Ann. Zool. Fennici 42: 141–160 ISSN 0003-455X
Helsinki 26 April 2005

Phylogeny and evolution of parthenogenesis in Finnish bagworm moth species (Lepidoptera: Psychidae: Naryciinae) based on mtDNA-markers

Alessandro Grapputo*, Tomi Kumpulainen & Johanna Mappes

*Department of Biological and Environmental Science, P.O. Box 35, FI-40014 University of Jyväskylä, Finland (*e-mail: grapputo@cc.jyu.fi)*

Received 25 Oct. 2004, revised version received 8 Dec. 2004, accepted 6 Dec. 2004

Grapputo, A., Kumpulainen, T. & Mappes, J. 2005: Phylogeny and the evolution of parthenogenesis in Finnish bagworm moth species (Lepidoptera: Psychidae: Naryciinae) based on mtDNA-markers. — *Ann. Zool. Fennici* 42: 141–160.

We investigated species diversity and evolution of parthenogenesis among bagworm moth species of *Dahlica* and *Siederia* using mitochondrial DNA sequencing. Parthenogenesis is rare among Lepidoptera other than Psychidae. Genera *Dahlica* and *Siederia* form a confusing group with controversial species boundaries and widely overlapping morphological features that make species determination difficult. We evaluated the reliability of species determination based on wing scale morphology by comparing it with a phylogenetic tree obtained using mtDNA. Species determination based on morphological characteristics did not correspond to species determination based on mtDNA markers. On the basis of the molecular phylogeny, the status of these two genera is questionable. Our results indicate that parthenogenetic *D. fennicella*, *D. triquetrella* and *D. lichenella* evolved independently from different sexual ancestors suggesting that asexual reproduction is favoured in this group.

Introduction

Sexual reproduction is the dominant strategy in higher animals (and plants) making the paradox of sex one of the most fundamental questions in evolutionary research. One hypothesis is that females of sexually selected lineages are in a male-dependent trap and that the trap alone should often be enough to maintain sex since females need males for normal reproduction (West-Eberhard 2003). Depending on the species, females may rely upon males to stimulate ovulation, to initiate embryonic development, or to provide essential genetic transcripts, organelles, or essential genes. Among Lepidoptera, asexual reproduction is extremely rare and, in most known examples, parthenogenesis is a secondary reproductive strategy (Suomalainen 1962, Bell 1982). Among Naryciinae (Lepidoptera: Psychidae) however, several parthenogenetic species are known. These species can be classified as strictly asexual lineages since no males are known among them. Interestingly, both sexual and asexual species often co-exist and there are no reliable morphological or ecological cues to separate parthenogenetically and sexually reproducing females (Kumpulainen *et al.* 2004, Kumpulainen 2004). Thus, psychid moths

offer an attractive opportunity to investigate the evolution of parthenogenesis. If parthenogenesis has evolved several times in this group, we can assume that some ecological, physiological or environmental factors may favor parthenogenesis in this group.

Species determination, status and phylogenetic relationships of many species in this subfamily are, however, still controversial (Suomalainen 1970, Lokki *et al*. 1975, Suomalainen 1980, Hermann 1994, Hättenschwiler 1997, Kullberg *et al*. 2002). The group is characterised by high endemism in mountain ranges in southern Europe and central Asia. There is a great number of morphologically similar species with several problematic species pairs and still unresolved relationships between parthenogenetic and sexual forms, which have caused confusion in the nomenclature and ecological studies involving well known taxa as well.

In Europe, the tribus Dahlicini (Naryciinae) consist of 49 species in six genera (Sauter & Hättenschwiler 1991). To date, 22 species of bagworm moths (Dahlicini) have been recorded in Finland. Of these, seven species are included in two genera — *Dahlica* Enderlein and *Siederia* Meier — which formerly were grouped in the genus *Solenobia* Duponchel) (Suomalainen 1980). All *Dahlica* and *Siederia* moths are relatively small-sized (female body length 3–6 mm) and their larval cases are particularly similar in size. Most species have a wide range of similarities in both morphological and ecological features. *Dahlica* and *Siederia* are most abundant near forest edges and other semi-open parts of their habitats, where the microclimate is favourable and larval food plants (several moss genera) are abundant (Kumpulainen 2004). All species have a very short adult phase, only 3–6 days. The longest stage of their life cycle, from one to two years, consists of five phases of larval development (Hättenschwiler 1985, 1997). Larvae of *Dahlica* and *Siederia* moths usually live and feed freely on mosses on the forest floor, on stones or other similar semi-open habitat patches, often close to tree trunks.

Species determination among *Dahlica* and *Siederia* is very difficult due to the lack of distinct morphological or ecological features. Current attempts to classify the species are based on head scale morphology of female pupa and abdominal spines of adult females. Also, certain differences in the structures of the front legs of adult males have been given as grounds for separating the species (Hättenschwiler 1997). However, there is too much variation among the species in the head scale morphology of the pupa for clear species determination and all other feature differences are also insufficient to reliably determine all species (Hättenschwiler 1997). Genital indices have been described for most of the species but these also overlap considerably (Hättenschwiler 1997). So far, only the shape of wing scales from the central area of the tip of male fore wings (Suomalainen 1980) has been shown to contain sufficient variation between adult specimens of most species, even though the use of wing scales for species determination has led to controversial interpretation of species boundaries (Sauter 1956, Suomalainen 1980, Hättenschwiler 1997). Moreover, this feature is inadequate for species determination of different parthenogenetic species.

