogenetic study.

currence of convergent host-plant family shifts in *Gonioctena*. The first possible explanation is that host-plant shifts are easier among chemically similar plants (Ehrlich and Raven, 1965; Mitter and Farrell, 1991). Although the different plant families colonized by *Gonioctena* species are not phylogenetically closely related, these plants still may possess particular chemical similarities. We explored this possibility by examining the plant's secondary compounds as described in the literature. The secondary compounds from the different plant genera associated with *Gonioctena* showed no general chemical resemblance; instead, they are strikingly different. In general, the Salicaceae are characterized by a great diversity of phenolglucosides, the Fabaceae by a high diversity of alkaloids, and the Rosaceae by cyanogenic products (prunasin, amygdalin) (Hegnauer, 1973; Bruneton, 1987). The only chemical similarity we found is the presence of quercetin in the leaves of *Salix* and *Populus* (Salicaceae), of *Corylus avellana* and *Alnus* species (Betulaceae), and of *Sorbus* species (Rosaceae) (Hegnauer, 1964, 1973). This flavonoid substance is, however, widespread in many plant families (Vickery and Vickery, 1981) and is unlikely to explain a specific plant–insect association. The results of such a literature-based search should nevertheless be taken with caution because not all plant secondary compounds are reported. A

deeper knowledge of the chemical plant compounds would be useful to further test the chemical similarity hypothesis. Indeed, even if these host-plant families possess major differences in their main secondary metabolites, they still could share some minor compounds not mentioned in the literature. One could thus still hypothesize that host shifts between these different plant families have occurred on the basis of these similarities, despite the presence of very different, possibly toxic, major secondary substances.

As a second explanation, we could follow the suggestion that host-plant selection in phytophagous insects is mainly governed by the insect's chemosensory system and that the main role of secondary plant substances in plant–insect relationships is that they form the "fingerprint," i.e., the specific signal pattern by which the insect recognizes the plants (Jermy, 1984). Mutations affecting the insect chemosensory system would be responsible for the host-plant shifts that occurred during the evolutionary history of these herbivorous insects. The host-plant shifts would therefore be constrained by the limited possibilities of changes that can occur in the chemosensory system. Only some host-plant shifts would be possible, and some would be more likely than others. Still, a mutation affecting the chemosensory system could be responsible for a radical change in the insect's feeding behavior and thus could make this insect shift toward a very distantly related plant family. If, in addition, this particular mutation shows a relatively high probability of occurrence, it may have occurred several times in a genus, explaining convergent shifts. These suggestions remain very speculative and deserve further investigation.

A third explanation, suggested by Pasteels and Rowell-Rahier (1991), is that host-plant shifts in leaf beetles can simply be directed toward plant species available in the insect's environment, even if these plants are not phylogenetically or chemically related. Indeed, in the mountainous habitat of the four *Goniomena* species (Fig. 6), their host plants *Alnus viridis* and *Corylus avellana* (Betulaceae), *Salix caprea* (Salicaceae), and *Prunus padus* and *Sorbus aucuparia* (Rosaceae) are relatively abundant and occur side by side. These host shifts must, however, be subject to some constraints because only some of the plant species abundantly present in this environment were colonized. The unused plant species may be more difficult to colonize for physiological (lack of needed nutrients or presence of toxic compounds) or behavioral reasons. For instance, all subgenera *Gonioctena* and *Goniomena* species feed on trees rather than on herbaceous plants, whereas *Oreina* species (also belonging to the tribe Chrysomelini, with some species living in the same montainous habitat as *Gonioctena* and *Goniomena*) live only on Asteraceae or/and Apiaceae (herbaceous families well represented in their habitat), despite the presence of several tree species (Dobler et al., 1996). A behavioral barrier in *Gonioctena* and *Goniomena* could therefore exist that makes the host shifts between trees more likely than those between trees and herbs.

Similar interfamilial host-plant shifts are observed in other leaf beetle genera and even in other groups of insects. Host shifts between Salicaceae and Betulaceae have occurred in *Phratora* (between *Salix* and *Betula*) (Köpf et al., in press) and in *Chrysomela* (between *Salix* and *Alnus*) (Jolivet and Hawkeswood, 1995). In the lepidopteran genus *Yponomeuta* (Lepidoptera, Yponomeutidae), host-plant shifts have occurred from the Celastraceae to the Rosaceae (*Prunus, Crataegus*) and from the Rosaceae to the Salicaceae (*Salix*) (Menken et al., 1992). This pattern suggests that host shifts between those major plant families are not rare in phytophagous insects.

Ward and Spalding (1993) gathered all the available information on phytophagous insects and mites (183 families) and their host plants (127 families) from Great Britain. They established a list of the 30 plant families with which the largest number of insects are associated. It is remarkable that, among the first seven of a list of 30 plant families ranked by the number of insect species associated with each family, five

families are colonized by *Gonioctena*: in order, the Rosaceae (976 insect species), the Salicaceae (807 species), the Fagaceae (637 species), the Betulaceae (600 species), and the Fabaceae (594 species). The two other (nonutilized) families are herbaceous: the Poaceae (828 species) and the Asteraceae (924 species). The Ulmaceae, also colonized by *Gonioctena*, has the 15th position in this list, with 216 insect species. Thus, the plants colonized by *Gonioctena* also attract numerous other phytophagous insects. These plants probably offer particular advantages or at least they do not have the disadvantages that other plant families may have.

It is tempting to hypothesize that the ancestral species of the genus *Gonioctena* were associated with the Fabaceae (Fig. 6) and originated in warm temperate zones, where these plants are well represented. Although a large number of the extant *Gonioctena* species have kept this host-plant affiliation, our analyses suggest that two lineages moved independently toward cooler areas and subsequently colonized more abundant plant species in these habitats. Species of the subgenus *Goniomena* and all European species of the subgenus *Gonioctena* are localized in northern Europe and in high elevation areas of central and western Europe (e.g., the Alps), where they have colonized very different plant species that are more abundant in those regions. In the case of the genus *Gonioctena*, genetic constraints have clearly not prevented them from shifting toward very different plant families. However, not all the available abundant plant species in their environment are used. Moreover, *Gonioctena* and *Goniomena* subgenera have independently colonized not only the same host-plant families but four of the same plant genera (*Salix, Alnus, Sorbus, Prunus*). Some constraints are therefore likely to have limited the possibilities of interfamilial host-plant shifts for those phytophagous insects. Because major chemical differences between plants do not seem to have prevented these interfamilial host-plant shifts, other types of constraints may have played a role in the evolution of host-plant diet in the genus *Gonioctena*, e.g., the abundance of the plant in the insect's habitat, physiological parameters such as nutrient requirements of the insects, or behavioral parameters such as the search for a particular type of microhabitat (trees or herbs). To what extent these constraints have played a role in the evolution of the associations of *Gonioctena* with their host plants clearly requires further investigation.

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APPENDIX 1. Allele frequency data for *Gonioctena* and two outgroup species. Lowercase letters represent the alleles found for each population. When more than one allele was found within a population, allele frequencies are given in parentheses. Thirty individuals per population were sampled when there was more than one population per species, and 50 individuals per species were sampled when only one population was available. After the species name, numbers in parentheses refer to different populations (see Table 2).

	Alleles						
Population	*PEP-D*	*FDH*	*MDH-1*	*MDH-2*	*G3PDH*	*IDH*	*PGM*
G. variabilis (1)	n (0.90) u (0.10)	a (0.18) e (0.82)	f	b	a (0.88) b (0.12)	d (0.07) k (0.93)	e (0.02) i (0.96) k (0.02)
G. variabilis (2)	m (0.03) n (0.97)	e	f	b	a (0.60) b (0.37) g (0.03)	k	i (0.98) k (0.02)
G. olivacea (1)	h (0.93) i (0.04) j (0.03)	c (0.01) d (0.99)	f (0.94) m (0.06)	a	a (0.83) b (0.17)	i (0.01) l (0.99)	n (0.20) p (0.80)
G. olivacea (2)	h	d	f (0.98) m (0.02)	a	a (0.91) b (0.09)	l	n (0.40) p (0.60)
G. olivacea (3)	g (0.20) h (0.56) i (0.10) j (0.14)	c (0.26) d (0.74)	f (0.96) m (0.04)	a	a (0.70) b (0.30)	l	n (0.42) p (0.58)
G. viminalis (1)	n (0.88) v (0.12)	d	e	a	j	n (0.99) q (0.01)	a (0.19) b (0.81)
G. viminalis (2)	n (0.95) v (0.05)	c (0.01) d (0.98) g (0.01)	e	a	g (0.92) j (0.08)	j (0.08) n (0.90) q (0.02)	b
G. linnaeana	k	d (0.56) g (0.37) k (0.07)	d (0.04) h (0.94) i (0.02)	d	a	p (0.99) r (0.01)	b (0.07) d (0.90) f (0.03)
G. rufipes	e (0.98) f (0.02)	d (0.75) i (0.25)	f (0.99) l (0.01)	d	f (0.01) m (0.99)	a	f
G. holdausi	q (0.94) t (0.06)	b	i	a	j	g	c
G. quinquepunctata (1)	l	b	f	e	c (0.97) i (0.03)	e (0.02) j (0.98)	n
G. quenquepunctata (2)	l	b	c (0.01) f (0.99)	e	c (0.97) i (0.03)	e (0.05) j (0.95)	m (0.03) n (0.97)
G. quinquepunctata (3)	l	b	f (0.97) k (0.03)	e	c	e (0.33) j (0.67)	n
G. pallida (1)	o (0.12) r (0.88)	b	b (0.08) f (0.92)	f (0.04) h (0.96)	c (0.55) g (0.45)	j	j (0.09) n (0.87) q (0.04)
G. pallida (2)	o (0.13) r (0.80) s (0.07)	b b	b (0.07) f (0.93)	h	c (0.53) g (0.47)	j	j (0.18) n (0.77) q (0.05)
G. pallida (3)	r (0.89) s (0.11)		f	f (0.24) h (0.76)	c (0.50) g (0.50)	j	n
G. interposita (1)	r (0.97) s (0.03)	b	f	f (0.99) g (0.01)	c (0.55) d (0.01) h (0.30) k (0.14)	j	n
G. interposita (2)	r	b	f	f	c (0.72) g (0.28)	j (0.93) o (0.07)	n (0.98) o (0.02)
G. intermedia	l (0.99) m (0.01)	b	j	e	c (0.97) d (0.03)	j (0.52) m (0.48)	n

APPENDIX 1. Extended.

