Androconial Hairbrushes of the *Syntomis* (*Amata*) *phegea* (L.) Group (*Lepidoptera, Ctenuchinae*): A Synapomorphic Character Supported by Sequence Data of the Mitochondrial 16S rRNA Gene

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Taxonomy of *Amata/Syntomis*, Phylogeny of Syntominae, Arctiinae, Ctenuchini and Syntomini

Males of several palaearctic *Syntomis/Amata* species (Lepidoptera: Arctiidae) possess androconial hairbrushes in connection with the foreleg coxa. The cuticular structure of these potentially behaviour-related and pheromone dissipating brushes is described. Such male-specific organs and signals play a crucial role in the female choice procedure. The presence of hairbrushes was found in 17 out of 28 inspected species of the tribe Syntomini. All members of the *Syntomis phegea* group (Europe to Central Asia, as well as Caspian, Caucasian and near-middle East species) have these structures, and only three oriental and south Asian, but none of three African species, carry this trait. The common genetic base of this morphological character is supported by an analysis of mitochondrial 16S rRNA from 19 representative taxa; species with hairbrushes form a monophyletic clade and the brushes are a synapomorphic character. This genetic finding corroborates the ethological significance of these organs. Phylogenetic data show a substantial genetic divergence between the tribe Ctenuchini (New World species) and the Old World Syntomini. Furthermore, DNA sequence data suggest a split of the genus *Amata* (sensu Obraztsov, 1966) in two distinct genera, *Amata* (without hairbrushes) and *Syntomis* (with hairbrushes).

Introduction

Peculiar male structures in Lepidoptera have been known since the early 19th century. Although they were soon understood as being secondary sexual organs, their specific function remained enigmatic until Fritz Müller's report (1877; see also Möller, 1915–23) that these structures often emit scent, probably as a sexual signal. This hypothesis was generally accepted, but it took many years before it could eventually be corroborated (see for reviews: Boppré, 1984; Birch and Hefetz, 1987; Birch *et al.*, 1990). Any male signal, scent-touch-sound-visual-, which is used in courtship, is a factor in the female choice procedure that critically steers sexual selection.

Our present view of the "single common peculiarity of these structures" (Scudder, 1887) is that their components are scales (or "hairs") which often dissipate a volatile. We know today that the formative (trichogen) cells of such hairs are capable of producing these odourants or their precursors which are then transmitted to the hair (Clearwater, 1975 a,b; Weatherston and Percy, 1977). Such scents play, at least in the more closely studied cases, a decisive role during the courtship and mating behaviour and are thus male sexual pheromones. In principle, however, such structures do not need to dissipate scents but could instead, or in combination with a chemical stimulus, also act as visual or tactile signals. Danaines are good examples for butterflies: males of *Danaus gilippus* (Cramer) use their androconial brushes to touch (to "hairpencil") the antennae of their females and in this process deposit scented pheromone-transfer-particles close to the odour-sensi-

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tive sensilla of their partner (Pliske and Eisner, 1969; Schneider and Seibt, 1969; Boppré, 1984). Complex sexual interplay with chemical, mechanical and visual signals was also reported from several moth species. A tortricid and a noctuid are informative cases (Baker and Cardé, 1979; Leppla *et al.*, 1987). Scudder (1887) called these male structures "androconial organs", a now well established term (Boppré, 1984). Such organs occur in a variety of shapes with corresponding names (scale fields, hairbrushes, hairfringes, hairpencils, coremata etc.) and are found on different places on the body of males. Watson (1865) was the first to suggest that the androconial organs might be of taxonomical value, a statement which in principle is trivial. In practice, however, taxonomists of Lepidoptera made and still make very little use of such organs, not the least because these structures are not so easily recognisable in museum specimens. We are aware of two notable exceptions: Elliot (1993, p. 388) who stated in his work on the lycaenids "it is possible to subdivide the Eumaeini according to the presence and type of brush-organ" and of Speidel *et al.* (1996) who used hairbrushes in a study of noctuid phylogeny. In this situation, however, general evolutionary considerations challenge us to expect visible taxonomic signs in relation to behaviourally relevant structures. In this communication we will describe such a case.

The palaearctic members of the genus *Amata/Syntomis* were treated in a monograph by Obraztsov (1966) who proposed a "new classification" which he mainly based on structural similarities in male genitalia. These characters allowed him only to split the genus *Amata* in the two subgenera *Amata* and *Syntomis* because he had no arguments to create two separate genera. However, Obraztsov could not unambiguously decide whether species of the Aethiopian *alicicia*-group, the SE Asian *bicincta*-group, or the Oriental *cymatilis*-group are properly placed in either one of his subgenera of *Amata*. Our intention with this study is not a general revision of the *Amata* complex. Instead we have studied whether a behaviourally relevant structure correlates with the molecular phylogeny within the *Amata* complex or whether it is a convergent trait.

In the course of studies of the pheromone biology of moths we discovered androconial hairbrushes on the foreleg coxa of *Amata* (*Syntomis*) *phegea* L., 1758. Since these structures were missing in males of the North African species *Amata mogadorensis* (Blachier, 1908), the question arose whether the occurrence of these brushes finds its expression in the taxonomy of this group of moths and is therefore a useful systematic character. In his work, Obraztsov (1966) could not use these structures as signs of group distinction since they were obviously not known to him. In dry museum specimens these brushes are hidden between the prethoracal sternum and the coxa. Because our morphological and genetic findings (as presented in this communication) show that both taxa *Amata* and *Syntomis* represent valid genera, we use the taxonomy and genus names as given in Table I throughout this publication. The higher order relationships of Arctiid taxa as listed in Table I are adapted to Kitching and Rawlins (1999) but could, with different emphasis, also been taken from Bendib and Minet (1998).

For some time already, systematists ranked the former family Ctenuchidae as a subfamily of the Arctiidae (see for example Covell, 1984; Minet, 1986). After completion of our morphological study on the male hairbrushes in the genera *Amata/Syntomis*, we learned from an analysis of the mitochondrial 16S rRNA gene of members of the Arctiidae: 1. that our two experimental species *S. phegea* and *A. mogadorensis* show sufficient genetic divergence to explain the hairbrush differences, 2. that the former Ctenuchidae deserves indeed the status of a subfamily of the Arctiidae ; 3. that Ctenuchinae or Syntominae may be separated (at least) into two independent clades (Wink & von Nickisch-Rosenegk, 1997; Wink *et al.*, 1998). The statements (2) and (3) fit well to studies of Bendib and Minet (1998), who use the female pheromone glands to arrange the Arctiidae, and to the larger view of other authors as summarized by Kitching and Rawlins (1999). The genetic differences mentioned under (1) encouraged us to extend our study to a wider genetic research on the taxonomic relationships in the so-called "*Syntomis phegea*-group" and neighbouring taxa.

In this publication we describe 1. the general morphology of androconial hairbrushes and give a survey of their occurrence in closer related and in some distantly related species of the "*phegea* group", and 2. analyse their phylogenetic relationships based on partial sequences of the mito-

chondrial 16S rRNA gene, with special emphasis on secondary rRNA structures.

Material and Methods

Morphology

Syntomis phegea specimens were offspring of females originally caught in northern Italy (Torino area) and southern Switzerland (Ticino). A laboratory culture of *A. mogadorensis* derived from specimens collected in Morocco. The larvae were kept on a variety of herbaceous plants (Wink and Schneider, 1990). Imagines were observed in flight cages. Dry specimens of other members of this family were studied in the Zoologische Staatssammlung München (ZSM) (Table I). Usually, the presence or absence of the hairbrushes in dry specimens could be tested by breaking the frontlegs away from the thorax (Fig. 1 and 2). If such brushes exist, they can be seen to insert in the intersegmental membrane between coxa and thorax (Fig. 3). These studies were carried out under a dissecting microscope. For scanning electron microscopy a Jeol 6300 F at 3.0 kV was used, after gold coating of the preparation.

DNA analysis

Origin of samples

The specimens used in this study were either dry material from Museum collections, caught in the field, or taken from laboratory cultures. Species from the Arctiinae, Lithosiinae, and Thyretini were included to complement our study. In order to reconstruct a reliable phylogeny, we used nucleotide sequences of an appropriate marker gene (see Table I for sequenced taxa).

DNA extraction

Part of the abdomen (typically the anterior or middle section) of adults was dissected, the posterior part (genitalia) being avoided because of the presence of sclerified parts, and, in mated females, of a spermatophore of male origin. Extraction was performed by incubating ground samples for 3 hours at 37 °C in 300 µl of 0.1 M Tris-HCl pH 8.0, 10 mM Na_2EDTA, 100 mM NaCl, 0.1% sodium dodecyl sulfate, 50 mM dithiothreitol and 1.5 mg proteinase K (Kocher *et al.*, 1989). Homogenates were then extracted twice with phenol/chloroform and DNA was precipitated with ethanol after addition of 6 µl of a 0.25% solution of linear acrylamide (the latter step was included in order to improve DNA recovery from dried specimens). DNA pellets were then dissolved in 50 µl Tris-EDTA-buffer (TE).

Amplification and sequencing of DNA

A partial section of the 16S rRNA gene with 528 nucleotides (nt) was amplified by Polymerase Chain Reaction (PCR) using the oligonucleotide primers : 5′-CGCCTGTTTATCAAAAACAT-3′ and 5′-CCGGTTTGAACTCAGATCA-3′. PCR amplification was carried out in a volume of 50 µl Taq polymerase buffer containing 200 µM of each dNTP, 1 mM of each primer and approximately 1 µg total DNA. Addition to the buffer of bovine serum albumin at a final concentration of 10 to 20 µg/ml improved the efficiency of amplification from dried material. PCR was initiated by adding 5 units of Taq polymerase (Pharmacia) to samples that had been heated at 92 °C for 2 min. The cycling program included 5 cycles consisting of 10 s at 92 °C, 45 s at 42 °C and 2.5 min at 65 °C (a controlled rate of heating of 7 °C/min was used between 42 to 65 °C), followed by 40 cycles consisting of 10 s at 92 °C, 45 s at a more stringent reassociation temperature (50 °C) and 2.5 min at 65 °C; a relatively low polymerisation temperature was chosen because of the A+T richness of moth mitochondrial DNA.

Sequencing was carried out by „Cycle sequencing" using two internal overlapping fluorescent primers: 5′-GTGCAAAGGTAGCATAATC-3′ and 5′-TAAAACTCTATAGGGTCTTCTCG-3′. Thermo Sequenase labelled primer kit with 7-deaza-dGTP (Amersham LIFE SCIENCE) was employed, according to a protocol provided by the manufacturer. Sequencing was performed on an ALFexpress DNA sequencer, AMV 3.0 (Pharmacia) with the following running conditions – 800 V; 60 mA; 25 W and 55 °C. Sequencing was carried out using 5.5% acrylamide gels (5.75% Long ranger solution -FMC Corp.-, 7 M urea, 1X TBE). Sequences were further treated on the ALF WIN V 1.0 PC interface. Sequences are given in Table II and will be deposited at the EMBL sequence data library.