Current phylogenetic methods allow for testing the validity of morphological and ecological features in constructing phylogenetic trees for a given taxon. The well-known problems of distinguishing between homologous and homoplasious features have made it difficult to interpret the true phylogenetic relations of many systems (Brooks & McLennan 1991, Avise 1994). The use of genetic markers together with ecological and morphological features, however, has revealed new information on the phylogeny of many controversial groups of animals, such as pandas (O'Brien 1987), flightless birds (Haddrath & Baker 2001) and many insect groups (Brower 1997, Kruse & Sperling 2002). Among insects, and particularly among Microlepidoptera, many controversial groups of species still remain (Hättenschwiler 1997, Kruse & Sperling 2002). The phylogeny of such groups as well as the status of many species is subject to change when new studies applying more precise and diverse methods are published.

Due to the apparent lack of reliable morphological features for species determination in the genera *Dahlica* and *Siederia* we conducted a phylogenetic analysis based on partial DNA sequences of mitochondrial Cytochrome Oxidase genes and small ribosomal subunit gene (*12S*) to

examine species diversity of solenobid bagworm moths. We were particularly interested in comparing the classification based on wing scale morphology to the phylogeny based on mtDNA markers. We were also interested in studying the evolution of parthenogenesis in this group. MtDNA analysis provided a test of species determination independent of wing scale morphology, reproductive strategy and ecological data.

Materials and methods

For the phylogenetic study we included all seven species previously described in Finland. To obtain a more accurate phylogenetic analysis of Naryciinae, we also included samples of *Siederia rupicolella*, *Dahlica triquetrella* and *D. lichenella* from the Alps. These species also occur in Finland. Moreover we added several endemic species (*D. sauteri*, *D. wockei*, *D. vaudella* and *D. generosensis*) from the Alps. From Sweden we included samples of *D. triquetrella*, *S. rupicolella* and *S. listerella* and finally samples of *D. triquetrella* and *D. lichenella* from western Canada, which were introduced to Canada in the 1940s (Arnscheid 1985, Hättenschwiler 1985, 1997, Hermann 1994). Sample locations and the number of samples per location are given in Table 1. We collected bagworm moth larvae by placing tape traps on the trunks of trees, mostly silver birch (*Betula pendula* Roth) and Norway spruce (*Picea abies*), and in one population (Rauma) by searching for larvae in the shadow and underside of larger stones. In early spring, larvae of *Siederia* and *Dahlica* species climb up tree trunks and other similar places for pupation, as snow usually still covers the ground at this time of a year. Climbing larvae stick to tape traps and are easy to collect. After collection, we reared the larvae until adulthood in the laboratory. A brief description of the species used in this study is given below.

Study species

Dahlica triquetrella (Hübner, 1813)

D. triquetrella is widely distributed and occurs throughout most temperate areas of Europe, from Spain to Great Britain and Ireland in the west and to Finland in the north. In southern and central Finland, *D. triquetrella* is not rare, but can normally be found only in small numbers (Suomalainen 1980). This species has both sexually and parthenogenetically reproducing forms of which only the parthenogenetic, tetraploid form occurs in Finland (Suomalainen 1980). Lokki *et al.* (1975) found two genotypically different forms (an eastern and a western form) in Finland using electrophoresis. *D. triquetrella* occurs mainly on the edges of warmer forest habitats, possibly preferring sandy and often south-facing habitats, sometimes with a human influence (Arnscheid 1985, Hättenschwiler 1997). *D. triquetrella* is slightly larger than other *Dahlica* or *Siederia* species and as larvae it should be relatively easy to distinguish by its larger size and also by the triangulate larval case (Suomalainen 1980, Hättenschwiler 1997).

Dahlica lichenella (Linnaeus, 1761)

D. lichenella is sometimes considered to contain a sexual form *D. lichenella* f. *fumosella* and a parthenogenetic form *D. lichenella* f. *lichenella* (Sauter 1956, Arnscheid 1985, Hermann 1994, Hättenschwiler 1997). Some authors (Suomalainen 1980, Kullberg *et al.* 2002), however, consider *D. lichenella* to be purely parthenogenetic and sexual *D. lichenella* f. *fumosella* to belong to a separate species *Dahlica fumosella* (Heinemann, 1870) or *Dahlica lazuri* (Clerck, 1759). Both "forms" of this species are widely distributed in Europe, from Spain to Finland. The sexual form is relatively common in the Nordic countries (Suomalainen 1980, Hättenschwiler 1997). *D. lichenella* is often found in rocky habitats with mostly scarce vegetation (Suomalainen 1980). In Finland, *D. lichenella* is a strictly parthenogenetic and relatively rare species occurring only in the coastal areas of southern and south-western parts of the country (Suomalainen 1980). *D. lichenella* is quite difficult to distinguish from all other species of *Dahlica* and *Siederia* (except *D. triquetrella*) (Suomalainen 1980, Hättenschwiler 1997). The larval case is not triangulate (*see D. triquetrella*) and it is often

Table 1. Samples, number of individuals, specimen codes, location, sex, mode of reproduction, and mitochondrial classification of bagworm moths used in this study. Specimen codes were assigned on the basis of the morphological classification. Individuals originally classified as the same species and sharing the same haplotype are indicated by the same code followed by a different letter (e.g. SRp2, SRp2a and b represent three different specimens of the same species sharing the same haplotype SRp2).