Alleles									
AAT-1	*AAT-2*	*TPI*	*PEP-B*	*FUMH*	*PEP-LA*	*AK*	*ARK*	*GPI*	*DDH*
b (0.03) d (0.85) k (0.12)	j (0.98) n (0.02)	a (0.13) f (0.87)	d (0.02) e (0.98)	b	g	f (0.02) j (0.93) n (0.05)	d	m (0.02) r (0.98)	b
d (0.80) j (0.20)	j	f (0.98) h (0.02)	d (0.02) e (0.96) f (0.02)	f	g (0.98) m (0.02)	j	d	r	b
o (0.12) q (0.88)	j (0.97) m (0.03)	h	f (0.86) j (0.14)	f	i (0.13) m (0.87)	l (0.46) o (0.54)	d (0.99) f (0.01)	j	b
o (0.22) q (0.78)	j	h	f (0.98) h (0.02)	e (0.56) f (0.44)	m	l (0.73) o (0.27)	d	i (0.07) j (0.93)	b (0.97) c (0.03)
o (0.12) q (0.88)	j (0.86) m (0.14)	h	f (0.24) j (0.76)	f	i (0.06) m (0.92) o (0.02)	l (0.52) o (0.48)	d (0.68) f (0.32)	j	b (0.98) c (0.02)
p	e	q	i	k	b (0.67) c (0.33)	f	d	e (0.12) f (0.88)	g
p	d (0.03) e (0.97)	m (0.02) q (0.98)	i	i (0.01) k (0.99)	a (0.36) b (0.64)	f	d	e (0.98) f (0.02)	g
l (0.02) m (0.44) o (0.54)	a	g (0.16) k (0.81) o (0.03)	i	h (0.11) k (0.25) m (0.64)	b	h	d	g (0.06) j (0.94)	e (0.02) g (0.98)
n	b	n	i	e	n	c	d	a	e (0.97) h (0.03)
r	c (0.97) e (0.03)	q	i	i (0.99) l (0.01)	n	e	d	c	j
e	g (0.58) h (0.42)	b	f (0.98) h (0.02)	d	l (0.98) p (0.02)	m	c	n	d
e	g (0.47) h (0.53)	b (0.97) d (0.03)	f (0.98) h (0.02)	d	l (0.94) p (0.06)	k (0.03) m (0.97)	c	k (0.14) n (0.86)	d
e	g (0.20) h (0.80)	b (0.97) d (0.03)	f (0.98) h (0.02)	d	k (0.12) l (0.76) p (0.12)	m	c	k (0.73) n (0.27)	d
e (0.34) h (0.66)	h	b	f (0.80) i (0.20)	b	l (0.96) o (0.04)	k (0.17) m (0.83)	c	o (0.98) q (0.02)	d
h	h	b	f	b	l (0.80) o (0.20)	k (0.05) m (0.95)	c	o	d
h	h	b	f (0.70) i (0.30)	b	l (0.99) o (0.01)	m	c	o	d
h (0.99) i (0.01)	h	b	f	d	l	m	c (0.99) e (0.01)	p	e
h	h	b	f	d	l	m	c	p	e
f	e (0.38) g (0.35) h (0.27)	b	f	d	h (0.05) l (0.88) n (0.07)	m	c	n	d

APPENDIX 1. Continued.

Population	Alleles						
	PEP-D	*FDH*	*MDH-1*	*MDH-2*	*G3PDH*	*IDH*	*PGM*
G. fornicata	p	b	d	i	e	c	h
Oreina cacaliae	b (0.97) d (0.03)	f (0.04) h (0.54) j (0.42)	h	k	g (0.13) l (0.87)	b (0.01) g (0.99)	i (0.98) j (0.02)
Chrysomela tremula	a (0.02) c (0.90) d (0.08)	e	g	c	g	f	j (0.03) l (0.95) n (0.02)

APPENDIX 1. Continued, extended.

Alleles									
AAT-1	*AAT-2*	*TPI*	*PEP-B*	*FUMH*	*PEP-LA*	*AK*	*ARK*	*GPI*	*DDH*
g	i	b	c	f	j	m	b	l	b
c (0.97) h (0.03)	f (0.06) k (0.94)	e (0.04) g (0.96)	f (0.70) i (0.30)	c (0.02) h (0.97) j (0.01)	g	g	c	d (0.05) e (0.94) h (0.01)	e (0.83) f (0.17)
h (0.09) j (0.91)	g (0.04) l (0.67) m (0.29)	c (0.09) i (0.87) l (0.04)	b	g	c (0.10) d (0.78) e (0.12)	a (0.74) b (0.14) d (0.12)	a	d (0.16) e (0.12) h (0.72)	e

APPENDIX 2

Aligned DNA fragments of the COII, 16S rRNA, and 12S rRNA genes sequenced for different *Gonioctena* and two outgroup species. For one 16S region, four alternative, equally parsimonious alignments are shown. The alignments of the 16S and 12S fragments were performed using Malign 2.5 (command "build," randorderns 10, align swap) (Wheeler and Gladstein, 1995). Gaps were coded as single characters irrespective of their length and added to the nucleotide data set. When gaps of different lengths overlapped, each size was considered a different character state.

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1) COII

G. viminalis (1)        CTTTTTTTAGAAATTTCTACATGAAAAACTTTAATACTCCAAGATAGAGCTTCCCCATTAATAGAGCAATTAGCCTATTTTCATGATCATGCTTTAATAA
G. olivacea (1)         CTTTTTTTAGAAATTTCTACCTGAAAAACTTTAATACTTCAAGATAGGGCATCTCCTTTAATAGAACAGCTAGCTTATTTCCATGACCATGCATTAATAA
G. rufipes              CTTTTTTTAGAAATTTCTACATGAAAAACTTTAATACTCCAAGATAGGGCTTCCCCATTAATAGAACAATTAGCATACTTTCATGATCACGCTTTAATAA
G. linnaeana            CTTTTTTTAGAAATTTCTACATGAAAAACTTTAATACTTCAAGACAGAGCTTCCCCACTAATAGAACAATTAGCTTATTTTCACGATCATGCTCTAATAA
G. variabilis (1)       CTTTTTTTAGAAATTTCTACATGAAAAACTTTAATATTACAAGATAGGGCATCTCCCTTAATAGAACAATTAGCATATTTTCATGATCATGCATTAATAA
G. fornicata            CTTTTTTTAGAAATTTCTACATGAAAAACTTTAATACTACAAGATAGAGCTTCACCTTTAATAGAACAATTAGCTTACTTTCATGACCATGCTCTAATAA
G. fornicata            CTTTTTTTAGAAATTTCTACATGAAAAACTTTAATACTACAAGATAGAGCTTCACCTTTAATAGAACAATTAGCTTACTTTCATGACCATGCTCTAATAA
G. occidentalis         CTTTTTTTAGAAATTTCTACATGAAAAACTTTAATACTCCAAGATAGGGCTTCTCCATTAATAGAACAATTAGCTTATTTTCATGATCATGCTTTAATAA
G. interposita (1)      CTTTTTTTAGAAATTGCTACATGAAAAACATTAATACTTCAAGATAGGGCATCCCCTTTAATAGAACAATTAGCTTATTTCCATGATCATGCTTTAATAA
G. interposita (2)      CTTTTTTTAGAAATTGCTACATGAAAAACATTAATACTTCAAGATAGGGCATCCCCTTTAATAGAACAATTAGCTTATTTCCATGATCATGCTTTAATAA
G. intermedia           CTTTTTTTAGAAATTGCTACATGAAAAACATTAATACTTCAGGATAGGGCTTCCCCTTTAATAGAACAATTAGCTTATTTCCATGACCATGCTTTAATAA
G. quinquepunctata (2)  CTTTTTTTAGAAATTGCTACATGAAAAACATTAATTCTCCAAGATAGGGCTTCCCCTTTAATAGAACAATTAGCTTATTTCCATGACCATGCTTTAATAA
G. holdausi             CTTTTTTTAGAAATTTCTACATGAAAAACTTTAATACTCCAAGATAGAGCTTCCCCATTAATAGAGCAATTAGCTTATTTTCATGATCATGCTTTAATAA
G. pallida (2)          CTTTTTTTAGAAATTGCTACATGAAAAACATTAATACTTCAAGATAGGGCATCCCCTTTAATAGAACAATTAGCTTATTTCCATGATCATGCTTTAATAA
G. pallida (3)          CTTTTTTTAGAAATTGCTACATGAAAAACATTAATACTTCAAGATAGGGCATCCCCTTTAATAGAACAATTAGCTTATTTCCATGATCATGCTTTAATAA
G. tredecimmaculata     CTTTTTTTAGAAATTGCAACATGAAAAACCTTAATACTTCAAGATAGGGCATCTCCATTAATAGAACAATTAGCTTACTTTCATGACCATGCTTTAATAA
G. nigroplagiata        CTTTTTTTAGAAATTGCAACATGAAAAACCCTTATACTCCAAGATAGAGCTTCCCCCTTAATAGAACAATTAGCTTATTTTCACGATCATGCTTTAATAA
G. kamikawai            CTTTTTTTAGAAATTGCTACATGAAAAACTTTAATACTCACAAGATAGAGCTCACCCTTCTATAGAACAATTAGCTTATTTTCACGATCATGCCCTTATAA
G. rubripennis          CTTTTTTTAGAAATGATAACATGAAAAAATATTACATTACAAGATAGTGCCTCACCTTTGATAGAACAATTAAATTACTTCCATGACCATACTTTAATGA
Oreina cacaliae         CTTTTTTTAGAAATCCCAACATGAAAAACTTTGATACTACAAGATAGAACCTCCCCTTTAATAGAACAACTTTCATAATTTTCATGATCATACCTTAATAA
Chrysomela tremula      CTTTTTTTAGAAATCCCAACATGAAAAACTTTGATACTACAAGATAGAACCTCCCCTTTAATAGAACAACTTTCATAATTTTCATGATCATACCTTAATAA