The sequence alignment (Table II) was deduced from the secondary structure constraints described in Maidak *et al.* (1996), and according to the rules of nucleotide linkages as in Michel and Costa (1998), and the reference sequence and secondary structure of *Papilio machaon* (Lepidoptera) given in Aubert *et al.* (1999).

Phylogenetic analyses

Many systematists and especially cladists (Brower *et al.*, 1996) prefer the character state method Maximum Parsimony (MP) for tree-building. It would be out of the scope of this publication to discuss the extensive, often acrimonious and contradictory battle which can be found in the literature on the advantage and disadvantage of character state versus distance methods (see publications cited in Felsenstein, 1988; Stewart, 1993; Nei, 1996). The same applies to the employment of bootstrap analysis (Felsenstein, 1985) to obtain a statistical estimate, i.e., whether a furcation is significantly supported by the data or not (see discussion in Nei, 1996; Sanderson, 1995).

However, we prefer a pragmatic way and use several methods, such as MP (Maximum Parsimony), ME (Minimum Evolution distance methods) and ML (Maximum Likelihood) to reconstruct phylogenetic relationships. In our experience, data sets based on a balanced sampling usually produce trees of almost congruent topology. We agree with Nei (1996) who stated "...it is now clear that any method (...of tree building) is not almighty, and there are situations in which one method is more efficient than others in obtaining the true tree and that, unless the evolutionary rate varies drastically with evolutionary lineages, all the three methods (MP, ME and ML) considered here generally give the same or similar topologies". If a clade is supported by these independent methods which employ different mathematical algorithms, we are confident in the results; ambiguous bifurcations rather indicate, that the topology cannot be resolved with a present data set.

All analyses were performed on a Pentium 166 PC with test version 4.0d64 of PAUP*, written by David L. Swofford. All heuristic searches for optimal trees were carried out by TBR (Tree-bissection-reconnection) branch swapping with option MULPARS in effect.

1. Parsimony-based analyses. Starting trees were obtained by stepwise addition. We checked that random addition of taxa did not lead to alternative, more parsimonious trees. For each bootstrap replicates, 10 heuristic searches were performed with random addition of taxa.
2. Distance- (minimum evolution) based analyses. A log-determinant (LogDet) distance measure was chosen since this transformation is robust and involves no assumptions on rates of substitution. Use of starting trees obtained by either neighbour-joining or random addition resulted in identical final topologies.
3. Analyses based on Maximum Likelihood. Starting trees were obtained by neighbour-joining (using a LogDet distance option, see above); a new optimal tree was sought and the process was repeated until a stable topology was achieved (different starting trees led to the same final topology, as it was the case without any starting tree). Rates for variable sites were always assumed to follow a Gamma distribution, and both the shape of this distribution and the fraction of invariable sites were estimated.

Results and Discussion

Cuticular structures of the androconial organ

The androconial hairbrushes are located on the caudal side of the interjoint membrane between the coxa and the thorax (Fig. 1). The brushes fan out when the front leg is actively or passively lifted from the resting position. With male moths walking in the observation cage, when pursuing a female, the hairbrushes were seen opening and closing like a fan, depending on the position of the

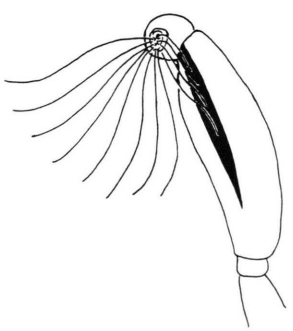

Fig. 1. *Syntomis phegea* hairbrush (length of 1.2 mm).

coxa. Whether or not males have control over the opening of the hairbrushes without moving the leg is not known. The brush hairs of *S. phegea* are bright yellow and very apparent when exposed. Microscopical inspection showed that the "resting locality" of the hairs is a furrow in the coxa. This is schematised in Fig. 1. The number of hairs per brush is about 60, the length of hairs approximately 1.2 mm and their diameter (in the middle part) 5–8 μm.

The hairs of the brush insert as a group in a shallow, collared pit of the interjoint membrane (Figs. 2 and 3). The hairs have prominent, segmented, longitudinal ribs. Between the ribs, highly structured zones of variable patterns appear (Figs. 4–6). The ribs converge towards the tip of the hairs. The areae between the ribs have many pits of 0.1–0.5 μm in diameter. At the bottom of these pits (Fig. 6) fine holes of 50–100 nm open to the lumen of a hair. In accordance with these perforations, fractured hairs show that they have a double wall (Fig. 5).

The hairbrushes of *Syntomis* species are located at the base of the front legs. There are several examples of similar coxal organs in the Syntominae, Sphingidae, and Noctuidae (Barth, 1966). Hairbrushes on the legs of male moths are known from primitive as well as highly developed moths yet only rarely has their fine structure, chemistry, and function been identified. It remains to be seen whether or not the *S. phegea* hairbrushes fit the criteria of a pheromone organ as: 1. the trichogen cell must also be the gland cell; 2. the odourant (in *S. phegea* so far not detected, let alone identified) should be emitted from the hair, and 3. elicit an olfactorial and behavioural response in females (and males?). To date, chemical extracts of the brushes have not shown any sign of a volatile compound (W. Francke unpublished) and we saw no electroantennogram (EAG) responses (in either sex) to air blown over the brushes to the antennae. However, we observed clear EAG reactions to artificially exposed female gland tubes in male antennae of *S. phegea, S. kruegeri*, and *A. mogadorensis* (Schneider unpublished). Female pheromones were chemically identified in *S. phegea* and *Dysauxes punctata* by Szöcs *et al.* (1987), and in the ctenuchines *Empyreuma mucro*, and *Syntomeida epilais* by Descoins *et al.* (1989). The active components are related polyunsaturated hydrocarbons, typical for Arctiidae.

The fine structure of the *S. phegea* brush-hairs are in essence similar to the more common type of androconial hairs in male Lepidoptera (see Boppré and Vane-Wright, 1989; Wunderer *et al.*, 1986; and Schneider *et al.*, 1992). They are rodlike organs ("hairs" in low power microscopy) composed, like the body scales and wing scales of a cuticular framework which combines rigidity with elasticity. Clearly, these delicate scales, with their very high surface area, appear to be ideally suited to hold and, upon exposure of the organ, to dissipate volatiles.

Occurrence of the androconial organ in Amata/ Syntomis in relation to zoogeographic distribution

The occurrence of the hairbrush organ in *Amata/Syntomis* is presented in Table I. All members of the genus *Amata* (grouped as subgenus A. (*amata*) in Section 1 by Obraztsov, 1966) are devoid of hairbrushes. These taxa are distributed in the Australasian region. Equally, species of Section 2 from Africa and Asia (see Table I), (including one of three specimens *S. divisa),* do not carry hairbrushes. In Section 3, all members of the phegea group have hairbrushes, only *S. bicincta* and *S. cymatilis*, members of other groups, do not have them. The closely related *phegea-ragazzi-kruegeri* group have a mainly Mediterranean distribution. *S. nigricornis* can be regarded as the eastern "extension" of the inner "*phegea*-group" and *S. bactriana* and *S. cocandica* as their Asian representatives. Another group of species with hairbrushes connects, albeit in rather wide zoogeographical terms, the European to central Asian representatives with species in the Caspian-Caucasian region and further on to species in Asia Minor: *S. maracandina- banghaasi- caspia- aequipunctata- antiochena- libanotica*. Interestingly, *S. germana* and *S. lucerna*, both members of Section 1 of the genus *Syntomis* have hairbrushes as well as two of the three males of *S. divisa* from different localities. A reevaluation of *S. divisa* is therefore needed. The two species of section 1, *S. germana* and *S. lucerna* (both with hairbrushes), do also reach far into the South-Oriental region. They are, with S. and S. E. Asian *S. divisa*, the only two *Syntomis* taxa with these androconical characters beyond Section 3, the *S. phegea* group. s.str.