Species according to morphology	*N*	Specimen code	Locality	Year of collection	Sex	Reproduction	Species according to mtDNA
S. rupicolella	1	SR1	Martigny, Switzerland	1997	Female	Sexual	*S. rupicolella*
S. rupicolella	3	SR2, SR2a, SR2b	Jyväskylä, Finland	2000/1	Female/Male	Sexual	*S. listerella*
S. rupicolella	2	SR3, SR4	Stockholm, Sweden	2004	Male	Sexual	*S. rupicolella* (1) /*S. listerella* (1)
S. rupicolella asex	4	SRp1, SRp2; SRp2a, SRp2b	Jyväskylä, Finland	2000/1	Female	Parthenogenetic	*D. fennicella*
S. listerella	1	SL3	Jyväskylä, Finland	2000	Female	Sexual	*S. listerella*
S. listerella	2	SL1, SL2	Stockholm, Sweden	2004	Male	Sexual	*S. listerella*
D. vaudella	1	DV	St. Cergue, Switzerland	1994	Female	Sexual	*D. vaudella*
D. sauteri	2	DS1, DS2	Steyr, Austria	2003	Male	Sexual	*D. sauteri*
D. wockei	3	DW1, DW2, DW3	Dürnstain, Austria	2003	Male	Sexual	*D. wockei*
D. triquetrella	3	DTs1, DTs2, DTs3	Linz, Austria	2003	Male	Sexual	*D. triquetrella*
D. triquetrella	1	DTp1	Stanserhorn, Switzerland	1994	Female	Parthenogenetic	*D. triquetrella*
D. triquetrella	3	DTp2, DTp2a, DTp3	Uster, Switzerland	1994–2003	Female	Parthenogenetic	*D. triquetrella*
D. triquetrella	7	DTp4, DTp5, DTp7, DTp7a, DTp7b, DTp8, DTp9	Kroisbach, Austria	2003	Female	Parthenogenetic	*D. triquetrella*
D. triquetrella	1	DTp6	Steyr, Austria	2003	Female	Parthenogenetic	*D. triquetrella*
D. triquetrella	2	DTp6a, DTp10	Jyväskylä, Finland	2001	Female	Parthenogenetic	*D. triquetrella*
D. triquetrella	2	DTp6c, DTp12	Åminsby, Finland	2004	Female	Parthenogenetic	*D. triquetrella*
D. triquetrella	1	DTp6b	Turku, Finland	2004	Female	Parthenogenetic	*D. triquetrella*
D. triquetrella	1	DTp11	Öland, Sweden	2004	Female	Parthenogenetic	*D. triquetrella*
D. lichenella	2	DLi4, DLi4a	Sorgan Banhof, Switzerland	2003	Female	Parthenogenetic	*D. lichenella*
D. lichenella	2	DLi1, DLi2	Agassis BC, Canada	1991	Female	Parthenogenetic	*D. lichenella*
D. lichenella	1	DLi3	Yvonand, Switzerland	1983	Female	Parthenogenetic	*D. lichenella*
D. lichenella	2	DLi4b, DLi5	Rauma, Finland	2002	Female	Parthenogenetic	*D. lichenella*
D. fennicella	4	DF1, DF1a, DF2, DF3	Jyväskylä, Finland	2001	Female	Parthenogenetic	*D. fennicella*
D. fennicella	2	DF4, DF4a	Åminsby, Finland	2004	Female	Parthenogenetic	*D. fennicella*
D. charlottae	4	DC1, DC1a, DC1b, DC2	Jyväskylä, Finland	2000/1	Male	Sexual	*D. charlottae*
D. lazuri	3	DL1, DL2, DL3	Jyväskylä, Finland	2000	Male	Sexual	*D. lazuri* (2) /*D. charlottae* (1)
D. lazuri	2	DL4, DL4a	Stockholm, Sweden	2004	Male	Sexual	*D. charlottae*
D. lichenella f. *fumosella*	2	DFu1, DFu2	Kroisbach, Austria	2003	Male	Sexual	*D. lichenella* f. *fumosella*
D. generosensis	4	DG1, DG2, DG3, DG4	Altanssee, Austria	2003	Male	Sexual	*D. generosensis*

more dark-coloured than that of *D. fennicella* or other *Dahlica* species (Suomalainen 1980).

Dahlica fennicella (Suomalainen, 1980)

This species was previously considered to be a form of *D. lichenella*, but was described as a separate species by Suomalainen (1980). However, it is still possible that tetraploid, parthenogenetic *D. fennicella* could be an asexual form of a known sexual *Dahlica* species (Suomalainen 1980). In Finland, *D. fennicella* is reported to be rare with only a few known populations in southern Finland and in the southern parts of central Finland (Suomalainen 1980). It is not known to occur in other parts of Finland or in other countries. *D. fennicella* is a small species, which is difficult to separate from other *Dahlica* species and, as larvae, also from *Siederia* species. The shape and size of the larval case can be very similar to those of *D. lazuri* and *S. rupicolella* (Sauter 1954) (Suomalainen 1980).

Dahlica lazuri (Clerck, 1759)

The status of this species is confusing (Arnscheid 1985) as *D. lazuri* is possibly a synonym for *D. fumosella* (Heinemann 1870), which also occurs in central Europe where some authors consider it to be a sexual form (f. *fumosella*) of *D. lichenella* (Hermann 1994, Hättenschwiler 1997). However, other authors treat it as an independent sexual species (Suomalainen 1980, Kullberg *et al*. 2002). This species is widespread in central Europe, but seems to be more common in the northern than in the southern Europe (Hermann 1994, Hättenschwiler 1997). In Finland, *D. lazuri* is a very common sexual species occurring throughout the whole country (Suomalainen 1980). This species is morphologically very similar to *D. charlottae* but, according to Hättenschwiler (1997) and Suomalainen (1980), it can be separated by the shape of male wing scales (*see* Fig. 1). The larval case of *D. lazuri* is often very similar to *D. fennicella* and *S. rupicolella*. This species can be found in many different forest habitats, but may prefer sites with more than average air humidity (Hättenschwiler 1997).