G. viminalis (1)        TTTTAGTTATTATTACAGTTTTAGTTGGACAATTAATAATAACATTATTTTTTTAATAAATTTTCACATCGATACCTTTTAGAAGGGCAATTAATTGAAAT
G. olivacea (1)         TTTTAGTTATTATTACTGTATTAGTTGGACAATTAATGATAACTTTTATTTTTTAATAAATTTACTCATCGATATTTACTAGAAGGACAATTAATTGAAAT
G. rufipes              TTTTAGTTATTATTACAGTCTTAGTTGGACAATTAATAGTAACATTATTTTTTCAATAAATTCACCCATCGATACCTTTTAGAAGGACAATTAATTGAAAT
G. linnaeana            TTCTAGTAATCATTACAGTTCTAGTTGGACAATTAATAATAACATTATTTTTTCAATAAATTTACCCATCGATATCTTTTAGAGGGTCAAATAATTGAAAT
G. variabilis (1)       TTTTAGTTATTATTACTGTACTTGTAGGACAATTAATAATAACTTTATTTTTTCAATAAATTTACTCATCGATATTTATTAGAAGGTCAAATAATTGAAAT
G. fornicata            TTTTAGTTATTATTACTGTATTAGTAGGACAATTAATAGTAACCCTTTTTTTTAATAAATTTACACATCGTTATTTATTAGAAGGTCAAATAATTGAAAT
G. fornicata            TTTTAGTTATTATTACTGTATTAGTAGGACAATTAATAGTAACCCTTTTTTTTAATAAATTTACACATCGTTATTTATTAGAAGGTCAAATAATTGAAAT
G. occidentalis         TTTTAGTTATTATTACAGTCTTAGTTGGACAATTAATAATAACATTATTTTTTAATAAATTTTCACACCGATATCTTTTAGAAGGACAATTAATTGAAAT
G. interposita (1)      TTTNAGTTATTATCACAGTATTAGTAGGCCAATTAATAGCAGCTTTATTTTTTCAATAAATTTACCCATCGGTATTTATTAGAGGGTCAAATAATTGAAAT
G. interposita (2)      TTTTAGTTATTATCACAGTATTAGTAGGCCAATTAATAGCAGCTTTATTTTTTCAATAAATTTACCCATCGGTATTTATTAGAGGGTCAAATAATTGAAAT
G. intermedia           TTTTAGTTATTATTACAGTATTAGTAGGTCAACTAATAACAACTTTATTTTTTTAATAAATTTACTCATCGATATTTATTAGAAGGTCAATTAATTGAAAT
G. quinquepunctata (2)  TTTTAGTTATTATTACAGTATTAGTAGGTCAATTAATAGTAACTTTATTTTTTTAATAAATTTACTCATCGATATTTATTAGAAGGTCAATTAATTGAGAT
G. holdausi             TTTTAGTTATTATTACAGTTTTAGTTGGACAATTATTAATAACATTATTTTTTTAATAAATTCTCACACCGATATCTCTTAGAGGGACAATTAATTGAAAT
G. pallida (2)          TTTTAGTTATTATTACAGTATTAGTAAGCCAATTAATAACAGCTTTATTTTTCAATAAATTTACCCATCGATATTTACTAGAGGGTCAAATAATTGAAAT
G. pallida (3)          TTNNNNNNATTATTACAGTATTAGTAGGCCAATTAATAGCAGCTTTATTTTTCAATAAATTTACCCATCGATATTTACTAGAGGGTCAAATAATTGAAAT
G. tredecimmaculata     TTCTAGTTATTATTACAGTATTAGTTGGTCAATTAATAATAACATTATTTTTTAATAAATTTACCCATCGAAATTTATTAGAAGGACAAATGATTGAAAT
G. nigroplagiata        TTTTAGTTATTATTACAGTTTTAGTTGGACAATTAATAATAACATTTATTTTTAATAAATTTACACATCGTTACTTACTTGAAGGTCAATTAATTGAAAT
G. kamikawai            TTTTAGTTATTATTACTGTATTAGTTGGACAATTAATAATAACATTATTTTTTAATAAATTTACACATCGATATTTATTAGAAGGACAAATAATTGAAAT
G. rubripennis          TTTTAGTTATTATTACTGTATTAGTGGGTCAATTAATATTAACCTTATTTTATAATAAATTTACCCATCGAAATTTATTAGAAGGACAAATAATTGAAAT
Oreina cacaliae         TTTTGTTAATTATTACAGTTTTAGTTGCCCAACTAATAATTAGTTTATTTTACAATAAATTTACACATCGATATTTATTAGAAGGACAAATTATTGAAAT
Chrysomela tremula      TCCTAATAATCATCACAGTATTAGTATCTCAATTAATAATCTCCTTATTTCTAAATAAATTTCCCACCGTTTCTATTAGAAGGTCAAATAATTGAAGT

G. viminalis (1)        TATTTGAACAGTTTTACCAACTTTAACCCTTATTTTTATTGCTATTCCATCTTTACGATTAATTTATATTTCTAGACGAAGTTAATAATCCTATAATTACT
G. olivacea (1)         TATTTGAACTATTCTACCAACACTAACGCTTATTTTTATTGCTATCCCATCTTTACGATTAATCTATATTTTAGATGAAATTAACAATCCAATAATTACA
G. rufipes              TATTTGAACAATTTTACCAACCCTAACACTTATTTTTATTGCTATTCCATCATTACGATTAATTTATATTTTAGATGAAGTTAATAATCCTATAATTACC
G. linnaeana            TATTTGAACAATTCTACCAACTTTAACATTAATCTTTATTGCTATCCCATCTTTACGATTAATTTATATTTTAGATGAAGTTAATAACCCTATAATTACT
G. variabilis (1)       TATTTGAACTATTCTACCAACATTAACACTAATTTTTATTGCAATTCCGTCATTACGTTTAATTTATATTTTAGATGAAATTAATAACCCAATAATTACT
G. fornicata            TATTTGAACTATTTTACCAACATTAACATTAATTTTTATTGCTATTCCTTCTTTACGATTAATTTACATTTTAGATGAAATTAATAATCCAATAATCACA
G. fornicata            TATTTGAACTATTTTACCAACATTAACATTAATTTTTATTGCTATTCCTTCTTTACGATTAATTTACATTTTAGATGAAATTAATAATCCAATAATCACA
G. occidentalis         TATTTGAACAATTCTACCAACTTTAACCCTTATTTTTATTGCTATCCCATCTTTACGATTAATTTATATTCTAGATGAAATTAATAATCCTATAATTACT
G. interposita (1)      TATTTGAACAATTTTACCTACATTAACTTTAATTTTTATTGCTATTCCTTCTTTACGATTAATCTATATTTTAGATGAAGTTAATAACCCTATAATTACC
G. interposita (2)      TATTTGAACAATTTTACCTACATTAACTTTAATTTTTATTGCTATTCCTTCTTTACGATTAATCTATATTTTAGATGAAGTTAATAACCCTATAATTACC
G. intermedia           TATTTGAACAATTTTACCAACATTAACATTAATTTTTATTGCTATTCCTTCTTTACGATTAATTTATATTTTAGATGAAGTTAATAACCCTATAATTACC
G. quinquepunctata (2)  TATTTGAACAATTTTACCAACATTAACATTAATTTTTATTGCTATTCCTTCTTTACGATTAATTTATATTTTAGATGAAGTTAATAACCCGATAATTACC
G. holdausi             TATTTGAACAATTTTACCAACTTTAACTCTTATTTTTATTGCTATTCCATCTTTACGATTAATTTATATTCTAGACGAAGTTAATAATCCTATAATTACC
G. pallida (2)          TATTTGAACAATTTTACCTACATTAACCTTAATTTTTATTGCTATTCCTTCTTTACGCTTAATCTATATTTTAGATGAAGTTAATAACCCTATAATTACC
G. pallida (3)          TATTTGAACAATTTTACCTACATTAACCTTAATTTTTATTGCTATTCCTTCTTTACGCTTAATCTATATTTTAGATGAAGTTAATAACCCTATAATTACC
G. tredecimmaculata     TATTTGAACTATTTTACCTACACTAACTCTAATTTTTATTGCTATCCCCTCCTTACGACTAATTTATATTTCTTGATGAAGTAAATAATCCAATAATTACT
G. nigroplagiata        TATTTGAACTATTTTACCAACATTAACATTAATTTTTATTGCTATTCCTTCTTTACGATTAATTTATATTTTAGATGAAATTAATAATCCAATAATTACA
G. kamikawai            TATTTGAACAGTTTTACCTACGTTAACTCTTATTTTTATTGCTATTCCTTCCTTACGATTAATTTATATTTTAGATGAAGTTAATAATCCTATAATTACT
G. rubripennis          TATTTGAACAATTTTACCAACATTAACTCTTATCTTTATTGCTATTCCATCATTACGATTAATTTATATTTTAGATGAAGTTAATAATCCAATAATTACT
Oreina cacaliae         TATCTGAACTATTTTACCTACTTTAACCTTAATTTTTATTGCTATTCCTTCCTTACGATTAATTTATATCTTAGATGAAATTAATAATCCTATAATTACA
Chrysomela tremula      AATTTGAACAATTTTACCTACACTTATTTTAATTTTGATTGCAATTCCCTCTTTACGATTAATTTATGTCCTAGATGAAGTTAATAGCCCTTTAGTCACA