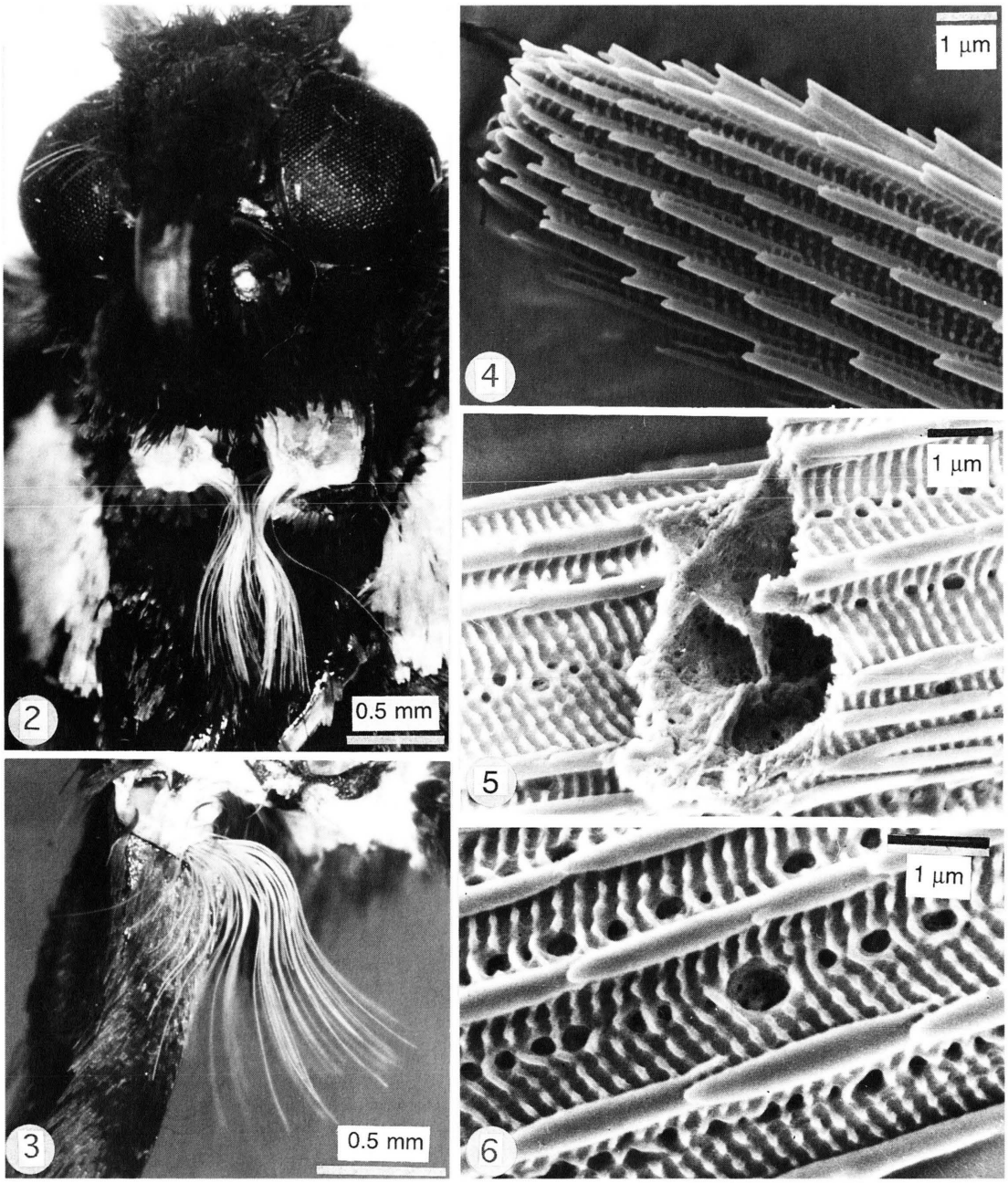

Fig. 2. Macrophotograph of *S. phegea* : Ventral overview of head and prothorax
The two hairbrushes are shown exposed after removal of the front legs and coxae.
Fig. 3. Macrophotograph of *S. phegea* : Right coxa with some of the hairs still in the fold, others exposed.
Fig. 4. Scanning electron micrographs of *S. phegea* showing the fine structure of the hairs
Tip area
Fig. 5. Scanning electron micrographs of *S. phegea* showing the fine structure of the hairs: Fractured hair. Note complex doublefold wall.
Fig. 6. Scanning electron micrographs of *S. phegea* showing the fine structure of the hairs: Middle part with ribs, many small pits and one large pit (photos by C. Bock).

Table I. List and systematics of the moths studied.
Brush: (+) = hairbrushes present, (−) = hair brushes missing, (?) = hair brushes questionable or not checked. 16S: (+) = mitochondrial 16S rRNA gene successfully sequenced. The general order of subfamilies and tribes follows Kitching and Rawlins (1999). The organisation of the *Syntomis/Amata* group is a result of this study and differs from that of Obraztsov (1966) who introduced the Group and Sections in the second and third column.

Organisms			Distribution	Brush	16S
Subfamily Arctiinae					
Tribus Arctiini					
Arctia caja (L.)			Holarctic	−	+
Tribus Ctenuchini					
Ctenucha venosa (Kir.)			SW USA, Mexico	−	+
Subfamily Lithosiinae					
Lithosia quadra L.			Western Europe	−	+
Subfamily Syntominae					
Tribus Thyretini					
Meganaclia perpusilla			West central Africa	−	+
Tribus Syntomini					
Genus *Dysauxes*					
Dysauxes punctata (F.)			South Europe, N Africa	−	+
Genus *Hydrusa*					
Hydrusa diaphana (Leach)			Sumatra, Borneo	−	+
Genus *Amata*					
Amata huebneri (B.)		Section 1	SE Asia, N Australia	−	+
Amata sperbius (F.)	*cingulata* group	Section 1	S China, India, Thailand	−	+
Amata mogadorensis 1/2 (Bla.)	*alicia* group	Section 2	Morocco, Hoggar Mts	−	+
Amata alicia (Btlr.)	*alicia* group	Section 2	Africa south of Sahara	−	+
Amata bicincta (Koll.)	*bicincta* group	Section 3	Kashmir, Himalaya, S China	−	+
Amata cymatilis Swinhoe	*cymatilis* group	Section 3	Philippines	−	
Amata damarensis (Grund.)	*alicia* group	Section 2	NE Africa, Yemen	−	
Amata sinensis (Roth.)		Section 2	S China	−	
Amata emma (Btlr.)		Section 1	Mongolia, China, Assam, Burma	−	
Amata cingulata (Web.)	*cingulata* group	Section 1	S China, India, Thailand	−	
Genus *Syntomis*					
Syntomis germana (Fldr.)		Section 1	Ussuri, China, Japan, Taiwan, Java	+	+
Syntomis lucerna (Wilem.)		Section 1	Tibet, Yunan, Sechuan, Taiwan	+	
Syntomis divisa (Wkr.)		Section 2	Nepal, S China, Assam, Burma	**+ (2x)**	
			S-China	**− (1x)**	
Syntomis phegea (L.)	*phegea* group	Section 3	NW Europe	+	
Syntomis phegea ligata 1/2 (Mull.)	*phegea* group	Section 3	Spain, SW Europe	+	+
Syntomis ragazzi (Trti.)	*phegea* group	Section 3	S Italy	+	+
Syntomis albionica (Duf.)	*phegea* group	Section 3	SE France	?	+
Syntomis kruegeri (Ragusa)	*phegea* group	Section 3	SW Ukraine, Balkans, Italy	+	+
Syntomis aequipunctata (Trti.)	*phegea* group	Section 3	S Turkey to Syria, Lebanon	+	+
Syntomis nigricornis (Alph.)	*phegea* group	Section 3	Central Russia, to Caucasus	+	+
Syntomis bactriana (Ersch.)	*phegea* group	Section 3	Russian central Asia	+	
Syntomis cocandica (Ersch.)	*phegea* group	Section 3	Central China to Central Asia	+	
Syntomis antiochena (Ld.)	*phegea* group	Section 3	Syria	+	
Syntomis libanotica (A. B.-H.)	*phegea* group	Section 3	Lebanon	+	
Syntomis maracandina (Ersch.)	*phegea* group	Section 3	S Russia, Central Asia, N Afghanistan	+	
Syntomis banghaasi (A. B.-H.)	*phegea* group	Section 3	Transcaucasia, Central Asia,NEChina	+	
Syntomis minutissima (Obr.)	*phegea* group	Section 3	Transcaspia	+	
Syntomis caspia (Stgr.)	*phegea* group	Section 3	NE Caspia to SW Siberia	+	+

Phylogeny of the genera Amata and Syntomis based on nucleotide sequences of the mitochondrial 16S rRNA gene

Among the 29 species of the tribe Syntomini analysed for the presence of hair brushes, DNA of 15 species was amplified and sequenced successfully. Furthermore, two specimens each of *Syntomis phegea* and *Amata mogadorensis* were sequenced to corroborate the reproducibility of our experimental design; as expected, specimens from the same species produced identical sequences. Three distantly related species were included in our study: *Lithosia quadra*, *Meganaclia perpusilla*, and

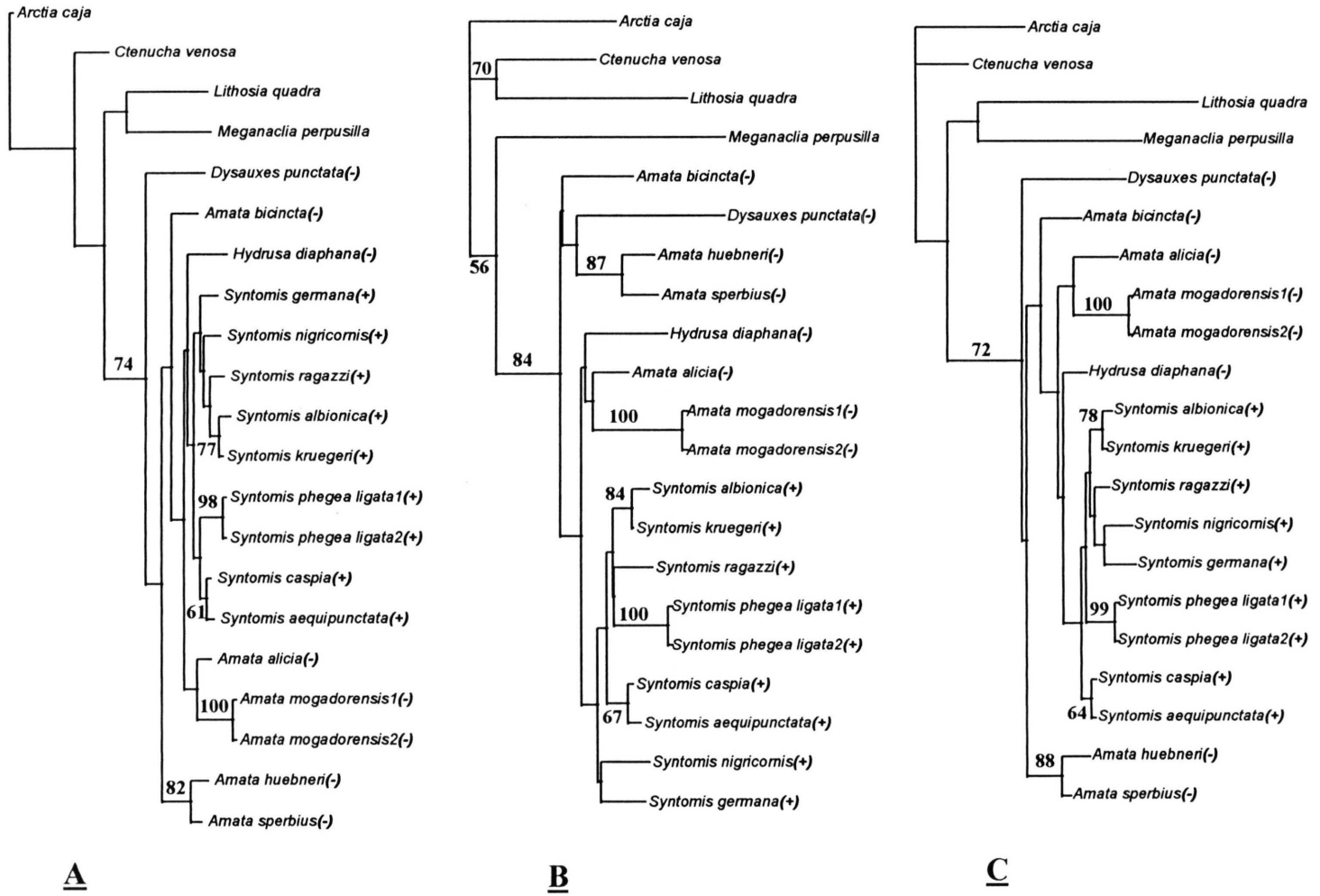

Fig. 7. Reconstruction of the phylogeny of the Syntominae inferred from nucleotide partial sequence of the mitochondrial 16S rRNA gene. A. Maximum parsimony (MP) tree (1 of the 2 most parsimonious trees found), Length=238 steps; CI=0.6513; RI=0.6278; RC=0.4089; HI= 0.3487; CI (exc. uninform characters) = 0.5489; HI (exc. uninform characters) = 0.4511. Bootstrap (X1000 replicates) values are given when higher than 50%; B. Minimum evolution (ME) tree : Length=0.43554, Logdet/paralinear distance algorithm. Heuristic search using NJ starting tree. Bootstrap (X1000 replicated) values indicated when superior to 50%; C. Maximum likelihood (ML) tree. Estimated 0.468 fraction of variable sites, whose rates of evolution were assumed to follow a gamma distribution with shape parameter 0.598 (4 rate categories represented by mean; rates of substitution were assumed to obey a six parameter, general time-reversible model, with rAC=1.90; rAG=44.17; rAT=13.55; rCG=4.10^{-8}; rCT=3.76; rGT=1. Bootstrap (X 1000 replicates) values are given when higher than 50%.