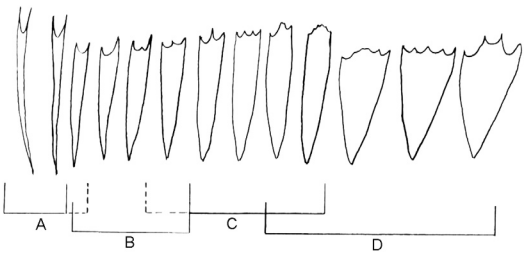

Fig. 1. The male wing scale morphology from the central area of the tip of the front wing (redrawn from Sauter (1956) and Suomalainen (1980)).

Dahlica charlottae (Meier, 1957)

D. charlottae is distributed over the eastern parts of northern and central Europe (Hermann 1994, Hättenschwiler 1997). In Finland, this sexual species is fairly common all over the country, but is usually not very abundant (Suomalainen 1980). According to Suomalainen (1980) and Hättenschwiler (1997), this species can be separated from other *Dahlica* and *Siederia* species by the narrow shape of wing scales and by the number of spines per wing scale (Fig. 1), and sometimes by the relatively triangulate shape of the larval case also. *D. charlottae* occurs in different forest habitats but can most commonly be found in habitats with pine (*Pinus sylvestris*), swamps and sandy ridges (Suomalainen 1980, Hermann 1994).

Siederia listerella (Linnaeus, 1758)

This sexual species has also been known as *S. pineti* (Zeller, 1852) and some authors have also used the name *S. cembrella* (Linnaeus, 1761). *S. listerella* is widely distributed in Europe from England to north-western Russia (Hermann 1994), especially in the northern parts of the continent (Hättenschwiler 1997). In Finland, *S. listerella* is quite rare, occurring mainly in the southern parts of the country (Suomalainen 1980). This species is morphologically very similar to *S. rupicolella* but, according to Hättenschwiler (1997) and Suomalainen (1980), can be distinguished by the wider shape of the male wing scales (Fig. 1). This species occurs in various forest habitats, often with Norway spruce

(*Picea abies*), and it may prefer habitats with a warmer microclimate (Hermann 1994).

Siederia rupicolella (Sauter, 1954)

This species is considered to be sexual (Suomalainen 1980, Hättenschwiler 1997) although many collectors and taxonomists have assumed it also has a parthenogenetic form in northern Europe. *S. rupicolella* is known to occur in mountainous regions (in the Alps in Switzerland, Austria, and Germany) and in Scandinavia (Hermann 1994, Hättenschwiler 1997). It is considered rare in central Europe (Hermann 1994, Hättenschwiler 1997), but in Finland, *S. rupicolella* is reported to be quite common and has spread almost throughout the country (Suomalainen 1980). It occurs in many different forest habitats, often with spruce and is often most abundant at forest edges, although it is not known to prefer any certain forest type (Suomalainen 1980, Hermann 1994), except in northern Finland, where it can be found in pine swamps (J. Itämies pers. obs.). Males of this species have narrower wing scales than *S. listerella* (*see* Fig. 1) (Suomalainen 1980, Hättenschwiler 1997). The larval case is often difficult to distinguish from that of *S. listerella*, *D. lazuri* or *D. fennicella*.

Dahlica generosensis (Sauter, 1954)

This sexual species occurs in Austria, Italy and in southernmost Switzerland (Arnscheid 1985, Hättenschwiler 1997). Male wing scales resemble those of *D. lazuri* (Hättenschwiler 1997) but they can be distinguished because wings are wider and grey with slightly yellowish (Hättenscwhiler 1997) or brownish (Arnscheid 1985) shading. The species is mostly found in open grassy mountain habitats with rocks, stones and stone walls (Hättenschwiler 1997).

Dahlica vaudella (Hättenschwiler, 1990)

D. vaudella is known to occur only on the Swiss part of the Jura mountains. Wing scales are very wide and comparable to those of *Siederia rupicolella* (Hättenschiler 1997) but the front wings are narrow and have a slightly round wing tip. Wings are also dark coloured with irregular cream-coloured spots. The species occurs only on rocks (Hättenschwiler 1997).

Dahlica sauteri (Hättenschwiler, 1977)

D. sauteri is a relatively widely distributed species occurring in northern Switzerland, Germany and most of western and central Europe up to the North Sea (Arnscheid 1985, Hättenschwiler 1997, http://www.lepidoptera.bai.pl/start.php?lang=GB). The form of the male wings is variable but generally narrower at the base than in other species (Hättenschwiler 1997, Arnscheid 1985). Wings are grey with very small white spots. Wing scales are also variable but mostly of medium width with two or more spines (Arncheid 1985, Hättenschwiler 1997). *D. sauteri* inhabits sunny forest edges or sparse forests (Herrmann 1994) and is often found in the same habitats as those of *D. triquetrella* (Hättenschwiler 1997).

Dahlica wockei (Heinemann, 1870)

This species occurs in central Europe and has been recorded in Germany, Poland and possibly Austria (Arnscheid 1985, Herrmann 1994, http://www.lepidoptera.bai.pl/start.php?lang=GB). Male front wings have ground colouration, slightly yellowish and wide light coloured spots (*see* Arnscheid 1985). The species lives in relatively dry deciduous forests and feeds on lichens (Arnscheid 1985). It is the first *Dahlica* species to occur in early spring (Herrmann 1994).