G. viminalis (1)        ATTAAAACTATTGGGCATCAATGATATTGATCATATGAATATTCTGATTTCAAAAACATTGAATTTGATTCTTATATATTACCAACCAAAGAATTAAATA
G. olivacea (1)         ATTAAAACTATTGGACATCAATGGTACTGATCATATGAATAGTCGAATTTGATTCATATATAATACCAACCTCAGAATTAAATA
G. rufipes              ATTAAAACCATTGGTCATCAATGATACTGATCATATGAATATTCTGATTTCAAAAATATTGAATTTGATTCTTATATACTACCAACTCAAGAATTAAATA
G. linnaeana            ATTAAAACTATTGGACATCAATGATACTGATCATATGAGTATTCAGATTTCAAAAATATTGAATTTGATTCTTACATATTACCAACTAAAGAATTAAATA
G. variabilis (1)       ATTAAAACTATTGGACACCAATGATATTGATCTTATGAATATTCTGATTTTAAAAATATTGAATTTGATTCTATATATACTACCAACCATAGAATTAAATA
G. fornicata            ATTAAAACTATTGGACATCAATGATATTGATCTTATGAATATTCTGATTTTAAAAATATTGAATTTGATTCTTACATATTACCAACTAGAGAATTAAATA
G. fornicata            ATTAAAACTATTGGACATCAATGATATTGATCTTATGAATATTCTGATTTTAAAAATATTGAATTTGATTCTTACATATTACCAACTAGAGAATTAAATA
G. occidentalis         ATTAAAACTATCGGGCATCAATGATATTGATCATACGAATATTCTGATTTTAAAAATATTGAATTTGATTCTTATATATTACCAACTAAAGAATTAAATA
G. interposita (1)      ATTAAAACTATTGGACATCAATGATACTGATCTTATGAATATTCAGATTTTAAAAATATTGAATTTGATTCCTATATACTCCCAACTGCAGAATTAAATA
G. interposita (2)      ATTAAAACTATTGGACATCAATGATACTGATCTTATGAATATTCAGATTTCAAAAATATTGAATTTGATTCATATATACTCCCAACTGCAGAATTAAATA
G. intermedia           ATTAAAACTATTGGACACCAATGATACTGATCCTATGAATATTCCGACTTCAAAAATATTGAATTCGATTCTTATATACTACCAACTATAGAGTTAAATA
G. quinquepunctata (2)  ATTAAAACTATTGGACACCAATGATATTGATCCTATGAATATTCTGACTTCAAGAATATTGAATTCGACTCTTACATACTACCAACCGCAGAATTAAATA
G. holdausi             ATTAAAACTATTGGACATCAATGATATTGATCATATGAATATTCTGATTTTAAAAACATTGAATTTGATTCTTATATACTACCAACCAAAGAATTAAATA
G. pallida (2)          ATTAAAACTATTGGACATCAATGATACTGATCTTATGAATATTCAGATTTCAAAAATATTGAATTTGATTCTTATATACTCCAACTGTAGAATTAAATA
G. pallida (3)          ATTAAAACTATTGGACATCAATGATACTGATCTTATGAATATTCAGATTTCAAAAATATTGAATTTGATTCCTATATACTCCCAACTGCAGAATTAAATA
G. tredecimmaculata     ATTAAAACTATCGGGCACCAATGATACTGATCTTACGAATATTCCGATTTTAAAAATATTGAATTTGATTCGTATATATTACCAACTACAGAATTAAATA
G. nigroplagiata        ATTAAAACTATTGGGCATCAATGATATTGATCTTATGAATATTCAGACTTCAAAAATATCGAATTTGATTCATATATATTACCAACTAGAGAATTAAATA
G. kamikawai            ATCAAAACAATTGGTCATCAATGATACTGATCCTATGAATACTCAGATTTTAAAAATATTGAATTTGATTCATACATACTCCCTACTAATGAATTAAAAA
G. rubripennis          ATTAAAACAATTGGACATCAATGATATTGATCATATGAATATTCAGATTTTAAAAATATTGAATTTGATTCTTATATATTACCAACAAGAGAACTTAATA
Oreina cacaliae         ATTAAAACTATTGGACATCAATGATATTGATCATATGAATATTCAGATTTTAAAAATATTGAATTTGATTCTTACATAATTCCTACTAATGAATTAAAAA
Chrysomela tremula      ATCAAAACAATTGGGCATCAATGATATTGATCCTACGAATACTCTGACTTCAAAAATATTGAATTTGATTCTTACATAACTCCAATTAATGAATTAAATA
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G. viminalis (1)        ATTTTAATTTTCGACTTTTAGATGTAGATAATCGAATTGTTGTACCATTTGAATCCCAAATTCGCATATTAGTATCTGCTGCAGATGTAATTCATTCTTG
G. olivacea (1)         AATTCAATTTTCGTCTTTTAGACGTTGATAATCGAGTAGTAGTACCTTTTGAATCTCAAATTCGAATATTAGTAACTGCGGCAGATGTAATTCACTCATG
G. rufipes              ATTTTAACTTTCGGCTATTAGATGTTGATAATCGAATTATTGTACCTTTTGAATCCCAAATTCGAATATTGGTATCTGCAGCAGATGTAATTCATTCTTG
G. linnaeana            ATTTTAATTTTCGACTTCTAGATGTAGATAACCGAATTGTTGTACCATTTGAATCTCAAATTCGAATATTAGTAAGTGCAGCAGACGTTATTCACTCCTG
G. variabilis (1)       AATTTAATTTTCGTTTATTAGATGTAGATAATCGAGTAGTTGTACCATTTGAATCACAGATTCGAATATTAGTTACTGCAGCAGATGTAATTCACTCTTG
G. fornicata            AATTTAATTTTCGCCTATTAGATGTAGATAATCGAATTGTAGTACCTTTTGAATCCCAAATTCGAATATTAGTAACTGCTGCAGACGTAATTCATTCATG
G. fornicata            AATTTAATTTTCGCCTATTAGATGTAGATAATCGAATTGTAGTACCTTTTGAATCCCAAATTCGAATATTAGTAACTGCTGCAGACGTAATTCATTCATG
G. occidentalis         ATTTTAACTTTCGACTTTTAGATGTAGATAATCGAATTGTTGTACCATTTGAATCCCAAATCCGCATATTAGTATCTGCAGCAGATGTAATCCATTCTTG
G. interposita (1)      AATTTAATTTTCGTTTATTAGATGTGGATAATCGAATTGTTATCCCCTTTGAATCACAAATTCGGATACTGGTAACTGCAGCTGATGTAATTCACTCATG
G. interposita (2)      AATTTAATTTTCGTTTATTAGATGTGGATAATCGAATTATTATCCCCTTTGAATCACAAATTCGGATACTAGTAACTGCAGCTGATGTAATTCACTCATG
G. intermedia           AATTTAACTTTCGTTTATTAGATGTAGATAATCGAATCGTTATTCCCCTTCGAATCACAAATTCGAATATTGGTAACTGCAGCTGATGTCATTCACTCCTG
G. quinquepunctata (2)  AATTTAATTTTCGTTTATTAGATGTAGATAACCGAATTGTTGTACCATTTGAATCCCAAATTCGAATATTGGTAACTGCTGCTGATGTAATTCACTCTTG
G. holdausi             CTTTTAATTTTCGACTTTTAGATGTAGACAATCGAATTGTTGTACCATTTGAATCCCAAATTCGAATATTGGTATCTGCTGCAGATGTAATTCATTCTTG
G. pallida (2)          AATTTAATTTTCGTTTATTAGATGTAGATAATCGAATTATTATTCCATTTGAATCACAAATTCGAATACTGGTAACTGCAGCTGATGTAATTCACTCATG
G. pallida (3)          AATTTAATTTTCGTTTATTAGATGTGGATAATCGAATTGTTATTCCCTTTGAATCACAAATTCGAATACTGGTAACTGCAGCTGACGTAATTCACTCATG
G. tredecimmaculata     AATTTAATTTTCCGCCTATTAGATGTAGATAACCGAATTGTAATCCCATTTGAGTCTCAAATTCGTATATTAGTAACTGCTGCAGATGTTATTCACTCTTG
G. nigroplagiata        AATATAACTTTCGTCTATTTAGATGTAGACAACCGAGTAATTGTTCCTTTTGAATCTCAAATTCGAATTAGTTACAGCAGCTGATGTTATTCATTCGTG
G. kamikawai            ATTTTAATTTTCCGGCTACTTGATGTCGACAATCGAATTGTTATTCCATTTGAATCCCAAATCCGAATATTAGTAACTGCTGCAGATGTAATTCATTCTTG
G. rubripennis          AATTTAATTTTCGGCTATTAGATGTTGATAATCGAATTGTAATTCCATTTGAATCACAAATTCGTATATTAGTAACTGCTGCCGATGTAATTCACTCTTG
Oreina cacaliae         TTTTTAATTTTCGTCTTTTAGATGTTGATAATCGAATTATTGCCCCATTCCAATCACAAATCCGTATATTAATTACGGCTACAGATGTTATCCACTCATG
Chrysomela tremula      AATTTAATTTTCGTCTTTTAGATGTAGATAACCGTGTTATTATCCCATTTGAATCCCAAATTCGTATATTAATTACTGCTGCTGATGTAGTTCATTCATG

G. viminalis (1)        AACTATCCCATCCTTAGGAGTTAAAATTGATGCAACCCCTGGACGGTTAAATC
G. olivacea (1)         AACAATTCCATCTTTAGGAGTAAAAATTGATGCAACTCCAGGACGATTAAATC
G. rufipes              AACTATTCCATCATTAGGTGTTAAAATTGATGCAACTCCTGGACGACTAAATC
G. linnaeana            AACTATCCCATCTTTAGGAGTTAAAATTGATGCCACTCCTGGGCGACTTAATC
G. variabilis (1)       AACAATCCCGTCATTAGGAGTCAAAATTGATGCTACCCCTGGACGATTGAATC
G. fornicata            AACAATTCCTTCACTAGGAGTAAAAATTGATGCAACCCCTGGACGACTAAATC
G. fornicata            AACAATTCCTTCACTAGGAGTAAAAATTGATGCAACCCCTGGACGACTAAATC
G. occidentalis         AACTATTCCATCCTTGGGAGTTAAAATTGATGCAACTCCTGGACGATTAAATC
G. interposita (1)      AACAATCCCTTCTTTAGGGGTAAAAATTGATGCTACACCAGGACGATTAAATC
G. interposita (2)      AACAATCCCTTCTTTAGGAGTAAAAATTGATGCTACACCAGGACGATTAAATC
G. intermedia           AACTATCCCTTCTTTAGGTGTTAAAATTGATGCTACCCCAGGTCGATTAAACC
G. quinquepunctata (2)  AACTATCCCTTCTTTAGGTGTAAAAATTGATGCAACCCCGGGTCGGTTAAATC
G. holdausi             AACTATTCCATCATTAGGGGTTAAAATTGATGCAACTCCTGGACGATTAAATC
G. pallida (2)          AACAATCCCTTCTTTAGGAGTAAAAATTGATGCTACACCAGGACGATTGAANN
G. pallida (3)          GACAATCCCTTCTTTAGGAGTAAAAATTGATGCTACACCAGGACGATTAAATC
G. tredecimmaculata     AACTATCCCATCATTAGGAGTAAAAATTGATGCTATACCAGGCCGCCTTAATC
G. nigroplagiata        AACAATTCCATCCTTAGGAGTAAAAATTGATGCGACACCTGGTCGATTAAATC
G. kamikawai            AACAATCCCATCTTTAGGAGTAAAAATTGACGCAACTCCAGGACGTCTAAACC
G. rubripennis          AACTATTCCATCTTTAGGAGTAAAAATTGATGCTACTCCTGGTCGTCTAAATC
Oreina cacaliae         GACAATCCCTTCTTTGGGAGTTAAAATTGATGCTACCCCTGGACGTTTAAATC
Chrysomela tremula      AACTATCCCTTCTTTAGGAGTAAAAATTGATGCAATACCTGGCCGATTAAATC