Ctenucha venosa, which are now, according to Kitching and Rawlins (1999) placed in the Lithosiinae, Thyretini, and Ctenuchini, respectively. *Dysauxes punctata* and *Hydrusa diaphana* were also investigated to elucidate their relative relationships with *Amata/Syntomis*, finally an unchallenged representative of the Arctiinae, *Arctia caja*, was chosen as outgroup (Fig. 7).

The subfamily Syntominae consists (depending on the view of different authors, e.g., Kitching and Rawlins (1999) of two tribes, i.e., the Syntomini, and Thyretini, whereas Ctenuchini – with or without Euchromiini – are considered a tribe in the subfamily Arctiinae. However, some morphological and preliminary molecular data imply that phylogenetic relationships of the subfamily Arctiinae are in fact much more complex than hitherto assumed (Legal and Wink, unpublished results).

Members of the tribe *Syntomini* (Fig. 7) appear as a monophyletic assemblage including *Hydrusa* and *Dysauxes*. The African tribe Thyretini clusters as the sister group of Syntomini in MP, ME, and ML reconstructions. This indicates that Thyretini are not a distinct family (Pinhey, 1975) but a tribe within the Syntominae as suggested by Kitching and Rawlins (1999) and Bendib and Minet (1998). Using our restricted data set it was not possible to determine unambiguously whether Lithosiinae + Thyretini are a sister group of Syntomini or if Lithosiinae forms a sister group to Thyretini + Syntomini (the latter hypothesis is favoured by a larger data set (Legal and Wink in prep.) and by some other taxonomists Bendib and Minet (1998). Except when a phenetic approach was used (Fig. 7B), it is evident that New World Ctenuchini are closer to Arctiinae than to the Lithosiinae-Thyretini-Syntomini clade, confirming that the New World Ctenuchini and Old World Syntomini are not closely related as hitherto assumed. This finding would support the classification of Kitching and Rawlins (1999), who placed the Ctenuchini as a tribe of the subfamily Arctiinae.

Dysauxes punctata was generally found to be the basal taxon within the Syntomini clade in MP and ML trees (Fig 7A,C) and is not closer related to Thyretini, as suggested by Kitching and Rawlins (1999). The genus *Amata* (sensu Obraztsov, 1966) is represented in this analysis by the two species *Amata sperbius* and *Amata huebneri*. According to morphological characters (sensu Obraztsov, 1966) and the lack of hairbrushes, they appear to be sibling species. The absolute distance between these two taxa is 1.7% (if a large insertion found for *A. huebneri* rRNA is deleted; see Table II) and they cluster together in every kind of our reconstructions with significant bootstrap values (Fig. 7A-C). Remarkably, these two species are quite distant from other members of Syntomini (divergence approximately 6%). Notably, *Syntomis (*now *Amata) bicincta* appears much closer to *Amata sperbius* and *Amata huebneri* than to *S. phegea* group where it was placed by Obraztsov (1966). Since this taxon lacks hairbrushes, its isolated position at the base of the *Amata/Syntomis* complex appears plausible. Another group may be also separated from the *S. phegea* complex; namely the African species *Syntomis (*now *Amata) alicia* and *A. mogadorensis* which always cluster together outside the *S. phegea* complex (Fig. 7 A-C). The same holds true for the S.-Asian *Syntomis (*now *Amata) bicincta*, which also lacks hair brushes.

Within the *S. phegea* complex, unambiguous resolution is more difficult to obtain, as these species are closely related and our marker gene is at its resolution limit. However, a few sibling species were recognized. For example, *S. kruegeri/ S. albionica, S. caspia/ S. aequipunctata* and *S. nigricornis/ S. germana*. Considering the small divergence between *S. kruegeri* and *S. albionica* (0.8%), our study confirms that these two taxa are extremely close relatives, but whether they are distinct species remains a matter of debate (Rougeot and Viette, 1978; Leraut, 1992). The divergence between *A. mogadorensis* or *A. bicincta* and other members of the genera *Syntomis* (including *S. germana*) ranges between 3.6% and 6.1% which exceeds significantly (t-test, $p<0.01$) the absolute distances within the *Syntomis* complex carrying hairbrushes (i.e., 3.3%; Table III); this could be another argument to attribute these species, which were formerly placed in the genus *Syntomis*, to the genus *Amata*.

The position of *Hydrusa diaphana* (Fig. 7) separating the former genus *Amata* in two parts, i.e. those with and without hair brushes, comes as a surprise, as a position outside the *Amata/Syntomis* clades would have been more likely.

Besides a sequence analysis illustrated in Fig. 7, we have started to compare the secondary structure of 16S rRNA. According to frame-

Table II. Aligned nucleotide sequences of the 16S rRNA gene.
The following characters are indicated by a star (i) constant characters, (ii) uninformative characters in terms of parsimony and (iii) when applicable, excluded characters. Excluded characters (in italics) were chosen according to ambiguities in the alignment. 44 characters out of 528 nucleotides were excluded. Putative regions involved in secondary structure are highlighted in bold (see also material and methods section). Complementary sequences forming each stems share a same number. Arrows indicate the respective length of complementary parts of the different stems (variable among species). In **Bold** are those nucleotides which base paired in secondary structure (Fig. 8).

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Ctenucha venosa             TTTTTGAAAATAATTTAAAGTCTAATCTGCCCACTGATTAT-ATAATTAAAGGGCTGCAGTA
Lithosia quadra             TTTTTGTAAATAATTTAAAGTCTAATCTGCCCACTGATAA---TAATTAAAGGGCTGCAGTA
Meganaclia perpusilla       TTTTTGTAGATGATTTAAAGTCTAATCTGCCCACTGATTT---TTATTAAAGGGCTGCAGTA
Dysauxes punctata           TTTTTGTAAATAATATAAAGTCTAATCTGCCCACTGATTTATAAAATTAAAGGGCTGCAGTA
Hydrusa diaphana            TTTTTGAAAATAATATAAAGTCTAATCTGCCCACTGATTT-AATAATTAAAGGGCTGCAGTA
Syntomis albionica          TTTTTGAAAATAATATAAAGTCTAATCTGCCCACTGATTT--TTAATTAAAGGGCTGCAGTA
Syntomis kruegeri           TTTTTGAAAATAATATAAAGTCTAATCTGCCCACTGATTTTTTTAATTAAAGGGCTGCAGTA
Syntomis ragazzi            TTTTTGAAAATAATATAAAGTCTAATCTGCCCACTGATTTTTTTAATTAAAGGGCTGCAGTA
Syntomis phegea ligata1     TTTTTGAAAATAATATAAAGTCTAATCTGCCCACTGATTT--TTAATTAAAGGGCTGCAGTA
Syntomis phegea ligata2     TTTTTGAAAATAATATAAAGTCTAATCTGCCCACTGATTT--TTAATTAAAGGGCTGCAGTA
Syntomis caspia             TTTTTGAAAATAATATAAAGTCTAATCTGCCCACTGATTT-ATTAATTAAAGGGCTGCAGTA
Syntomis aequipunctata      TTTTTGAAAATAATATAAAGTCTAATCTGCCCACTGATTTTTTTAATTAAAGGGCTGCAGTA
Syntomis nigricornis        TTTTTGAAAATAATATAAAGTCTAATCTGCCCACTGATTTTATTAATTGAAGGGCTGCAGTA
Syntomis germana            TTTTTGAAAATAATATAAAGTCTAATCTGCCCACTGATTTTTTTAATTAAAGGGCTGCAGTA
Amata bicincta              TTTTTGAAAATAATATAAAGTCTAATCTGCCCACTGATTT--ATAATTAAAGGGCTGCAGTA
Amata mogadorensis1         TTTTTGAAAATAATATAAAGTCTAATCTGCCCACTGATTT---TAATTAAAGGGCTGCAGTA
Amata mogadorensis2         TTTTTGAAAATAATATAAAGTCTAATCTGCCCACTGATTT---TAATTAAAGGGCTGCAGTA
Amata alicia                TTTTTGAAAATAATATAAAGTCTAATCTGCCCACTGATTT---TAATTAAAGGGCTGCAGTA
Amata huebneri              TTTTTGAAAATAATATAAAGTCTAATCTGCCCACTGATTATTTTAATTAAAGGGCTGCAGTA
Amata sperbius              TTTTTGAAAATAATATAAAGTCTAATCTGCCCACTGATTATTATAATTGAAGGGCTGCAGTA