Species identification

We primarily used males for identification of sexual species. However, for *S. rupicolella,* we also used sexual females. We classified males into different species using the wing scales (Fig. 1). For sexual females, we used mate choice and acceptance to reliably determine the species.

S. rupicolella females were paired with males (one at a time) starting with those males whose larval case was morphologically most similar to their own. We observed the reactions of the males towards offered females. When presented to females of their own species, males reacted immediately to the female pheromone and tried to initiate copulation. Reactions towards females of other species vary from avoidance to no reaction at all. We were able to confirm successful fertilisation when fertilised eggs hatched. In our analysis we used only sexual females that had successfully copulated with males that had already been identified according to their wing scale morphology. We determined parthenogenetic females on the basis of their behaviour. After hatching, all females climb on top of their own larval case. Parthenogenetic females soon start to lay eggs inside their larval case whereas sexual females stay on their larval case and begin to release pheromones to attract males. Once parthenogenesis was confirmed, we also used the form of the larval case and the distribution area as identification methods. *D. triquetrella* has a large larval case with an easily recognisable triangular shape. It was more problematic to identify the small sized, non-triangular larval case of parthenogenetic *Dahlica*. We assigned other *Dahlica* parthenogenetic forms to the different species according to the form and material forming the sack and their geographic location. All specimens collected from the south-western coast of Finland were assigned to *D. lichenella*. This species is not known to occur in central Finland. Parthenogenetic specimens collected in central Finland were considered asexual *S. rupicolella* according to collectors' common belief. Moreover, *D. fennicella*, the other possible parthenogenetic species, is considered very rare and to occur only in southern Finland (Suomalainen 1980). Finally, specimens collected in Åminsby, the location where Suomalainen (1980) first described *D. fennicella*, were classified as *D. fennicella*. Endemic species from the Alps (Switzerland and Austria) were obtained and classified by P. Hättenschwiler and E. Hauser. Species from Sweden, Canada and the Alps that are also present in Finland were classified by P. Hättenschwiler, E. Hauser and G. Palmqvist and were confirmed by one of the authors (TK) with the methods described above. The outgroup species, *Psyche norvegica* (Heylaerts, 1882), *Diplodoma laichartingella* (Goeze, 1783) and *Taleporia borealis* (Wocke, 1862), were determined by the shape, material and size of their larval cases. All outgroup species are very easy to determine, particularly when compared with *Dahlica* and *Siederia* species (Hättenschwiler 1997).

We assigned a specimen code to each sample (Table 1) based on the morphological classification. Following the sequence analysis, specimens originally classified as the same species and sharing the same haplotypes were assigned to the same code followed by a different letter (a, b, c etc.).

Laboratory procedures

Samples were frozen at –20 °C until DNA extraction. Additional samples from Switzerland and Canada were preserved in 70% ethanol. We isolated total genomic DNA from the entire individual with a solution of 5% Chelex chelating resin (Pearce *et al*. 1997). A fragment of about 600 bp spanning from the end of the COI to the COII was amplified using the primers S2792 (Brower 1994) and C2-N-3389 (Simon *et al*. 1994). Moreover, a fragment of about 400 bp of the *12S* rRNA gene was amplified using the primers SR-J-14233 and SR-N-14588 (Simon *et al*. 1994). We carried out the amplifications in a total volume of 25 μl, with the use of 10 mM of Tris-HCl, 1.5 mM of $MgCl_2$, 5 pmoles of each primer, 200 μM of each dNTPs, 1 unit of Taq polymerase (Boehringer Mannheim) and 20–50 ng of DNA. The forward and reverse primers labelled with a fluorescent dye (IDR-800 and IDR-700 respectively; Li-Cor Inc.) were used with the Thermo Sequenase DYEnamic Direct cycle sequencing kit (Amersham) as described in the technical bulletin #71 (Li-Cor Inc.). We then loaded the sequencing reactions in a Li-Cor DNA bidirectional sequencer 4200 and ran them overnight. COI and COII sequences translated correctly. We, therefore, feel confident that the sequences were from the mitochondrial DNA genome. The sequences reported here have been deposited in GenBank (data accession numbers: AY449388–AY449457).

Sequence analyses

We aligned the sequences with ClustalX (Thompson *et al*. 1997) using the "full multi-alignment" option and dynamic programming (slow-accurate pairwise alignment method). Gap opening penalty was 15 and gap extension penalty 6.66 in both pairwise and multiple alignment parameters. In the latter, delay divergent sequence was 30% and DNA transition weight 0.50. The alignment was checked visually. A gap of 16 bases followed by a T and another gap of 6 bases were introduced by the alignment program between the COI and $tRNA_{leu}$ genes of the TB1, TB2 and DLa1 and DLa2 sequences. However, the T was clearly the first T of the $tRNA_{leu}$, thus we manually modified the alignment creating a unique gap of 22 bases in these four sequences.

Test of stationary across sequence was performed with the program Treefinder (Jobb *et al*. 2004) on all sites. The sequence of *Psyche norvegica* did not pass the stationary test and so it was excluded from the analyses.

Phylogenetic reconstructions were obtained with Bayesian inference. The method was chosen because Bayesian inference allows the implementation of specific models of DNA substitution and it also performs exceptionally well in supporting correct grouping as compared with traditional ML and MP methods (Alfaro *et al*. 2003). However, we need to note that the full meaning of Bayesian support values is still under debate (Simmons *et al*. 2004).