2) 12S

G. viminalis (1)        GCATATTTTAGAGCTAAAATCAAATCAATTAATTAATTAAAATTACTTTCAAATCCAAATTCATATTACTTTAAAAATAATATTTCCTAATTTTAG-TTA
G. olivacea (1)         GCATATTTTAGAGCTAAAATCAAATTAATTAATTAATTAAAATTACTTTCAAATCCAAATTCATTATATTTTAAAAATAAAAATCTAAAATTATAT-TTA
G. rufipes              ACATATTTTAGAGCTAAAATCAAATTAATTAATTAATTAAAATTACTTTCAAATCCAAATTCTTATTATTTTAAAAATAATAATCCCTAATTATTA-TTA
G. linnaeana            GCATATTTTAGAGCTAAAATCAAATTAAATAATTAATCAATATTACTTTCAAATCCAAATTCATATTACTTTAAAAATAATAATCCCTAATTTTAA-TTA
G. variabilis (1)       GCATATTTTAGAGCTAAAATCAAATCAACTAATTAATTAAAATTACTTTCAAATCCAAATTCATTTTATATTACAAATAAAAATCCTAAATTATAT-TTA
G. fornicata            GCATATTTTAGAGCTAAAATCAAATTAATAAATTAATTAAAATTACCTTCAAATCCAAATTCATATTACTTTAAAAATAATAATCCTTCAATTATTT-TTA
G. fornicata            GCATATTTTAGAGCTAAAATCAAATTAATAAATTAATTAAAATTACCTTCAAATCCAAATTCATATTACTTTAAAAATAATAATCCTTCAATTATTT-TTA
G. occidentalis         GCATATTTTAGAGCTAAAATCAAATTAATTAATTAATTAAAATTACTTTCAAATCCACATTCATATTACTTTAAAAATAATAATCCTTAAATTTAA-TTA
G. interposita (1)      ACATATTTTAGAGCTAAAATCAAATTAACAAAATAATTAAAATTACTTTCAAATCCAAATTCATATTATTTTAAAAATAATAATCCTTAAATTATAT-TTA
G. interposita (2)      ACATATTTTAGAGCTAAAATCAAATTAACAAAATAATTAAAATTACTTTCAAATCCAAATTCATATTATTTTAAAAATAATAATCCTTAAATTATAT-TTA
G. intermedia           GCATATTTTAGAGCTAAAATCAAATTAACAAATTAATTAAAATTACTCTCAAATCCAAATTCATATTATTTTAAAAATAATAATCCTTAAATTATAT-TTA
G. quinquepunctata (2)  GCATATTTTAGAGCTAAAATCAAATTAATAAATTAATTAAAATTACTCTCAAATCCAAATTCATATTATTTTAAAAATAATAATCTTAAATTATAT-TTA
G. holdausi             GCATATTTTAGAGCTAAAATCAAATTAATTAATTAATTAAAATTACTTTCAAATCCAAATTCATATTATTTTAAAAATAATAATCCTTCAAATTAAA-TTA
G. pallida (2)          ACATATTTTAGAGCTAAAATCAAATTAACAAAATAATTAAAATTACTTTCAAATCCAAATTCATATTATTTTAAAAATAATAATCCTTAAATTATAT-TTA
G. pallida (3)          ACATATTTTAGAGCTAAAATCAAATTAACAAAATAATTAAAATTACTTTCAAATCCAAATTCATATTATTTTAAAAATAATAATCCTTAAATTATAT-TTA
G. tredecimmaculata     GCATATTTTAGAGCTAAAATCAAATTAATGAATTTATTAAAATTACCCTCAAATCCAAATTCATATTATTTTAAAAATAATAATCCCTAAATTTAT-TTA
G. nigroplagiata        GCATATTTTAGAGCTAAAATCAAATTAATTAATTAATTAAAATTACTTTCAAATCCAAATTCATATTATTTTAAAAATAATAATCCTAAATTATTATTA
G. kamikawai            GCATATTTTAGAGCTAAAATCAAATTAATTAATTAATTAAAATTACTTTCAAATCCAAATTTATATTACTTTAAAAATAATAATCCTTAATTTTTTT-TTA
G. rubripennis          GCATATTTTAGAGCTAAAATCAAATTAATTAATTAATTAAAATTACTTTCAAATCCAAATTCATATTATTTTAAAAATAATAATCCTAAATTTTTT-TTA
Oreina cacaliae         ACATATTTTAGAGCTAAAATCAATTTAATTAATTAATTAAATTTACTCTCAAATCCAAATTCATATTATTTTATAAATAATAATCCTTAAATAATT-TTA
Chrysomela tremula      ACATATTTTAGAGCT-AAATCATTTTAATTAATTAATTTAAATTACTGTCAAATCCTTATTCAAATTACTTTAAAAGTAACCATCCCAAAATTTTA-TTA

G. viminalis (1)        CTGTAACCCATTTCTACTTTTGTATAAACTACACCTTGATCTNATTTA-TTTTTATTATAGTAAATTTCGAATATTAAAATTCTTATAAAATATTCAAATT
G. olivacea (1)         ATGTAACCCATTTCTACTTTTTTTATAAACTACACCTTGATCTGATTTA-TTTTTTTTATTAAAAATCTTGAATATTATAATTCTTATAAAATATTC-AATT
G. rufipes              TTGTAACCCATTTCTACTTTTTTTATAAACTACACCTTGATCTGATTTA-TTTTTATGATAATAAATTTCGAATATTAAAATTCTTATAAAATATTCAAATT
G. linnaeana            ATGTAACCCATTTCTACTTTTTTTATAAACTACACCTTGATCTGATTTA-TTTTTATAATAATAAATCTTGAATATTAAAATTCTTATAAAATATTCAAATT
G. variabilis (1)       ATGTAACCCATTTCTTCTTTTTTATAAACTACACCTTGATCTGATTTA-TTTTTTTTATAAAAAATCTTGAATATTATTATTCTTATAAAATATTCAAATT
G. fornicata            ATGTAACCCATTTCTTCTTTTTATATAAACTACACCTTGATCTGATTTA-TTTTATTATCAAAATTCTTGAATATTAAAATTCTTATAAAATATTCAAATT
G. fornicata            ATGTAACCCATTTTCTTCTTTTTATATAAACTACACCTTGATCTGATTTA-TTTTATTATCAAAATTCTTGAATATTAAAATTCTTATAAAATATTCAAATT
G. occidentalis         ATGTAACCCATTTCTACTTTCTTATAAACTACACCTTGATCTGATTTA-TTTTATTATAATAAATTTTGAATATTAAAATTCTTATAAAATATTCAAATT
G. interposita (1)      ATGTAACCCATTTCTTCTTTTCCATATACTACACCTTGATCTGATTTA-TTTTTTTATAAAAATTCTTGAATATTATAATTCTTATAAAATATTCAAATT
G. interposita (2)      ATGTAACCCATTTCTTCTTTTCCATATACTACACCTTGATCTGATTTA-TTTTTTTATAAAAATTCTTGAATATTATAATTCTTATAAAATATTCAAATT
G. intermedia           TTGTAACCCATTTCTTCTTTTATCATAAACTACACCTTGATCTGATTTA-TTTTTTTATAAAAATTCTTGAATATTAAAATTCTTATAAAATATTCAAATT
G. quinquepunctata (2)  TTGTAACCCATTTCTTCTTTCCCATAAACTACACCTTGATCTGATTTA-TTTTTTTTATAAAAATTCTTGAATATTAAAATTCTTATAAAATATTCAAATT
G. holdausi             TTGTAACCCATTTCTACTTTCATATAAACTACACCTTGATCTGATTTA-TTTTATTATAATAAATTTTGAATATTAAAATTCTTATAAAATATTCAAATT
G. pallida (2)          ATGTAACCCATTTCTTCTTTTCCATATACTACACCTTGATCTGATTTA-TTTTTTTTATAAAAATTCTTGAATATTATAATTCTTATAAAATATTCAAATT
G. pallida (3)          ATGTAACCCATTTCTTCTTTTCCATATACTACACCTTGATCTGATTTA-TTTTTTTTATAAAAATTCTTGAATATTATAATTCTTATAAAATATTCAAATT
G. tredecimmaculata     TTGTAACCCATTTCTTCTTTTTTATAAACTACACCTTGATCTGATTTA-CTTTTATTCTAATACATCTTGAATATTTAAATTCTTATAAAATATTCAAATT
G. nigroplagiata        ATGTAACCCATTTCTTCTTTCTTATAAACTACACCTTGATCTGATTTA-TTTTTTTTATTAAAATTTTTAAATATTAAAATTCTTATAAAATATTTAATTT
G. kamikawai            ATGTAACCCATTTCTTCTTCTGTATAAACTACACCTTGATCTGATTTA-TTTTATTATAATAAATCTTGAATATTAAAATTCTTATAAAATACTCAAATT
G. rubripennis          TTGTAACCCATTTCTTCTTTTTTATAAACTACACCTTGATCTGATTTA-TTTTATTATAGTAAATTTTGAATATTAAAATTCTTATAAAATATTCAAATT
Oreina cacaliae         TTGTAACCCATTTCTTCTTTTTTATAAACTGCACCTTGATCTGATTTATTTTATTATAAAAAATCTTGAATATTATTATTCTTATAAAATATTCAAATT
Chrysomela tremula      TAGTAACCCATTTCTTCTTACTAATAAACTACACCTTGATCTGATTTTT-TTTTATTATAATAAATCTTGAATATTTAAATTCTAATAAAATATTCAAACT
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G. viminalis (1)        ACGACGATATGTAAACTAATAAAAAAAGTAAATTAAATCGTGGACTGTCAATTACAGAACAGGTTCCTCTGAATAGACTAAAATACCGCCAAAATCTTTA
G. olivacea (1)         ACGATGATATGTAAATTAATAAAAAAAGTAAATTAAATCGTGGACTATCGATTACAGAACAAGTTCCTCTGAATAGACTAAAATACCGCCAAAATCTTTA
G. rufipes              ACGACGATATGCAAACTAATAAAAAAAGTAAATTAAATCGTGGATTGTCAATTATAGAACAGGTTCCTCTGAATAGACTAAAATACCGCCAAAATCTTTA
G. linnaeana            ACGACGATATGTAAACTAATAAAAAAAGTAAATTAAATCGTGGATTATCAATTATAGAACAGGTTCCTCTGAATAGACTAAAATACCGCCAAAATCTTTA
G. variabilis (1)       ACGATGATATGTAAATTAATAAAAAAAGTAAATTAAATCGTGGATTATCGATTATAGAACAGGTTCCTCTGAATAGACTAAAATACCGCCAAAATCTTTA
G. fornicata            ACGACGATATGTAAATTAATAAAAAAAGTAAATTAAATCGTGGATTATCGATTACAGAACAGGTTCCTCTGAATAGACTAAAATACCGCCAAAATCTTTA
G. fornicata            ACGACGATATGTAAATTAATAAAAAAAGTAAATTAAATCGTGGATTATCGATTACAGAACAGGTTCCTCTGAATAGACTAAAATACCGCCAAAATCTTTA
G. occidentalis         ACGACGATATGTAAACTAATAAAAAAAGTAAATTAAATCGTGGACTGTCAATTACAGAACAGGTTCCTCTGAATAGACTAAAATACCGCCAAAATCTTTA
G. interposita (1)      ACGACGATATGTAAATTAACAAAAAAAGTAAATTAAATCGTGGATTATCGATTACAGAACAGGTTCCTCTGAATAGACTAAAATACCGCCAAAATCTTTA
G. interposita (2)      ACGACGATATGTAAATTAACAAAAAAAGTAAATTAAATCGTGGATTATCGATTACAGAACAGGTTCCTCTGAATAGACTAAAATACCGCCAAAATCTTTA
G. intermedia           ACGACGATATGTAAATTAATAAACAAAGTAAATTAAATCGTGGATTATCGATTACAGAACAGGTTCCTCTGAATAGACTAAAATACCGCCAAAATCTTTA
G. quinquepunctata (2)  ACGACGATATGTAAATTAATAAAAAAAGTAAATTAAATCGTGGATTATCGATTACAGAACAGGTTCCTCTGAATAGACTAAAATACCGCCAAAATCTTTA
G. holdausi             ACGACGATATGTAAACTAATAAAAAAAGTAAATTAAATCGTGGACTGTCAATTACAGAACAGGTTCCTCTGAATAGACTAAAATACCGCCAAAATCTTTA
G. pallida (2)          ACGACGATATGTAAATTAATAAAAAAAGTAAATTAAATCGTGGATTATCGATTACAGAACAGGTTCCTCTGAATAGACTAAAATACCGCCAAAATCTTTA
G. pallida (3)          ACGACGATATGTAAATTAATAAAAAAAGTAAATTAAATCGTGGATTATCGATTACAGAACAGGTTCCTCTGAATAGACTAAAATACCGCCAAAATCTTTA
G. tredecimmaculata     ACGACGATATGTAAACTAATAAAAAAAGTAAATTAAATCGTGGATTATCAATTACAGAACAGGTTCCTCTGAATAGACTAAAATACCGCCAAAATCTTTA
G. nigroplagiata        ACGATGATATGTAAATTAATAAAAAAAGTAAATTAAATCGTGGATTATCAATTACAGAACAGGTTCCTCTGAATAGACTAAAATACCGCCAAAATCTTTA
G. kamikawai            ACGACGATATGTAAGCTAATAAAAAAAGTAAATTAAATCGTGGATTATCAATTATAGAACAGGTTCCTCTGAATAGACTAAAATACCGCCAAAATCTTTA
G. rubripennis          ACGATGATATGTAAACTAATAAAAAAAGTAAATTAAATCGTGGACTATCAATTATAGAACAGGTTCCTCTGAATAGACTAAAATACCGCCAAAATCTTTA
Oreina cacaliae         ACGACGATATACAAACTAATAAAAAAAGTAAATTAAATCGTGGATTATCAATTATAGAACAGGTTCCTCTGAATAGACTAAAATACCGCCAAAATCTTTA
Chrysomela tremula      ACGACGATATGTAAACTAATATAGTAAGTGAATTAAATCGTGGATTATCAATCACAGAACAGGTTCCTCTGAATAGACTAAAATACCGCCAAAATCTTTA