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Syntomis albionica          TTTTGACTGTACAAAGGTAGCATAATAAATAGTCATTTAATTGATGACTTGAATGAAAGATT
Syntomis kruegeri           TTTTGACTGTACAAAGGTAGCATAATAAATAGTCATTTAATTGATGACTTGAATGAAAGATT
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Amata mogadorensis2         TTTTGACTGTACAAAGGTAGCATAATCAATAGTCATTTAATTGGTGACTTGAATGAAAGATT
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Syntomis ragazzi           AAGTTTAAATAAATTAAAAAGACGAGAAGACCCTATAGAGTTTTATAAATAATTAAATTTAA
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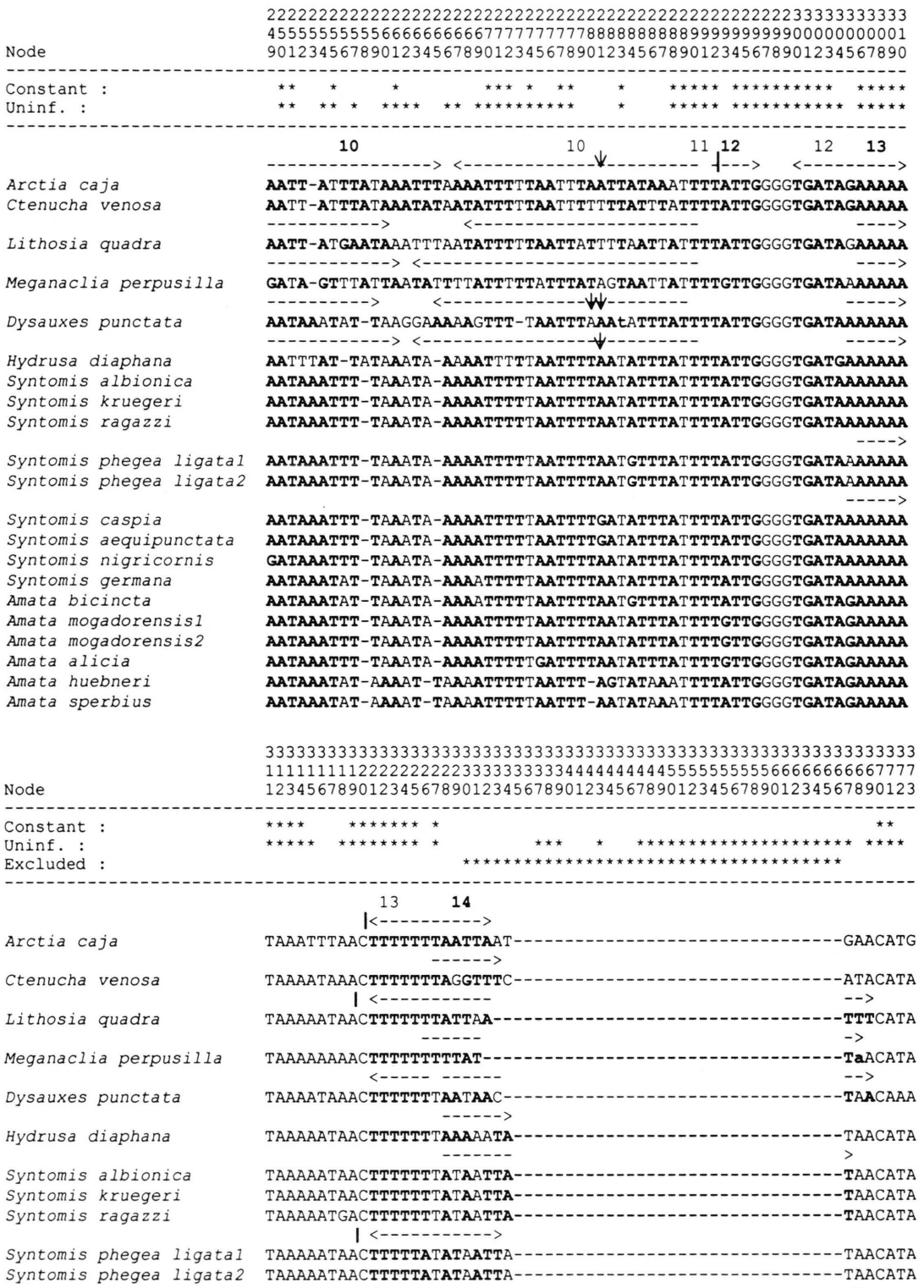
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Syntomis ragazzi
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Syntomis aequipunctata
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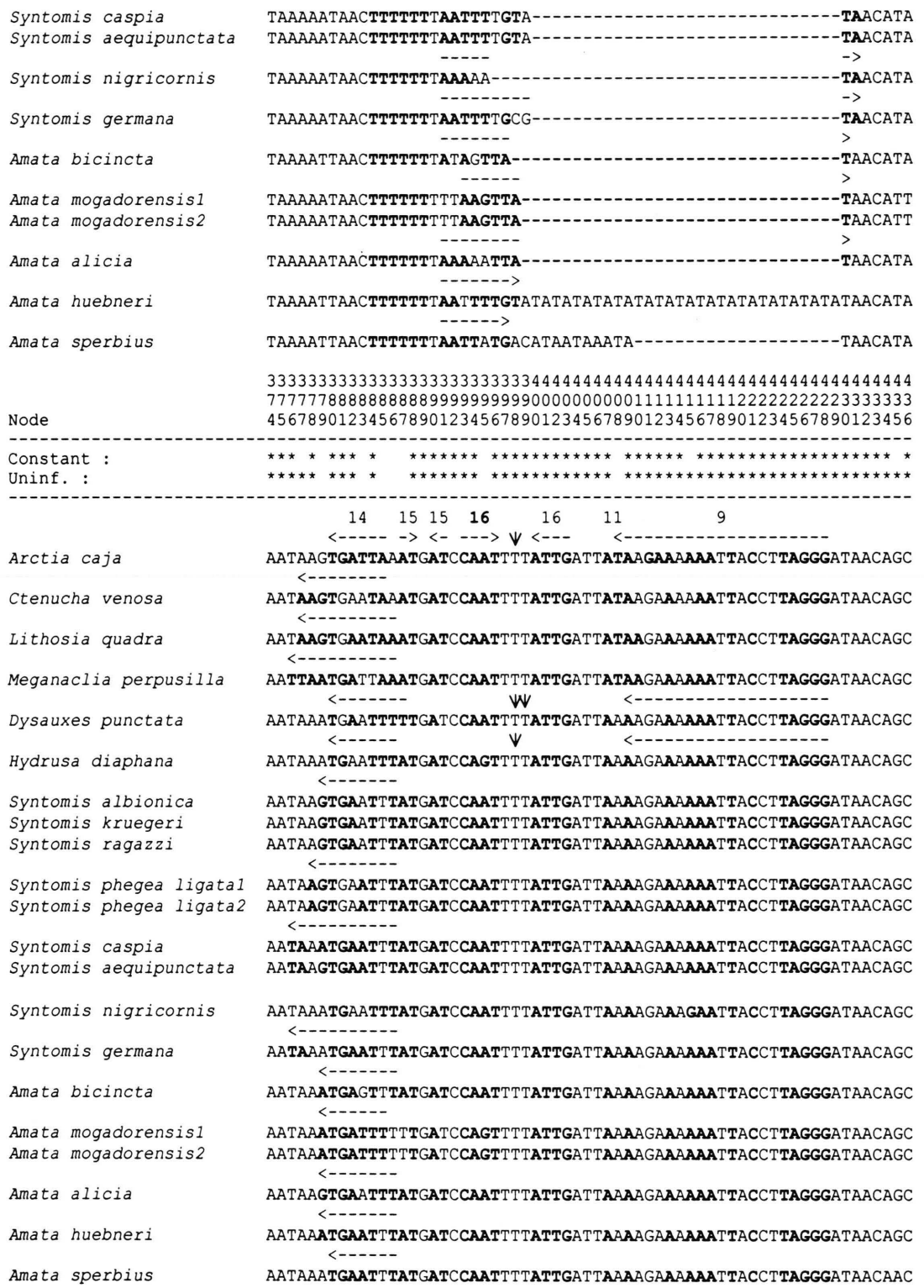
Amata sperbius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Syntomis ragazzi
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Amata alicia
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Amata sperbius

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Syntomis germana          GTAATTTATTTTTTTTAGTTCTTATAAAAAAAATGAGTTTGCGACCTCGATGTTGGATTAAGATA
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Amata huebneri            GTAATTTATTTTTTTTAGTTCATATAAAAAAAATAAGTTTGCGACCTCGATGTTGGATTAAGATA
Amata sperbius            GTAATTTATTTTTTTTAGTTCATATAAAAAAAATAAGTTTGCGACCTCGATGTTGGATTAAGATA

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Lithosia quadra           AAAATTAAATGTAGAAGTTTAAA-TTTTT
Meganaclia perpusilla     AAAATTAAATGCAAAAGTTTAAA-TTTTT
Dysauxes punctata         AAAATTAAATGCAAAAGTTTAAAGT-TTT
Hydrusa diaphana          AAAATTAAATGCAAAAGTTTAAA-TTTTT
Syntomis albionica        AAAATTAAATGCAAAAGTTTAAA-TTTTT
Syntomis kruegeri         AAAATTAAATGCAAAAGTTTAAA-TTTTT
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Syntomis aequipunctata    AAAATTAAATGCAAAAGTTTAAA-TTTTT
Syntomis nigricornis      AAAATTAAATGCAAAAGTTTAAA-TTTTT
Syntomis germana          AAAATTAAATGCAAAAGTTTAAA-TTTTT
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Amata alicia              AAAATTAAATGCAAAAGTTTAAA-TTTTT
Amata huebneri            AAAATTAAGTGCAAAAGTTTAAA-TTTTT
Amata sperbius            AAAATTAAATGCAAAAGTTTAAA-TTTTT
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works already determined in former studies (see Aubert *et al.*, 1999 and related references), we tried to select those secondary structures which allowed the largest number of Watson-Crick and non Watson-Crick interactions. The complete secondary structure was determined for all samples; an illustration is shown in Fig. 8A. The most informative part of the rRNA structure lies in stem "9" (see Table II). This portion is shown for the following species: *C. venosa*; *L. quadra*;

Table III. Pairwise distances (numbers of observed substitutions) based on nucleotide sequences of the partial 16S rRNA gene.
Below diagonal absolute distances, above diagonal % divergence (1=100%). Bold printed values are based a data set whose alignment is unambiguous (see data matrix in Table III for the list of excluded characters). Lower values represent distances which include all characters.