To select the model of DNA substitution that best fitted the data, we employed the hierarchical likelihood ratio test (Huelsenbeck & Crandall 1997) implemented in MODELTEST 3.06 (Posada & Crandall 1998). For the combined data set of COI, $tRNA_{leu}$, COII and *12S*, the Hasegawa, Kishino and Yano model of nucleotide substitution, with the gamma distribution parameter to correct for the rate variation among sites (HKY + γ) (Hasegawa *et al*. 1985), was the most appropriate based on the hierarchical likelihood ratio test. Under the Akaike information criterion (Akaike 1974), the K81uf + γ model of nucleotide substitution (Tamura & Nei 1993) was the best model.

Bayesian analyses were performed with MAC5 (written by McGuire and Agapow and available at: http://www.agapow.net/software/mac5/) which allow gaps to be treated as 5th state and MrBayes 3.0 (Huelsenbeck & Ronquist 2001) where gaps are treated as missing data. The effects of different models of nucleotide substitutions were explored. In MAC5 both the Jukes and Cantor (Jukes & Cantor 1969) (JC) and the F84 model of substitution (Kishino & Hasegawa 1989), which is equivalent to the HKY model, were applied, while in MrBayes the JC, HKY and the general time reversible (GTR) (Lanave *et al*. 1984) models with γ were applied. An analysis setting of one million generations, sampling every 100 trees and with a burn-in of 200 000 was applied for both programs. In MAC5 the weighting option was set either to off (all gaps treated equally) or on (for down weighting gap positions in the alignment. The weight is the reciprocal of the average gap length at that column of the alignment; if there are no gaps, the weight is one). Three independent runs were used to check that the chains had converged properly.

Results

We used a concatenated sequence of COI (188 bp), intergenic sequence (27 bp), $tRNA_{leu}$ (68 bp), COII (295 bp) and *12S* (364 bp) to determine the diversity of bagworm moth species in Finland. A total of 271 (28.8%) of the sites were variable, of which 235 (24.9%) were parsimony-informative. The variable sites in the different genes were: 83 (44%) in the COI fragment, 9 (13%) in the $tRNA_{leu}$, 79 (27%) in the COII segment and 91 (25%) in the *12S* fragment. The TS: TV ratio was estimated to be 2.7 for the entire sequence. Corrected sequence divergence values calculated with the HKY + G model are shown in Table 2. The gamma shape parameter was 0.21. All *D. triquetrella* sequences were characterised by an insertion of three bases (CTA or CCA in DTp1) in the intergenic sequence between COI and $tRNA_{leu}$.

The trees produced by the Bayesian methods of estimating the phylogenetic relationships among bagworm moths with JC and F84 models of nucleotide substitutions and with a gap treated as 5th state (either gap no-weighted or weighted in MAC5) are shown in Figs. 2, 3, 4 and 5. The

trees obtained by treating gaps as missing data (MrBayes) are shown in Figs. 6 (JC + γ model) and 7 (HKY + γ model). The GTR + γ model produced an identical tree to the HKY + γ tree.

In all trees the haplotypes of the parthenogenetic *D. lichenella* form a monophyletic group which was the sister group of the haplotypes of the sexual *D. fumosella*. *D. lichenella* showed very low intraspecific variation although it included samples from Canada (Table 2). *D. generosensis* haplotypes also formed a monophyletic group which was basal to the *D. lichenella/fumosella* groups. *D. fennicella* and all samples classified as the parthenogenetic form of *S. rupicolella* clustered in the same group. Intraspecific variation was very low (0% to 0.3% divergence) and these species shared the most common haplotypes (DF1). *D. fennicella* group was the sister group of two haplotypes (DL1 and DL2) of the sexual species *D. lazuri*. The cluster *D. fennicella/rupicolella/lazuri* was the sister cluster of *D. fumosella/lichenella/generosensis* group. All the relationships listed above were highly supported in all trees. *D. lazuri* haplotypes did not form a monophyletic group. Three more specimens (DL3, DL4 and DL4a) clustered with *D. charlottae* haplotypes. The *D. charlottae/lazuri* cluster was the sister group of *D. wockei* in all trees but in the tree obtained using the model F84 + gap and no gap weight in MAC5 (Fig. 3). *D. wockei* haplotypes always formed a monophyletic group. The *D. wockei/charlottae/lazuri* group was the sister group of the *D. lichenella/fennicella* cluster when the F84 model with gap weight was used in Mac5 (Fig. 5). With the same model but no gap weight *D. charlottae/lazuri* was the basal group of the cluster formed by *D. wockei/fennicella/lichenella* (Fig. 3). Intraspecific variation among *D. triquetrella* was higher than that observed for the other species, with a divergence of up to 4.2% between Austrian and Swiss haplotypes (DTp1 and DTp4 respectively) (*see* Table 2). *D. triquetrella* formed a monophyletic group when gaps were treated as 5th state (Figs. 2, 3, 4 and 5). When gaps were treated as missing data, the relationships among *D. triquetrella* haplotypes were unresolved forming a polytomy with those of the *S. rupicolella/listerella/D. sauteri/vaudella* group (Figs. 6 and 7). In MAC5, using the F84 model both with gap and with no gap weight, *D. triquetrella* was the sister group of the *D. wockei/fennicella/lichenella* cluster. With the JC model and no gap weight, *D. triquetrella* haplotypes were the sister group of the *D. charlottae/lazuri/wockei* group (Fig. 2). When the gap weight was applied in the JC model, the tree obtained was similar to those obtained with the gap treated as missing data: *D. triquetrella* haplotypes were the sister group of the *S. rupicolella/listerlla/vaudella/sauteri* group (Fig. 4). Basal to this group was the *D.wockei/charlottae/lazuri* group. In MrBayes trees this relationship was resolved only with the JC + γ model (Fig. 6), while with the more complex model of nucleotide substitution the relationships among major clusters were unresolved (Fig. 7). The position of *Siederia* haplotypes in the trees varied along with the model of nucleotide substitution implemented and how gaps were treated. Nevertheless, *Siederia* always formed two well-supported distinct groups (Figs. 2, 3, 4, 5, 6 and 7). A Swiss and a Swedish *S. rupicolella* haplotypes formed one cluster (SR1 and SR3 respectively) and all other specimens from Finland and Sweden classified as *S. rupicolella* and *S. listerella* formed another cluster. We found very little genetic variation among the different sexual *Siederia* in Finland and Sweden, whereas we observed high divergence (10%) between the two *Siederia* groups. In all analyses with gaps treated as missing data and with the JC model + gaps (Figs. 2, 4, 6 and 7), the two *S. rupicolella* haplotypes (SR1 and 3) were basal to all the *Dahlica* and *Siederia* species. In contrast, using the F84 model + gaps and no gaps weight the other *S. rupicolella* and *S. listerella* haplotypes were basal to the tree (Fig. 5). The position of *D. vaudella* and *D. sauteri* also varied according to the model used in the phylogenetic reconstruction (*see* e.g., Figs. 3 and 4).