G. viminalis (1)        ACTTTCAAGAACATAGCTAATACTAACTTAGTCTAAATA-AATACACTTATA 011001
G. olivacea (1)         ATTTTAAAGAACATAACTATTACTAATCTAGCATTATAA-ATTACATTTATA 011101
G. rufipes              ATTTTCAAGAACATAACTGATACTAAATTAGCCTAAATA-AATACGCTTATA 011001
G. linnaeana            ATTTTCAAGAACATAACTAATACTAACTTAG-TCAAATA-AATACATTTATA 011011
G. variabilis (1)       ATTTTCAAGAACATAACTAATACTAATTTAGTTATTTAA-ATTACATTTATA 011001
G. fornicata            ATTTTCAAGAACATAACTAATACTAATTTAGTTTTATAA-ATTACATTTAAA 011001
G. fornicata            ATTTTCAAGAACATAACTAATACTAATTTAGTTTTATAA-ATTACATTTAAA 011001
G. occidentalis         ACTTTCAAGAACATAACTAATATTAACTCAGCTTAAATA-AATACACTTATA 011001
G. interposita (1)      ATTTTCAAGAACATAACTATTACTAATTTAGTTTTTAAA-ATTACACTTAAA 011001
G. interposita (2)      ATTTTCAAGAACATAACTATTACTAATTTAGTTTTTAAA-ATTACACTTAAA 011001
G. intermedia           ATTTTCAAGAACATAACTAATACTAATTTAGCTTTTAAA-ATTACATTTAAA 011001
G. quinquepunctata (2)  ATTTTCAAGAACATAACTAATACTAATTTAGCTTTTAAA-ATTACATTTAAA 011001
G. holdausi             ACTTTCAAGAACATAACTAATACTAACTTAGCTTAAACA-AATACACTTATA 011001
G. pallida (2)          ATTTTCAAGAACATAACTATTACTAATTTAGTTTTTAAA-ATTACACTTAAA 011001
G. pallida (3)          ATTTTCAAGAACATAACTATTACTAATTTAGTTTTTAAA-ATTACACTTAAA 011001
G. tredecimmaculata     ATTTTCAAGAACATAACTAATACTAATTTAGCTTTAATATAATACATTTATA 011000
G. nigroplagiata        ATTTTCAAGAACATAACTAATACTAATTTAGCTTAATAA-ATTACATTTATA 001001
G. kamikawai            ATTTTCAAGAACATAACTAATACTAATTTAGCATAATAA-TATACATTTAAA 011001
G. rubripennis          ATTTTCAAGAACATAACTAATACTAATTTAGTTTTTAAT-AATACATTTAGA 011001
Oreina cacaliae         ATTTTCAAGAACATAACTAATACTAATTTAGCATATAAA-AATACATTTAAA 010001
Chrysomela tremula      ATTTTTGAGAATATACCTTATACTAATTTAGTCTATAAA-AATACATTTTAA 111001
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3) 16S

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G. viminalis (1)        AGACCTAATA-TTTTAGCTTCTACACCAAAAATTAATTTTAATCCAACATCGAGGTCGCAATCTTTTTTTTTCAATATGAACTTTCTAAAAAAATTACGCT
G. olivacea (1)         NGACCCAATA-TTTTAGCTTCTACACCAAAAATTAACTTTAATCCAACATCGAGGTCGCAATCTTTTTTTTTCAATATGAACTTTCTAAAAAAATTACGCT
G. rufipes              NNNCCTAATA-TTTCAGCTTCTGCACCGAAAATTAATTTTAATCCAACATCGAGGTCGCAATCTTTTTTTTTCAATTTGAACTTTCTAAAAAAATTACGCT
G. linnaeana            AGACCTAATA-TTTTAGCTTCTACACCAAAAATAAACTTTAATCCAACATCGAGGTCGCAATCTTTTTTTTTCAATTTGAACTTTCTAAAAAAATTACGCT
G. variabilis (1)       AGACCTAATA-TTTTAGCTTCTACACCAAAAATTAATTTTAATCCAACATCGAGGTCGCAATCTTTTTTTTTCAATATGAACTTTCTAAAAAAATTACGCT
G. fornicata            AGACCTAATA-TTTTAGCTTCTACACCAAAAATTAATTTTAATCCAACATCGAGGTCGCAATCTTTTTTTTTCAATATGAACTTTCTAAAAAAATTACGCT
G. fornicata            AGACCTAATA-TTTTAGCTTCTACACCAAAAATTAATTTTAATCCAACATCGAGGTCGCAATCTTTTTTTTTCAATATGAACTTTCTAAAAAAATTACGCT
G. occidentalis         AGACCTAATA-TTTTAGCTTCTACACCAAAAATTAATTTTAATCCAACATCGAGGTCGCAATCTTTTTTTTTCAATATGAACTTTCTAAAAAAATTACGCT
G. interposita (1)      AGACCTAATC-TTTCAGCTTCTGCACCAAAAATTAATTTTAATCCAACATCGAGGTCGCAATCTTTTTTTTTCAATTTGAACTTTCTAAAAAAATTACGCT
G. interposita (2)      AGACCTAATC-TTTCAGCTTCTGCACCAAAAATTAATTTTAATCCAACATCGAGGTCGCAATCTTTTTTTTTCAATTTGAACTTTCTAAAAAAATTACGCT
G. intermedia           AGACCTAATT-TTTTAGCTTCTACACCAAAAATTAATTTTAATCCAACATCGAGGTCGCAATCTTTTTTTTTCAATTTGAACTTTCTAAAAAAATTACGCT
G. quinquepunctata (2)  NCACCTAATT-TTTTAGCTTCTACACCAAAAATTAATTTTAATCCAACATCGAGGTCGCAATCTTTTTTTTTCAATTTGAACTTTCTAAAAAAATTACGCT
G. holdausi             AGACCTAATA-TTTTAGCTTCTACACCAAAAATTAATTTTAATCCAACATCGAGGTCGCAATCTTTTTTTTTCAATATGAACTTTCTAAAAAAATTACGCT
G. pallida (2)          NGACCTAATA-TTTCAGCTTCTACACCAAAAATTAACTTTAATCCAACATCGAGGTCGCAATCTTTTTTTTTCAATTTGAACTTTCTAAAAAAATTACGCT
G. pallida (3)          NGACCTAATA-TTTCAGCTTCTACACCAAAAATTAACTTTAATCCAACATCGAGGTCGCAATCTTTTTTTTTCAATTTGAACTTTCTAAAAAAATTACGCT
G. tredecimmaculata     NNNNCTAATA-TTTTAGCTTCTACACCAAAAATAAATTTTAATCCAACATCGAGGTCGCAATCTTTTTTTTTCAATATGAACTTTCTAAAAAAATTACGCT
G. nigroplagiata        AGACCTAATT-TTTTAGCTTCTACACCAAAAATAAATTTTAATCCAACATCGAGGTCGCAATCTTTTTTTTTCAATTTGAACTTTCTAAAAAAATTACGCT
G. kamikawai            AGACCTAATA-TTTCAGCTTCTACACCAAAAATTTACTTTAATCCAACATCGAGGTCGCAATCTTTTTTTTTCAATATGAACTTTCTAAAAAAATTACGCT
G. rubripennis          AGACCTAATA-TCCTAGCTTCTACACCAAAAATTAATTTTAATCCAACATCGAGGTCGCAATCTTTTTTTTTCAATATGAACTTTCTAAAAAAATTACGCT
Oreina cacaliae         AGACCTAATATTTTTAGCTTCTACACCAAAAATTTATTTTAATCCAACATCGAGGTCGCAATCTTTTTTTTTTAATATGAACTTTCTAAAAAAATTACGCT
Chrysomela tremula      AGACCTAATA-TTTTAGCTCCTACACCAAAAATTAATTTTAATCCAACATCGAGGTCGCAATCTTTTTTTTTTGATTTGAACTCTAAAAAAAAATAACGCT