	Taxon	1	2	3	4	5	6	7
1	*A.caja*	–	**0.062**	**0.110**	**0.107**	**0.089**	**0.093**	**0.087**
			0.074	0.116	0.110	0.097	0.102	0.095
2	*C.venosa*	**30**	–	**0.099**	**0.099**	**0.091**	**0.087**	**0.081**
		39	–	0.110	0.114	0.104	0.097	0.093
3	*M.perpusilla*	**53**	**48**	–	**0.122**	**0.105**	**0.097**	**0.095**
		61	58	–	0.121	0.106	0.097	0.097
4	*D.punctata*	**52**	**48**	**59**	–	**0.076**	**0.072**	**0.068**
		58	60	64	–	0.078	0.076	0.070
5	*H.diaphana*	**43**	**44**	**51**	**37**	–	**0.043**	**0.041**
		51	55	56	41	–	0.044	0.042
6	*S.albionica*	**45**	**42**	**47**	**35**	**21**	–	**0.006**
		54	51	51	40	23	–	0.008
7	*S.kruegeri*	**42**	**39**	**46**	**33**	**20**	**3**	–
		50	49	51	37	22	4	–
8	*S.ragazzi*	**44**	**41**	**50**	**33**	**23**	**10**	**7**
		52	51	55	37	25	11	7
9	*S.phegea lig1*	**46**	**44**	**50**	**39**	**23**	**10**	**11**
		55	53	54	44	25	10	12
10	*S.phegea lig2*	**46**	**44**	**50**	**39**	**23**	**10**	**11**
		55	53	54	44	25	10	12
11	*S.caspia*	**43**	**39**	**46**	**33**	**17**	**10**	**9**
		52	50	53	40	23	16	15
12	*S.aequipunctata*	**42**	**38**	**49**	**33**	**20**	**11**	**8**
		50	49	56	39	27	17	13
13	*S.nigricornis*	**42**	**43**	**48**	**33**	**21**	**13**	**10**
		49	55	54	39	26	20	17
14	*S.germana*	**42**	**42**	**48**	**33**	**20**	**14**	**11**
		50	53	55	39	27	20	16
15	*A.bicincta*	**44**	**43**	**53**	**33**	**22**	**18**	**17**
		53	52	57	39	25	19	19
16	*A.mogadorensis1*	**48**	**49**	**44**	**39**	**23**	**20**	**19**
		57	60	49	45	27	23	23
17	*A.mogadorensis2*	**48**	**49**	**44**	**39**	**23**	**20**	**19**
		57	60	49	45	27	23	23
18	*A.alicia*	**42**	**39**	**45**	**36**	**20**	**12**	**11**
		50	50	50	41	23	15	15
19	*A.huebneri*	**37**	**41**	**55**	**33**	**32**	**26**	**23**
		75	82	92	69	69	62	58
20	*A.sperbius*	**33**	**39**	**55**	**31**	**31**	**29**	**26**
		50	61	72	46	47	46	42
21	*L.quadra*	**47**	**37**	**51**	**60**	**51**	**49**	**48**
		54	48	54	62	55	53	53

	Taxon	8	9	10	11	12	13	14
1	*A.caja*	**0.091**	**0.095**	**0.095**	**0.089**	**0.087**	**0.087**	**0.087**
		0.098	0.104	0.104	0.098	0.095	0.093	0.095
2	*C.venosa*	**0.085**	**0.091**	**0.091**	**0.081**	**0.079**	**0.089**	**0.087**
		0.097	0.100	0.100	0.095	0.093	0.104	0.100
3	*M.perpusilla*	**0.103**	**0.103**	**0.103**	**0.095**	**0.101**	**0.099**	**0.099**
		0.104	0.102	0.102	0.100	0.106	0.102	0.104
4	*D.punctata*	**0.068**	**0.081**	**0.081**	**0.068**	**0.068**	**0.068**	**0.068**
		0.070	0.083	0.083	0.076	0.074	0.074	0.074
5	*H.diaphana*	**0.048**	**0.048**	**0.048**	**0.035**	**0.041**	**0.043**	**0.041**
		0.047	0.047	0.047	0.044	0.051	0.049	0.051
6	*S.albionica*	**0.021**	**0.021**	**0.021**	**0.021**	**0.023**	**0.027**	**0.029**
		0.021	0.019	0.019	0.030	0.032	0.038	0.038
7	*S.kruegeri*	**0.014**	**0.023**	**0.023**	**0.019**	**0.017**	**0.021**	**0.023**
		0.013	0.023	0.023	0.028	0.025	0.032	0.030
8	*S.ragazzi*	–	**0.025**	**0.025**	**0.021**	**0.014**	**0.023**	**0.025**
		–	0.025	0.025	0.030	0.023	0.034	0.032
9	*S.phegea lig1*	**12**	–	**0.000**	**0.021**	**0.023**	**0.031**	**0.033**
		13	–	0.000	0.030	0.032	0.042	0.042
10	*S.phegea lig2*	**12**	**0**	–	**0.021**	**0.023**	**0.031**	**0.033**
		13	0	–	0.030	0.032	0.042	0.042

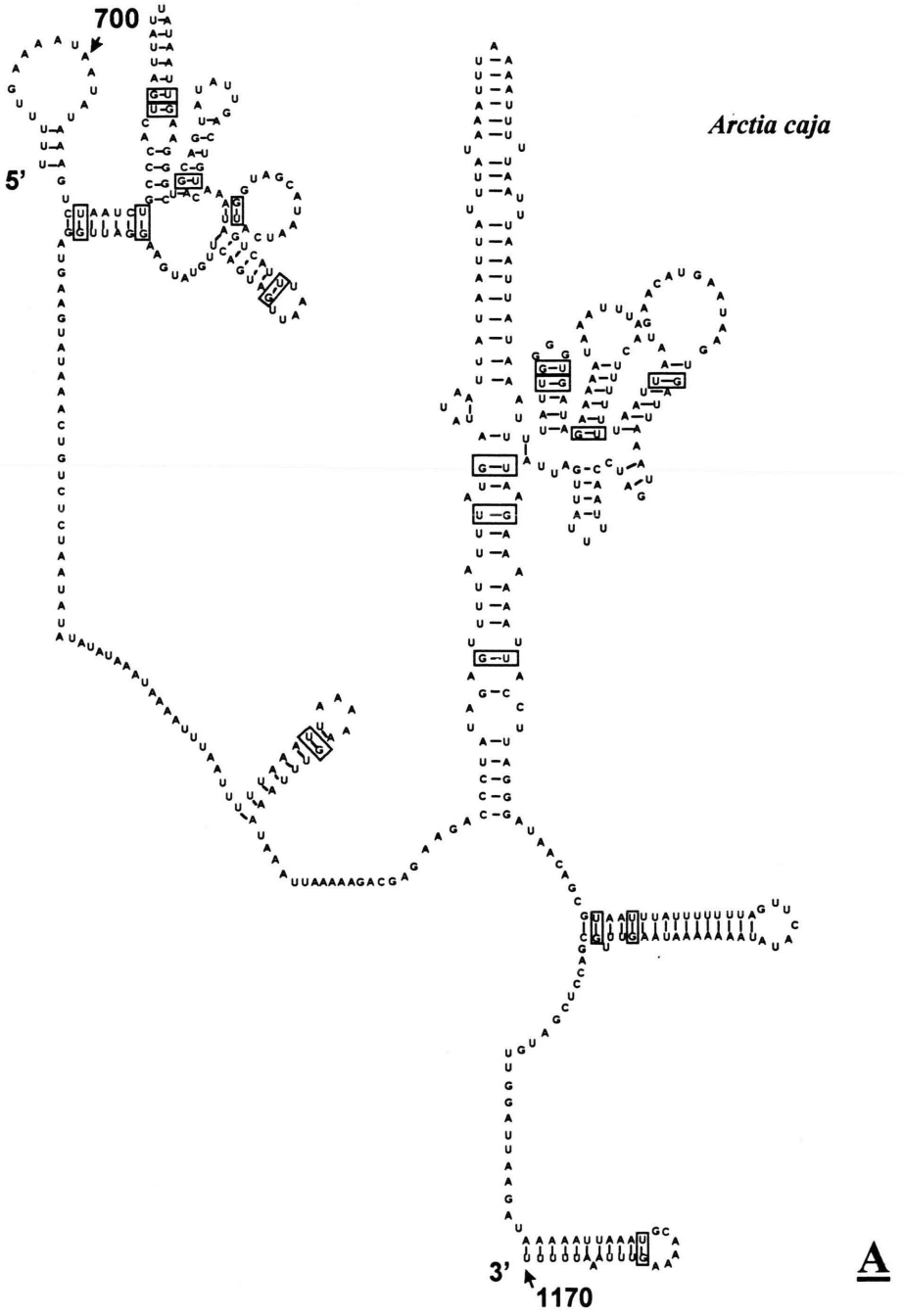
Table III. (continued).

	Taxon	8	9	10	11	12	13	14
11	*S.caspia*	**10**	**10**	**10**	**–**	**0.006**	**0.023**	**0.021**
		16	16	16	–	0.008	0.040	0.025
12	*S.aequipunctata*	**7**	**11**	**11**	**3**	**–**	**0.025**	**0.023**
		12	17	17	4	–	0.044	0.025
13	*S.nigricornis*	**11**	**15**	**15**	**11**	**12**	**–**	**0.023**
		18	22	22	21	23	–	0.042
14	*S.germana*	**12**	**16**	**16**	**10**	**11**	**11**	**–**
		17	22	22	13	13	22	–
15	*A.bicincta*	**20**	**18**	**18**	**16**	**19**	**21**	**16**
		22	19	19	22	25	29	22
16	*A.mogadorensis1*	**24**	**22**	**22**	**20**	**23**	**24**	**24**
		28	25	25	27	30	32	31
17	*A.mogadorensis2*	**24**	**22**	**22**	**20**	**23**	**24**	**24**
		28	25	25	27	30	32	31
18	*A.alicia*	**16**	**16**	**16**	**12**	**13**	**16**	**16**
		20	19	19	19	20	23	23
19	*A.huebneri*	**25**	**30**	**30**	**23**	**24**	**23**	**21**
		60	66	66	54	54	64	53
20	*A.sperbius*	**28**	**31**	**31**	**24**	**25**	**24**	**22**
		44	48	48	38	38	44	35
21	*L.quadra*	**49**	**51**	**51**	**49**	**50**	**52**	**53**
		54	55	55	56	57	57	60