Discussion

Finnish samples of bagworm moths clustered in six separate groups with all models of substitution used to infer their phylogenetic relationships. These relationships allowed us to compare the species' identity with the identifications based on their wing morphology, the most used charac-

Table 2. Pairwise sequence divergence among haplotypes used in this study based on the concatenated sequence of COI, tRNAleu, COII and *12S*. The genetic distances were calculated with the HKY + γ model of nucleotide substitution. Specimens not included in the matrix were: SL3 because it was identical to SR2, SRp1 and 2 because they were identical to DF1 and DL4, and DTs2 because it differed by one indel from DC1 and DTs1 respectively.

		PN	DLa1	SR1	SR2	DV	DS1	DS2	DW1	DW2	DTs1	DTs2	DTp1	DTp2	DTp3	DTp4	DTp5	DTp6	DTp7
Psyche norvegica	PN																		
Diplodoma laichartingella	DLa1	0.486																	
Siederia rupicolella	SR1	0.641	0.287																
Siederia rupicolella	SR2	0.682	0.334	0.113															
Dahlica vaudella	DV	0.640	0.354	0.101	0.106														
Dahlica sauteri	DS1	0.617	0.316	0.103	0.086	0.029													
Dahlica sauteri	DS2	0.625	0.320	0.105	0.084	0.030	0.001												
Dahlica wockei	DW1	0.644	0.322	0.110	0.098	0.107	0.108	0.106											
Dahlica wockei	DW2	0.647	0.323	0.110	0.091	0.103	0.101	0.099	0.005										
Dahlica triquetrella	DTs1	0.644	0.274	0.083	0.083	0.084	0.073	0.071	0.078	0.078									
Dahlica triquetrella	DTs2	0.679	0.287	0.090	0.089	0.090	0.079	0.077	0.078	0.084	0.002								
Dahlica triquetrella	DTp1	0.701	0.295	0.093	0.084	0.085	0.071	0.069	0.060	0.063	0.037	0.037							
Dahlica triquetrella	DTp2	0.712	0.275	0.073	0.061	0.076	0.051	0.049	0.057	0.057	0.032	0.032	0.035						
Dahlica triquetrella	DTp3	0.701	0.289	0.078	0.065	0.081	0.055	0.054	0.059	0.059	0.036	0.036	0.037	0.003					
Dahlica triquetrella	DTp4	0.703	0.300	0.095	0.095	0.096	0.084	0.082	0.083	0.089	0.008	0.008	0.041	0.036	0.040				
Dahlica triquetrella	DTp5	0.686	0.282	0.091	0.091	0.092	0.080	0.078	0.079	0.086	0.006	0.006	0.038	0.033	0.037	0.004			
Dahlica triquetrella	DTp6	0.654	0.269	0.085	0.078	0.079	0.074	0.072	0.073	0.073	0.007	0.009	0.037	0.032	0.037	0.013	0.011		
Dahlica triquetrella	DTp7	0.666	0.271	0.085	0.080	0.079	0.074	0.072	0.075	0.076	0.007	0.010	0.038	0.033	0.037	0.013	0.011	0.000	
Dahlica lichenella	DLi1	0.723	0.331	0.089	0.114	0.110	0.108	0.106	0.089	0.096	0.078	0.078	0.055	0.064	0.065	0.083	0.080	0.074	0.077
Dahlica lichenella	DLi2	0.726	0.326	0.086	0.111	0.106	0.105	0.103	0.086	0.093	0.076	0.076	0.053	0.061	0.063	0.081	0.077	0.072	0.074
Dahlica lichenella	DLi3	0.716	0.328	0.089	0.114	0.114	0.108	0.106	0.088	0.095	0.078	0.078	0.058	0.063	0.065	0.083	0.080	0.074	0.076
Dahlica lichenella	DLi4	0.701	0.322	0.086	0.110	0.110	0.104	0.102	0.085	0.092	0.075	0.075	0.055	0.061	0.063	0.080	0.077	0.071	0.074
Dahlica lichenella	DLi5	0.738	0.339	0.091	0.117	0.116	0.110	0.108	0.090	0.098	0.079	0.079	0.059	0.065	0.067	0.085	0.081	0.075	0.077
Dahlica fennicella	DF1	0.622	0.300	0.087	0.098	0.109	0.103	0.101	0.088	0.088	0.071	0.076	0.060	0.055	0.056	0.081	0.078	0.067	0.069
Dahlica fennicella	DF2	0.640	0.307	0.091	0.101	0.113	0.106	0.104	0.091	0.091	0.073	0.079	0.062	0.057	0.059	0.084	0.081	0.070	0.072
Dahlica fennicella	DF3	0.645	0.311	0.092	0.103	0.114	0.108	0.106	0.086	0.093	0.075	0.075	0.059	0.054	0.056	0.080	0.076	0.071	0.073
Dahlica charlottae	DC1	0.654	0.318	0.078	0.087	0.089	0.074	0.072	0.065	0.065	0.060	0.066	0.063	0.044	0.048	0.070	0.067	0.051	0.052
Dahlica lazuri	DC2	0.680	0.304	0.073	0.096	0.087	0.073	0.071	0.063	0.063	0.058	0.063	0.060	0.042	0.046	0.068	0.065	0.049	0.049
Dahlica lazuri	DC3	0.673	0.311	0.075	0.091	0.085	0.071	0.069	0.062	0.063	0.058	0.063	0.060	0.042	0.046	0.068	0.064	0.049	0.050
Dahlica lazuri	DL1	0.620	0.291	0.080	0.086	0.104	0.094	0.092	0.083	0.083	0.062	0.067	0.053	0.049	0.051	0.072	0.068	0.058	0.060
Dahlica lazuri	DL2	0.632	0.297	0.082	0.088	0.106	0.097	0.095	0.085	0.085	0.063	0.069	0.055	0.051	0.052	0.074	0.070	0.060	0.061
Dahlica lichenella f. *fumosella*	DFu1	0.686	0.319	0.087	0.115	0.112	0.106	0.104	0.087	0.093	0.074	0.074	0.057	0.062	0.064	0.079	0.075	0.070	0.072
Dahlica lichenella f. *fumosella*	DFu2	0.687	0.309	0.084	0.111	0.108	0.102	0.100	0.083	0.090	0.073	0.073	0.054	0.059	0.061	0.078	0.075	0.069	0.072
Dahlica generosensis	DG1	0.667	0.316	0.080	0.106	0.103	0.091	0.089	0.085	0.086	0.070	0.075	0.055	0.056	0.058	0.080	0.077	0.066	0.068
Dahlica generosensis	DG2	0.662	0.312	0.078	0.107	0.101	0.091	0.089	0.085	0.086	0.068	0.075	0.054	0.056	0.056	0.080	0.077	0.066	0.067