G. viminalis (1)        GTTATCCCTAAGGTAATTTTATCTTATAATC--AAAAAAATTGGATCAATAATTCATAAATCAATGTTATATTACAAAAAAAGTTAAATAAATTTTTTTAA
G. olivacea (1)         GTTATCCCTAAGGTAATTTTATCTTATAATC--AAAAAAATTGGATCAATAAATCATACATTAATGTTAAAATACAAAAAAAGTTAATTAAATTTTTTTAA
G. rufipes              GTTATCCCTAAGGTAATTATATCTTATAATC-AAAAAAAATTGGATCAATAATTCATAAATTAATGTTATTATACAAAAAAAGTTAATAATATTTTTCAA
G. linnaeana            GTTATCCCTAAGGTAATTTTATCTTATAATCAAAAAAAAAATTGGATCAATAATTCATAAATTAATGTTATATTACAAAAAAAGTTTATCATATTTTTCAA
G. variabilis (1)       GTTATCCCTAAGGTAATTTTATCTTATAATC--AAAATAATTGGATCAATTATTCATATAAATATGTAATAATACAAAAAAAGTTTAATAAATTTTTCAA
G. fornicata            GTTATCCCTAAGGTAATTTTATCTTATAATC--AAAATAATTGGATCAATAATTCATAAATTAATGTTATTATATAAAAAAAGTTAATTAAATTTTTTAA
G. fornicata            GTTATCCCTAAGGTAATTTTATCTTATAATC--AAAATAATTGGATCAATAATTCATAAATTAATGTTATTATATAAAAAAAGTTAATTAAATTTTTTAA
G. occidentalis         GTTATCCCTAAGGTAATTTTATCTTATAATC--AAAAAAATTGGATCAATAATTCATAAATCAATGTTATATTACAAAAAAAGTTAAATAAATTTTTTAA
G. interposita (1)      GTTATCCCTAAGGTAATTTTATCTTATAATC--AAAATAACTGGATCAATGATTCATAAATTAATGTTAAAATACAAAAAAAGTTTACTAAATTTTTTTAA
G. interposita (2)      GTTATCCCTAAGGTAATTTTATCTTATAATC--AAAATAACTGGATCAATGATTCATAAATTAATGTTAAAATACAAAAAAAGTTTACTAAATTTTTTTAA
G. intermedia           GTTATCCCTAAGGTAATTTTATCTTATAATC--AAAACTATTGGATCAATAACTCATAAATTAATGTTAAAATACAAAAAAAGTTTACTAAATTTTTTTAA
G. quinquepunctata (2)  GTTATCCCTAAGGTAATTTTATCTNATAATC--AAAATAATTGGATCAATAATTCATAAATTAATGTTAAAATACAAAAAAAGTTTAATAAATTTTTTTAA
G. holdausi             GTTATCCCTAAGGTAATTTTATCTTATAATC--AAAAAAATTGGATCAATAACTCATAAATTAATGGTATATTACAAAAAAAGTTAAATAAATTTTTTTAA
G. pallida (2)          GTTATCCCTAAGGTAATTTTATCTTATAATC--AAAATAACTGGATCAATAATTCATAAATTAATGTTAAAATACAAAAAAAGTTTACTAAATTTTTTTAA
G. pallida (3)          GTTATCCCTAAGGTAATTTTATCTTATAATC--AAAATAACTGGATCAATGATTCATAAATTAATGTTAAAATACAAAAAAAGTTTACTAAATTTTTTTAA
G. tredecimmaculata     GTTATCCCTAAGGTAATTTTATCTTGTAATC--AAAATAATTGGATCAATAATTCATAAATCAATGTTACTATATAATAAAAAAGTTTTCTAAATTTTCACAA
G. nigroplagiata        GTTATCCCTAAGGTAATTTTATCTTGTAATC--AAAATAATTGGATCAATAATTCATAAATTAATGTAATTATACAAAAAAAGTTTATTAAATTTTTTTAA
G. kamikawai            GTTATCCCTAAGGTAATTTTATCTTATAATC--AAAATTATTGGATCAAGAAATCATAAATTAATGAATAATTACAAAAAAAGTTAATTAAATTTTTTCAA
G. rubripennis          GTTATCCCTAAGGTAATTTTATCTTATAATC--AAAAAAATTGGATCAATAATTCATAAATTTATGTTACTATATAAAAAAAGTTAATTAAATTTTTTTAA
Oreina cacaliae         GTTATCCCTAAGGTAATTTTATCTTATAATC--AAAAATATTGGATCATATACTCATAAATTAATGTTTTAAAATAAAAAAAGTTAAATAAATTTTTCAA
Chrysomela tremula      GTTATCCCTAAGGTAATTTAATCTTATAATC--ACTAAATTTGGATCAAATATTCATTAATTAATGTTAAATTATAAAAAAAGTTAAATTAATTTTTTAA
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Ambiguous region

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G. viminalis (1)        TCGCCCCAATCAAATTATAAATCAAATTTTAATAAATAAATTCTAAAATAAAAAA-TATA-AATTATAATAAAACTCTATAGGGTCTTCTCGTCTTTTAAT
G. olivacea (1)         TCACCCCAATTAAATTATAAAAAAAAATGAATAAAAAAATACTAAAATAAAAAA-TTTA-TTTTAAAATTAAACTCTATAGGGTCTTCTCGTCTTTTAAT
G. rufipes              TCGCCCCAATCAAATTATAAATTAAATTTTAATAAATAAATTCTAAAATAAAAAA-TAAA-AACTATAATAAAACTCTATAGGGTCTTCTCGTCTTTCAAT
G. linnaeana            TCACCCCAATCAAATTATAGATCAAATTTAATAATTAAATTCTAAAATAAAAAA-TATG-TACTATAATAAAACTCTATAGGGTCTTCTCGTCTTTTAAT
G. variabilis (1)       TCACCCCAATCAAATTATAAAATAAA-TAAATAAATAAATACTAAAATATAAAA-TTAAATTTTATAATTAAACTCTATAGGGTCTTCTCGTCTTTTAAT
G. fornicata            TCACCCCAATTAAATTATAAAAAAAATTTAATAAATAAATTCTAAAATAAAAAACTTTA-TTTTATAATAAAACTCTATAGGGTCTTCTCGTCTTTTAAT
G. fornicata            TCACCCCAATTAAATTATAAAAAAAATTTTAATAAATAAATTCTAAAATAAAAAACTTTA-TTTTATAATAAAACTCTATAGGGTCTTCTCGTCTTTTAAT
G. occidentalis         TCGCCCCAATCAAATTATAAATCAAATTTAATAAATAAATTCTAAAATAAAAAA-TATA-AACTATAATAAAACTCTATAGGGTCTTCTCGTCTTTTAAT
G. interposita (1)      TCACCCCAATTAAATTATAGAAAAAA-TTAATAAATAAATACTAAAATAAAAAA-TTTA-TACTATAATTAAACTCTATAGGGTCTTCTCGTCTTTTAAC
G. interposita (2)      TCACCCCAATTAAATTATAGAAAAAA-TTAATAAATAAATACTAAAATAAAAAA-TTTA-TACTATAATTAAACTCTATAGGGTCTTCTCGTCTTTTAAC
G. intermedia           TCACCCCAATTAAATTATAAAAAAAA-TTAATAAATAAATACTAAAATAAAAAA-TATA-TTCTATAATTAAACTCTATAGGGTCTTCTCGTCTTTTAAT
G. quinquepunctata (2)  TCACCCCAATTAAATTATAAAAAAAA-TTAATAAATAAATACTAAAATAAAAAA-TATA-ATTTATAATTAAACTCTATAGGGTCTTCTCGTCTTTTAAT
G. holdausi             TCGCCCCAATCAAATTATAAATCAAATTTTAATACATAAATTCTAAAATAAAAAA-TATA-AATTATAATAAAACTCTATAGGGTCTTCTCGTCTTTTAAT
G. pallida (2)          TCACCCCAATTAAATTATAGAAAAAA-TTAATAAATAAATACTAAAATAAAAAA-TTTA-TACTATAATTAAACTCTATAGGGTCTTCTCGTCTTTTAAC
G. pallida (3)          TCACCCCAATTAAATTATAGAAAAAA-TTAATAAATAAATACTAAAATAAAAAA-TTTA-TACTATAATTAAACTCTATAGGGTCTTCTCGTCTTTTAAC
G. tredecimmaculata     TCACCCCAATTAAATTATAAACTAAATTTAATAATTAAATTCTAAAATACAAAA-TATA-AATTATAATAAAACTCTATAGGGTCTTCTCGTCTTTTAAC
G. nigroplagiata        TCACCCCAATTAAATTATAAAAAAAA-TTAATAAATAAATTCTAAAATAAAAAA-TTTATTTTTATAATTAAACTCTATAGGGTCTTCTCGTCTTTTAAT
G. kamikawai            TCACCCCAATCAAATTATAAATCAAATTTAATAAATAAATTCTAAAATATAAAA-TATA-ATATATAATTAAACTCTATAGGGTCTTCTCGTCTTTCAAT
G. rubripennis          TCACCCCAATTAAATTATAAATTAAATTTAATAATTAAATTCTAAAATAAAAAA-TTTA-AATTATAATAAAACTCTATAGGGTCTTCTCGTCTTTTAAC
Oreina cacaliae         TCACCCCAATTAAATTATTCAATAAAATAAATAAATAAATACTAAAATATATTT-TTTA-TTAAATAATTAAACTCTATAGGGTCTTCTCGTCTTTTAAT
Chrysomela tremula      TCACCCCAACTAAATAAATACCTAAATTAATAAAATTAATTCTAAATATAATAA-TTTA-TTTATATATAAAACTCTATAGGGTCTTCTCGTCTTTTAAT