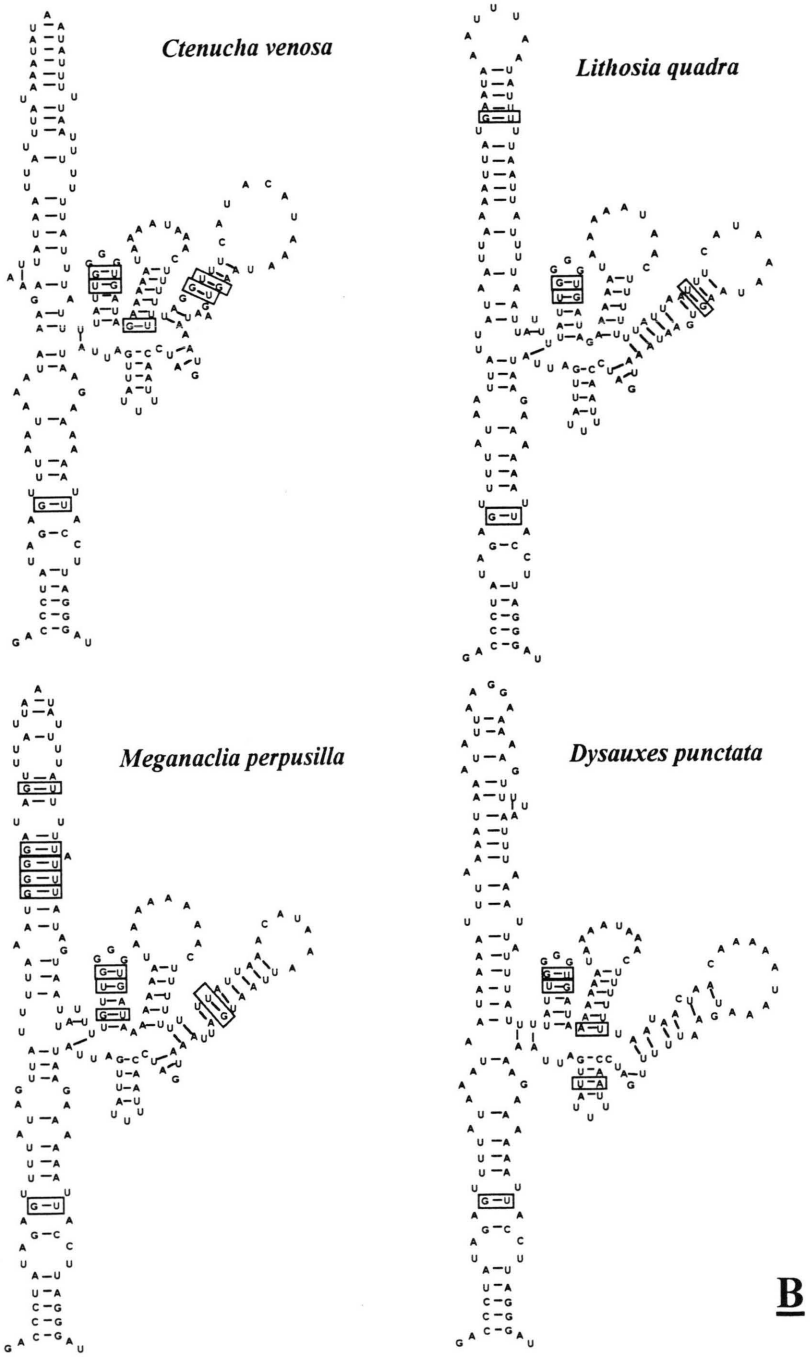
	Taxon	15	16	17	18	19	20	21
1	*A.caja*	**0.091**	**0.099**	**0.099**	**0.087**	**0.076**	**0.068**	**0.097**
		0.100	0.108	0.108	0.095	0.142	0.095	0.102
2	*C.venosa*	**0.089**	**0.101**	**0.101**	**0.081**	**0.085**	**0.081**	**0.076**
		0.098	0.114	0.114	0.095	0.155	0.116	0.091
3	*M.perpusilla*	**0.110**	**0.091**	**0.091**	**0.093**	**0.114**	**0.114**	**0.105**
		0.108	0.093	0.093	0.095	0.174	0.136	0.102
4	*D.punctata*	**0.068**	**0.081**	**0.081**	**0.074**	**0.068**	**0.064**	**0.124**
		0.074	0.085	0.085	0.078	0.131	0.087	0.117
5	*H.diaphana*	**0.045**	**0.048**	**0.048**	**0.041**	**0.066**	**0.064**	**0.105**
		0.047	0.051	0.051	0.044	0.131	0.089	0.104
6	*S.albionica*	**0.037**	**0.041**	**0.041**	**0.025**	**0.054**	**0.060**	**0.101**
		0.036	0.044	0.044	0.028	0.117	0.087	0.100
7	*S.kruegeri*	**0.035**	**0.039**	**0.039**	**0.023**	**0.048**	**0.054**	**0.099**
		0.036	0.044	0.044	0.028	0.110	0.080	0.100
8	*S.ragazzi*	**0.041**	**0.050**	**0.050**	**0.033**	**0.052**	**0.058**	**0.101**
		0.042	0.053	0.053	0.038	0.114	0.083	0.102
9	*S.phegea lig1*	**0.037**	**0.045**	**0.045**	**0.033**	**0.062**	**0.064**	**0.105**
		0.036	0.047	0.047	0.036	0.125	0.091	0.104
10	*S.phegea lig2*	**0.037**	**0.045**	**0.045**	**0.033**	**0.062**	**0.064**	**0.105**
		0.036	0.047	0.047	0.036	0.125	0.091	0.104
11	*S.caspia*	**0.033**	**0.041**	**0.041**	**0.025**	**0.048**	**0.050**	**0.101**
		0.042	0.051	0.051	0.036	0.102	0.072	0.106
12	*S.aequipunctata*	**0.039**	**0.048**	**0.048**	**0.027**	**0.050**	**0.052**	**0.103**
		0.047	0.057	0.057	0.038	0.102	0.072	0.108
13	*S.nigricornis*	**0.043**	**0.050**	**0.050**	**0.033**	**0.048**	**0.050**	**0.107**
		0.055	0.061	0.061	0.044	0.121	0.083	0.108
14	*S.germana*	**0.033**	**0.050**	**0.050**	**0.033**	**0.043**	**0.045**	**0.110**
		0.042	0.059	0.059	0.044	0.100	0.066	0.114
15	*A.bicincta*	**–**	**0.045**	**0.045**	**0.039**	**0.050**	**0.048**	**0.107**
		–	0.049	0.049	0.044	0.114	0.076	0.108
16	*A.mogadorensis1*	**22**	**–**	**0.000**	**0.031**	**0.066**	**0.066**	**0.112**
		26	–	0.000	0.030	0.131	0.091	0.112
17	*A.mogadorensis2*	**22**	**0**	**–**	**0.031**	**0.066**	**0.066**	**0.112**
		26	0	–	0.030	0.131	0.091	0.112
18	*A.alicia*	**19**	**15**	**15**	**–**	**0.054**	**0.054**	**0.099**
		23	16	16	–	0.119	0.078	0.098
19	*A.huebneri*	**24**	**32**	**32**	**26**	**–**	**0.017**	**0.105**
		60	69	69	63	–	0.066	0.167
20	*A.sperbius*	**23**	**32**	**32**	**26**	**8**	**–**	**0.112**
		40	48	48	41	35	–	0.133
21	*L.quadra*	**52**	**54**	**54**	**48**	**51**	**54**	**–**
		57	59	59	52	88	70	–

M. perpusilla; *D. punctata*; *A. huebneri*; *H. diaphana*; *A. mogadorensis* and *S. phegea*. We intend to use these structures as additional characters to discriminate genetic relationships (a complete detailed study of these secondary structures on a much wider data set will be published elsewhere ; Legal and Wink, in prep.).

Among the clearest differences found using secondary structures are the following (Table II and Fig. 8):



1. Length of stem 4 separated three groups of species: *A. caja* + *C. venosa* / *L. quadra* + *M. perpusilla* / and *A. huebneri* + *A. sperbius*
2. Length of stem 6 clearly separated Syntomini from all our outgroups.
3. Symmetry (centered around adenine 266) of stem 10 clearly separated *A. caja* + *C. venosa* from the other species, also *M. perpusilla* which clusters as a sister group to Syntomini (Fig. 7) has the same modification (symmetry



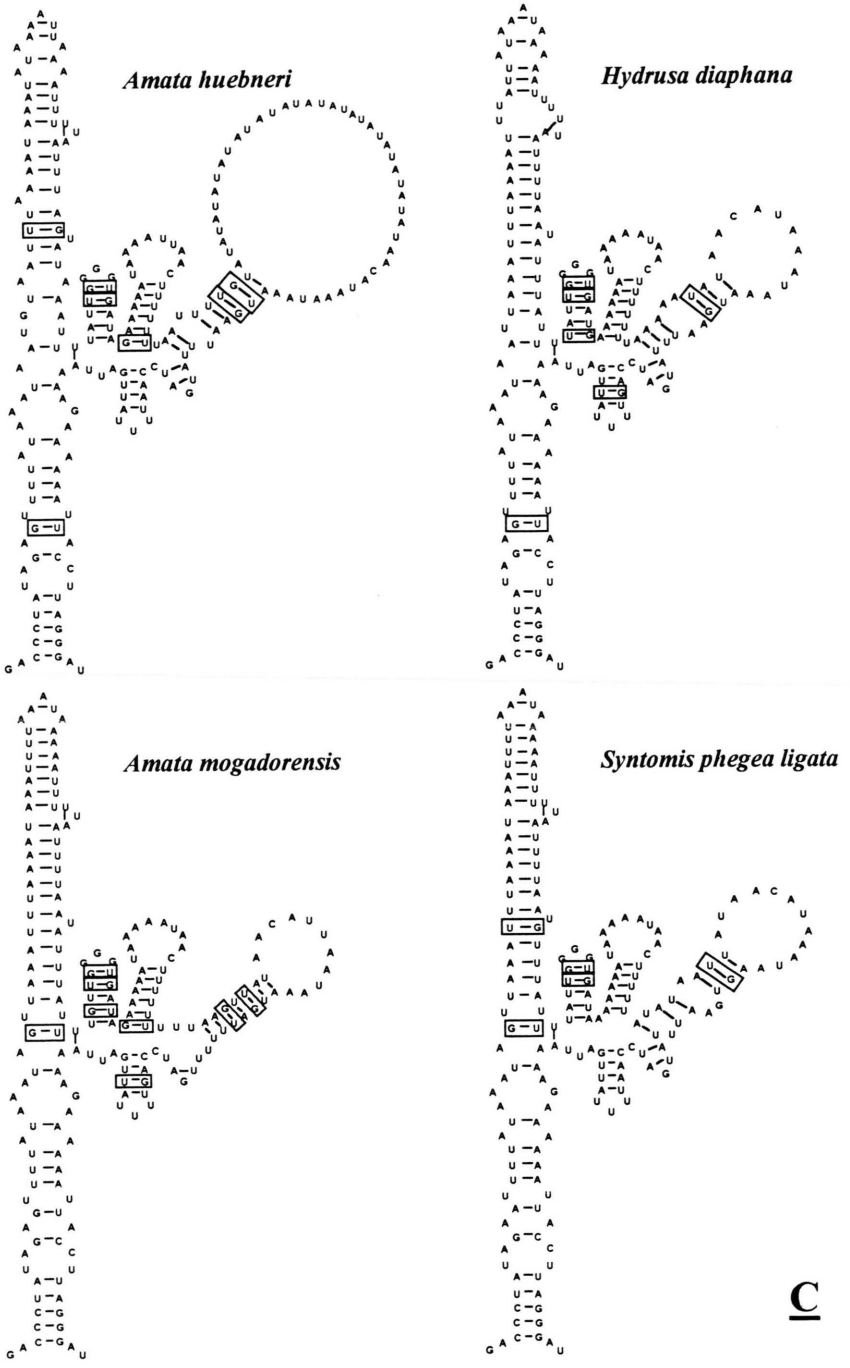


Fig. 8. Putative secondary structure of the mitochondrial 16S ribosomal RNA gene (partial, position 700 and 1170 indicated correspondance with *Drosophila melanogaster* sequence, Maidak *et al.*, 1996) of **A**: *Arctia caja.* Links represent the possible interactions between bases. Squared: non Watson-Crick G-U pairs which might be involved in the stabilisation of secondary structure (for a review see Michel *et al.* 1998). **B** and **C** : partial secondary structure of the sequenced part of 16S RNA gene (from position 215 to 430 according Table II). **B** : *C. venosa*, *L. quadra*, *M. perpusilla* and *D. punctata*; **C** : *A. huebneri*, *H. diaphana*, *A. mogadorensis* and *S. phegea*.

centered around the adenine 262) as all Syntomini.