G. viminalis (1)        TTAACTTAAGCTTTTTAACTTAAAAATAAAATTCTAATAAAATTAAATAGAGACAGTTATTTTTTTATCTAACCC-TTCATTCCAGCTTTCAATTAAAAA
G. olivacea (1)         ATTAATTAAGCTTTTTAACTTAAAAATAAAATTCTAATAAAATTAAATAGAGACAGTTATTTTCTTATCTAACCCTTTCATTCCAGTTTTTAATTAAAAA
G. rufipes              TTAATTTAAGCTTTTTAACTTAAAAATAAAATTCTAATAAAATTAAATAGAGACAGTTACTTTTTTATCTAACCC-TTCATTCCAGCTTTTAATTAAAAA
G. linnaeana            TTAAATTAAGCTTTTTAACTTAAAAATAAAATTCTAATAAAATTAAACAGAGACAGTTATTTTTTTATCTAACCC-TTCATTCCAGCTTTTAATTAAAAA
G. variabilis (1)       AAAATTTAAGCTTTTTAACTTAAAAATAAAATTATAATAAAATTAAATAGAGACAGTCATTTTCTTATATAACCC-TTCATTCCAGTTTTCTAATTAAAAA
G. fornicata            AACAATTAAGCTTTTTAACTTAAAAATAAAATTCTAATAAAATTAAATAGAGACAGTTATTTTCTTATCTAACCC-TTCATTCCAGTTTCTAATTAAGAA
G. fornicata            AACAATTAAGCTTTTTAACTTAAAAATAAAATTCTAATAAAATTAAATAGAGACAGTTATTTTCTTATCTAACCC-TTCATTCCAGTTTCTAATTAAGAA
G. occidentalis         TTAACTTAAGCTTTTTAACTTAAAAATAAAATTCTAATAAAATTAAATAGAGACAGTTATTTTTTTATCTAACCC-TTCATTCCAGCTTTCAATTAAAAA
G. interposita (1)      AATAATTAAGCTTTTTAACTTAAAAATAAAATTCTAATAAATTTAAATAGAGACAGCTATTTTCTTATCTAACCC-TTCATTCCAGTTTTTAATTAAAAA
G. interposita (2)      AATAATTAAGCTTTTTAACTTAAAAATAAAATTCTAATAAATTTAAATAGAGACAGCTATTTTCTTATCTAACCC-TTCATTCCAGTTTTTAATTAAAAA
G. intermedia           AATAATTAAGCTTTTTAACTTAAAAATAAAATTCTAATAAATTTAAATAGAGACAGTTATTTTTCTTATCTAACCC-TTCATTCCAGTTTTTAATTAAAAA
G. quinquepunctata (2)  AATAATTAAGCTTTTTAACTTAAAAATAAAATTCTAATAAATTTAAATAGAGACAGTTATTTTCTTATCTAACCC-TTCATTCCAGTTTTTAATTAAAAA
G. holdausi             TTAACTTAAGCTTTTTAACTTAAAAATAAAATTCTAATAAAATTAAATAGAGACAGTTATTTTTTTATCTAACCC-TTCATTCCAGCTTTCAATTAAAAA
G. pallida (2)          AATAATTAAGCTTTTTAACTTAAAAATAAAATTCTAATAAATTTAAATAGAGACAGTTATTTTCTTATCTAACCC-TTCATTCCAGTTTTTAATTAAAAA
G. pallida (3)          AATAATTAAGCTTTTTAACTTAAAAATAAAATTCTAATAAATTTAAATAGAGACAGTTATTTTCTTATCTAACCC-TTCATTCCAGTTTTTAATTAAAAA
G. tredecimmaculata     TAAACTTAAGCTTTTTAACTTAAAAATAAAATTCTAATAAAATTATATAGAGACAGCTATTTTCTTATCTAACCT-TTCATTCCAGATTTTAATTAAAAA
G. nigroplagiata        AATAATTAAGCTTTTTAACTTAAAAATAAAATTCTAATAAAATTAAATAGAGACAGCTATTTTCTTATCTAACCC-TTCATTCCAGTTTTTAATTAAAAA
G. kamikawai            TATAATTAAGCTTTTTAACTTAAAAATAAAATTCTAATAAAATTAAATAGAGACAGTTATTTTCTTATCTAACCC-TTCATTCCAGCTTTCAATTAAAAA
G. rubripennis          TAAACTTAAGCTTTTTAACTTAAAAATAAAATTCTAATAAAATTATACAGAGACAGTTATTTTCTTATCTAACCC-TTCATTCCAGCTTTCAATTAAAAA
Oreina cacaliae         AATAATTAAGCTTTTTAACTTAAAAATAAAATTCTAATTAAATTTAACAGAGACAGCTATTTTCTTATCCAACCT-TTCATTCCAGTTTTTAATTAAAAA
Chrysomela tremula      GAAATTTAAGCTTTTTAACTTAAATATAAAATTCTAATAAAATTAAATAAAGACAGTTATTTTTTTATCCAACCC-TTCATTCCAGCTTTCAATTAAAAA

G. viminalis (1)        ACTAATGATTATGCTACCTTAGCACGGTCAAAATACCACGGCCATTTAAATATTTCAGTGGGCAGGCCAGACCTAAAATTAACATC 110111000
G. olivacea (1)         ACTAATGATTATGCTACCTTAGCACGGTCAAAATACCACGGCCATTCAAAT-ACTCAGTGGGCAGGCCAGACCTTAAATTATAATC 110110010
G. rufipes              ACTAATGATTATGCTACCTTAGCACGGTCAAAATACCANGGCCATTTAAATAATTCAGTGGGNNNGCCAGACCTAAANTTAAATTC 120111000
G. linnaeana            ACTAATGATTATGCTACCTTAGCACGGTCAAAATACCACGGCCATTTAAATAATTCAGTGGGCAGGCCAGACCTAAAATTAAATNN 100111000
G. variabilis (1)       ACTAATGATTATGCTACCTTAGCACGGTCAAAATACCACGGCCATTCAAAT-ACTCAGTGGGCAGGCCAGACCTAAATTATAATC 111101010
G. fornicata            ACTAATGATTATGCTACCTTAGCACGGTCAAAATACCACGGCCCTTCAAAT-TTTCAGCGGGCAGGTCAGACCTTAAATTATAATC 110011010
G. fornicata            ACTAATGATTATGCTACCTTAGCACGGTCAAAATACCACGGCCCTTCAAAT-TTTCAGCGGGCAGGTCAGACCTTAAATTATAATC 110011010
G. occidentalis         ACTAATGATTATGCTACCTTAGCACGGTCAAAATACCACGGCCATTTAAATATTTCAGTGGGCAGGCCAGACCT-AAATTANNNNN 110111001
G. interposita (1)      ACTAATGATTATGCTACCTTAGCACGGTCAAAATACCACGGCCATTCAAAT-ACTCAGTGGGCAGGCCAGACCTTAAATTATAATC 111111010
G. interposita (2)      ACTAATGATTATGCTACCTTAGCACGGTCAAAATACCACGGCCATTCAAAT-ACTCAGTGGGCAGGCCAGACCTTAAATTATAATC 111111010
G. intermedia           ACTAATGATTATGCTACCTTAGCACGGTCAAAATACCACGGCCATTCAAAT-ACTCAGTGGGCAGGCCAGACCTTATATTATAATC 111111010
G. quinquepunctata (2)  ACTAATGATTATGCTACCTTAGCACGGTCAAAATACCACGGCCATTCAAAT-ACTCAGTGGGCAGGCCAGACCTTATATTATAATC 111111010
G. holdausi             ACTAATGATTATGCTACCTTAGCACGGTCAAAATACCACGGCCATTTAAATATTTCAGTGGGCAGGCCAGACCTAAAATTANNNNN 110111000
G. pallida (2)          ACTAATGATTATGCTACCTTAGCACGGTCAAAATACCACGGCCATTCAAAT-ACTCAGTGGGCAGGCCAGACCTTAAATTATAATC 111111010
G. pallida (3)          ACTAATGATTATGCTACCTTAGCACGGTCAAAATACCACGGCCATTCAAAT-ACTCAGTGGGCAGGCCAGACCTTAAATTATAATC 111111010
G. tredecimmaculata     ACTAATGATTATGCTACCTTAGCACGGTCAAAATACCACGGCCATTTAAAT-CTTCAGTGGGCAGGTCGGACCTTAAATCNNNNNN 110111010
G. nigroplagiata        ACTAATGATTATGCTACCTTAGCACGGTCAAAATACCACGGCCCTTCAAAT-CTTCAGTGGGCAGGCCAGACCTTAANNNNNNNNN 111101010
G. kamikawai            ACTAATGATTATGCTACCTTAGCACGGTCAAAATACCACGGCCATTTAAATATATCAGTGGGCAGGCCAGACCTTAAATTAAATTC 110111000
G. rubripennis          ACTAATGATTATGCTACCTTAGCACGGTCAAAATACCACGGCCATTTAAAAATTTCAGTGGGCAGGCCAGACCTTAAATTAAATTC 110111000
Oreina cacaliae         ACTAATGATTATGCTACCTTAGCACGGTCAAAATACCACGGCCATTTAAAA-TCTCAGTGGGCAGGTTAGACCTTAAATTATCNNN 010111010
Chrysomela tremula      ACTATTGATTATGCTACCTTAGCACGGTTAAAATACCGCGGCCATTC-AAT-TCTCATTGGGCAGGCTAGACTTTAAATNNNNNNN 110111110
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4) four different alignments of the ambiguous region of the 16S:

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                        alignment 1          alignment 2          alignment 3          alignment 4
G. viminalis (1)        TAAATCAAATTTAAT      TAAATCAAATTTAAT      TAAATCAAATTTAAT      TAAATCAAATTTAAT
G. olivacea (1)         TAAAAAAAAATGAAT      TAAAAAAAAATGAAT      TAAAAAAAAATGAAT      TAAAAAAAAATGAAT
G. rufipes              TAAATTAAATTTAAT      TAAATTAAATTTAAT      TAAATTAAATTTAAT      TAAATTAAATTTAAT
G. linnaeana            TAGATCAAATTTAAT      TAGATCAAATTTAAT      TAGATCAAATTTAAT      TAGATCAAATTTAAT
G. variabilis (1)       TAAAATAAA-TAAAT      TAAA-ATAAATAAAT      T-AAAATAAATAAAT      TAA-AATAAATAAAT
G. fornicata            TAAAAAAAATTTAAT      TAAAAAAAATTTAAT      TAAAAAAAATTTAAT      TAAAAAAAATTTAAT
G. fornicata            TAAAAAAAATTTAAT      TAAAAAAAATTTAAT      TAAAAAAAATTTAAT      TAAAAAAAATTTAAT
G. occidentalis         TAAATCAAATTTAAT      TAAATCAAATTTAAT      TAAATCAAATTTAAT      TAAATCAAATTTAAT
G. interposita (1)      TAGAAAAAA-TTAAT      TAGA-AAAAATTAAT      T-AGAAAAAATTAAT      TAG-AAAAAATTAAT
G. interposita (2)      TAGAAAAAA-TTAAT      TAGA-AAAAATTAAT      T-AGAAAAAATTAAT      TAG-AAAAAATTAAT
G. intermedia           TAAAAAAAA-TTAAT      TAAA-AAAAATTAAT      T-AAAAAAAATTAAT      TAA-AAAAAATTAAT
G. quinquepunctata (2)  TAAAAAAAA-TTAAT      TAAA-AAAAATTAAT      T-AAAAAAAATTAAT      TAA-AAAAAATTAAT
G. holdausi             TAAATCAAATTTAAT      TAAATCAAATTTAAT      TAAATCAAATTTAAT      TAAATCAAATTTAAT
G. pallida (2)          TAGAAAAAA-TTAAT      TAGA-AAAAATTAAT      T-AGAAAAAATTAAT      TAG-AAAAAATTAAT
G. pallida (3)          TAGAAAAAA-TTAAT      TAGA-AAAAATTAAT      T-AGAAAAAATTAAT      TAG-AAAAAATTAAT
G. tredecimmaculata     TAAACTAAATTTAAT      TAAACTAAATTTAAT      TAAACTAAATTTAAT      TAAACTAAATTTAAT
G. nigroplagiata        TAAAAAAAA-TTAAT      TAAA-AAAAATTAAT      T-AAAAAAAATTAAT      TAA-AAAAAATTAAT
G. kamikawai            TAAATCAAATTTAAT      TAAATCAAATTTAAT      TAAATCAAATTTAAT      TAAATCAAATTTAAT
G. rubripennis          TAAATTAAATTTAAT      TAAATTAAATTTAAT      TAAATTAAATTTAAT      TAAATTAAATTTAAT
Oreina cacaliae         TTCAATAAAATAAAT      TTCAATAAAATAAAT      TTCAATAAAATAAAT      TTCAATAAAATAAAT
Chrysomela tremula      ATACCTAAATTAATA      ATACCTAAATTAATA      ATACCTAAATTAATA      ATACCTAAATTAATA
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