4. Length and composition of stem 14 (the most variable part of the 16S rRNA gene analyzed) is informative in the genus *Syntomis* as it is linking *S. kruegeri*/*S. albionica*/ *S. ragazzi* with *S. caspia*/*S. aequipunctata.*
5. Internal structure of stem 10 (interaction between adenine 289 and uracil 291) is identical for *L. quadra* and *M. perpusilla*, which cluster as sister taxa in Fig. 7 A and B.

In conclusion, the morphological character "hairbrushes present/absent" is congruent with the molecular phylogeny based on 16S rDNA sequences. Thus, the acquisition of hairbrushes constitute an informative, derived synapomorphic character among *Syntomini* which helps to define the genera *Syntomis* and *Amata*. Therefore, we propose to reserve the name *Syntomis* for the monophyletic "*phegea* group" which has hairbrushes and to leave the name *Amata* to the rest without brushes, as listed in Table I.

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Aubert J., Legal L., Descimon H. and Michel M. (1999), Molecular phylogeny of swallowtail butterflies of the tribe Papilionini (Papilionidae, Lepidoptera). Mol. Phylogen. Evol. **12**, 156–167.

Baker T. C. and Cardé R. T. (1979), Courtship behaviour of the oriental fruit moth (*Grapholitha molesta*): experimental analysis and consideration of the role of sexual selection in the evolution of courtship pheromones in the *Lepidoptera*. Ann. Entomol. Soc. Am. **72**, 173–188.

Barth R. (1966), Orgaos odoriferos dos lepidopteros. Boletim N°7, 159 pp. Ministerio da Agricultura, Servicio Florestal, Parque Nacional do Itataia. Rio de Janeiro, Brasilia.

Bendib A. and Minet J. (1998), Female pheromone glands in Arctiidae (Lepidoptera). Evolution and phylogenetic significance. C. R. Acad. Sci. Paris, Sciences de la Vie, **321**, 1007–1014.

Birch M. C. and Hefetz A. (1987), Extrusible organs in male moths and their role in courtship behaviour. Bull. Ent. Soc. Am. **33**, 222–229.

Birch M. C., Poppy G. M. and Baker T. C. (1990), Scents and eversible scent structures of male moths. Annu. Rev. Ent. **35**, 25–58.

Boppré M. (1984), Chemically mediated interactions between butterflies. In: The Biology of Butterflies (Vane-Wright R. I. and Ackery P. R., ed.). London, Academic Press, pp. 259–277.

Boppré M. and Vane-Wright R. I. (1989), Androconial systems in Danainae (*Lepidoptera*): functional morphology of *Amauris*, *Danaus*, *Tirumala* and *Euploea*. Zool. J. Linn. Soc. **97**,101–133.

Brower A. V. Z., Desalle R. and Vogler A. (1996), Gene trees, species trees and systematics: a cladistic perspective. Annu. Rev. Ecol. Syst. **27**, 423–450.

Clearwater J. R. (1975a), Structure, development and evolution of the male pheromone system in some Noctuidae (Lepidoptera). J. Morphol. **146**,129–176.

Clearwater J. R. (1975b), Pheromone metabolism in male *Pseudaletia separata* (Walk.) and *Mamestra configurata* (Walk.) (Lepidoptera: Noctuidae). Comp. Biochem. Physiol. **50B**,77–82.

Covell C. V. (1984), A Field Guide to the Moths of Eastern North America. Houghton Mifflin Comp. Boston.

Descoins C. B., Lalanne-Cassou B., Ferot B., Malosse C. and Renou M. (1989) Etude comparative des sécrétions pheromonales emises par les femelles d'Arctiides et de Ctenuchides (insectes, Lepidoptera) de la zone néotropicale. C. R. Acad. SDci. Paris, **309**, Série III, 577–588.

Elliot J. N. (1973), The higher classification of the Lycaenidae (Lepidoptera): a tentative arrangement. Bull. Brit. Mus. Natl. Hist. Entomology **28**, 371–505.

Felsenstein J. (1985), Confidence limits on phylogenies: an approach using the bootstrap. Evolution **39**,783–791.

Felsenstein J. (1988), Phylogenies from molecular sequences: inference and reliability. Annu. Rev. Genet. **22**, 521–565.

Holloway J. D. (1988), The Moths of Borneo (Part 6): Family Arctiidae, Subfamilies Syntominae, Euchromiinae, Artiinae: Noctuidae misplaced in Arctiidae (Camptoloma; Aganainae). – Southdene Sdn. Bhd., Malysia.

Kitching I. J. and Rawlins J. E. (1999), The Noctuidea. In: Lepidoptera, Moths and Butterflies. Vol. **1** (N. P. Kristensen, ed.), Hdb. Zool., Vol. IV, Part 35. Walter de Gruyter, Berlin.

Kocher T. D., Thomas W. K., Meyer A., Edwards S. V., Pääbo S., Villablanca F. X. and Wilson A. C. (1989), Dynamics of mitochondrial evolution in animals: amplification and sequencing with conserved primers. Proc.Natl. Acad. Sci. U. S. A. **86**, 6196–6200.

Leppla N. C., Guy R. H., Heath R. R. and Dueben B. (1987), Laboratory studies of the courtship of the velvetbean caterpillar moth, *Anticarsia gemmatalis* (Hübner) (Lepidoptera: Noctuidae). Ann. Entom. Soc. Am. **80**, 27.

Leraut P. (1992), Les papillons dans leur milieu. Bordas, Paris.

Maidak B. L., Olsen G. J., Overbeek R., Mc Caughey M. J. and Woese C. R. (1996), The Ribosomal database project (RDP). Nucl. Acids Res. **24,** 82–85.

Michel F. and Costa M. (1998), Inferring RNA structure by phylogenetic and genetic analyses. In: RNA structure and function (R. W. Simons and M. Grunbberg-Manago, eds). Cold Spring Harbour Laboratory Press, N. Y., pp. 175–202.

Minet J. (1986), Ebauche d'une classification moderne de l'ordre des Lépidoptères. Alexanor. **14**, 291–313.

Möller A. (ed.), (1915–1923), Fritz Müller-Werke, Briefe und Leben. Bd. 1–3, G. Fischer, Jena, Germany.

Müller F. (1877), Os orgaos odoriferos nas pernas de certos Lepidopteros (incl. supplemento). (German translation in A. Möller pg. 1440ff.). Arch. do Museo Nacional, Rio de Janeiro **2**, 37–46.

Nei M. (1996), Phylogenetic analysis in molecular evolutionary genetics. Annu. Rev. Genet. **30**, 371–403.

Obraztsov N. S. (1966), Die palaearktischen *Amata* Arten. Veröff. Zool. Staatssammlung München **10**, 1–383.

Pinhey E. C. G. (1975), Moths of Southern Africa. Tafelberg Publ., Cape Town.

Pliske T. E. and Eisner T. (1969), Sex pheromone of the queen butterfly. Science **164**,1170–1172.

Rougeot P. C. and Viette P. (1978), Guide des papillons nocturnes d'Europe et d'Afrique du Nord. Delachaux & Niestlé, Neuchâtel.

Sanderson M. J. (1995), Objections to bootstrapping phylogenies: a critique. Syst. Biol. **44**, 299–320.

Schneider D., Schäfer W. and Wunderer H. (1992), Scent organ in male moth: morphology, alpha-keto-butyric acid content and evoked antennal responses. Zool. J. Physiol. **96**, 365–377.

Schneider D. and Seibt U. (1969), Sex pheromone of the queen butterfly: Electroantennogram responses. Science **164**,1173–1174.

Scudder S. H. (1887), Antigeny, or sexual diaphism in butterflies. Proc. Am. Acad. Arts Sci. **12**,150–158.

Simon C., Frati F., Beckenbach A., Crespi B., Liu H. and Flook, P. (1994), Evolution, weighting and phylogenetic utility of mitochondrial gene sequences and a compilation of conserved polymerase chain reaction primers. Ann. Entom. Soc. America. **87**, 651–701.

Speidel W., Fänger H. and Naumann C. M. (1996), The phylogeny of the Noctuidae (Lepidoptera). Systematic Entomology **21**, 219–251.

Stewart C. B. (1993), Powers and pitfalls of parsimony. Nature **361**, 303–307

Szöcs G., Toth M., Bestmann H. J., Vostroiwsky O., Heath R. R. and Tumlinson J. H. (1987), Polyenic hydrocarbons as sex attractants for geometrids and amatids (Lepidoptera) found by field screening in Hungary. Z. Naturforsch. **42c,** 165–168.

Watson J. (1865), On the microscopical examination of plumules. Entomologist's monthl. Mag. **2**,1–2.

Weatherston J. and Percy J. E. (1977), Pheromones of male Lepidoptera. In: Advances in Invertebrate Reproduction (K. G. Adiyo and R. G. Adiyodi, ed.). Vol **1**, Peralam-Kenoth, India. pp. 295–307.

Wink M. and von Nickisch-Rosenegk E. (1997), Sequence data of mitochondrial 16S rDNA of Arctiidae and Nymphalidae: Evidence for a convergent evolution of pyrrolizidine alkaloid and cardiac glycoside sequestration. J. Chem. Ecol. **23**,1549–1568.

Wink M. and Schneider D. (1990), Fate of plant derived secondary metabolites in three moth species (*Syntomis mogadorensis*, *Syntomeida epilais* and *Creatonotos transiens*). J. Comp. Physiol. B **160**, 389–400.

Wink M., von Nickisch-Rosenegk E. and Legal L. (1998), Response. J. Chem. Ecol. **24**, 1285–1291, 1998.

Wunderer H., Hansen K., Bell. T. W., Schneider D. and Meinwald J. (1986), Sex pheromones of two Asian moths (*Creatonotos transiens*, *C. gangis*; Lepidoptera-Arctiidae): behaviour, morphology, chemistry and electrophysiology. Exp. Biol. **46**, 11–27.