	PN	DLi1	DLi2	DLi3	DLi4	DLi5	DF1	DF2	DF3	DC1	DC2	DC3	DL1	DL2	DFu1	DFu3	DG1	DG2
Psyche norvegica	DLa1																	
Diplodoma laichartingella	SR1																	
Siederia rupicolella	SR2																	
Siederia rupicolella	DV																	
Dahlica vaudella	DS1																	
Dahlica sauteri	DS2																	
Dahlica sauteri	DW1																	
Dahlica wockei	DW2																	
Dahlica wockei	DTs1																	
Dahlica triquetrella	DTs2																	
Dahlica triquetrella	DTp1																	
Dahlica triquetrella	DTp2																	
Dahlica triquetrella	DTp3																	
Dahlica triquetrella	DTp4																	
Dahlica triquetrella	DTp5																	
Dahlica triquetrella	DTp6																	
Dahlica triquetrella	DTp7																	
Dahlica lichenella	DLi1																	
Dahlica lichenella	DLi2	0.003																
Dahlica lichenella	DLi3	0.002	0.003															
Dahlica lichenella	DLi4	0.001	0.002	0.001														
Dahlica lichenella	DLi5	0.002	0.003	0.002	0.001													
Dahlica fennicella	DF1	0.027	0.029	0.024	0.026	0.025												
Dahlica fennicella	DF2	0.029	0.031	0.026	0.027	0.026	0.001											
Dahlica fennicella	DF3	0.027	0.029	0.024	0.025	0.024	0.002	0.003										
Dahlica charlottae	DC1	0.076	0.074	0.076	0.073	0.077	0.069	0.072	0.073									
Dahlica lazuri	DC2	0.078	0.075	0.078	0.075	0.078	0.070	0.073	0.075	0.002								
Dahlica lazuri	DC3	0.073	0.071	0.073	0.070	0.074	0.066	0.069	0.071	0.001	0.001							
Dahlica lazuri	DL1	0.022	0.023	0.019	0.020	0.022	0.009	0.010	0.011	0.062	0.063	0.060						
Dahlica lazuri	DL2	0.023	0.025	0.020	0.022	0.023	0.010	0.012	0.013	0.064	0.065	0.061	0.001					
Dahlica lichenella f. *fumosella*	DFu1	0.007	0.008	0.007	0.006	0.007	0.027	0.028	0.026	0.075	0.076	0.072	0.027	0.029				
Dahlica lichenella f. *fumosella*	DFu2	0.004	0.006	0.004	0.003	0.005	0.024	0.026	0.024	0.071	0.073	0.069	0.025	0.026	0.002			
Dahlica generosensis	DG1	0.011	0.012	0.011	0.009	0.011	0.026	0.027	0.028	0.068	0.069	0.065	0.024	0.026	0.010	0.008		
Dahlica generosensis	DG2	0.009	0.011	0.009	0.009	0.010	0.024	0.027	0.028	0.066	0.068	0.064	0.023	0.024	0.010	0.008	0.002	

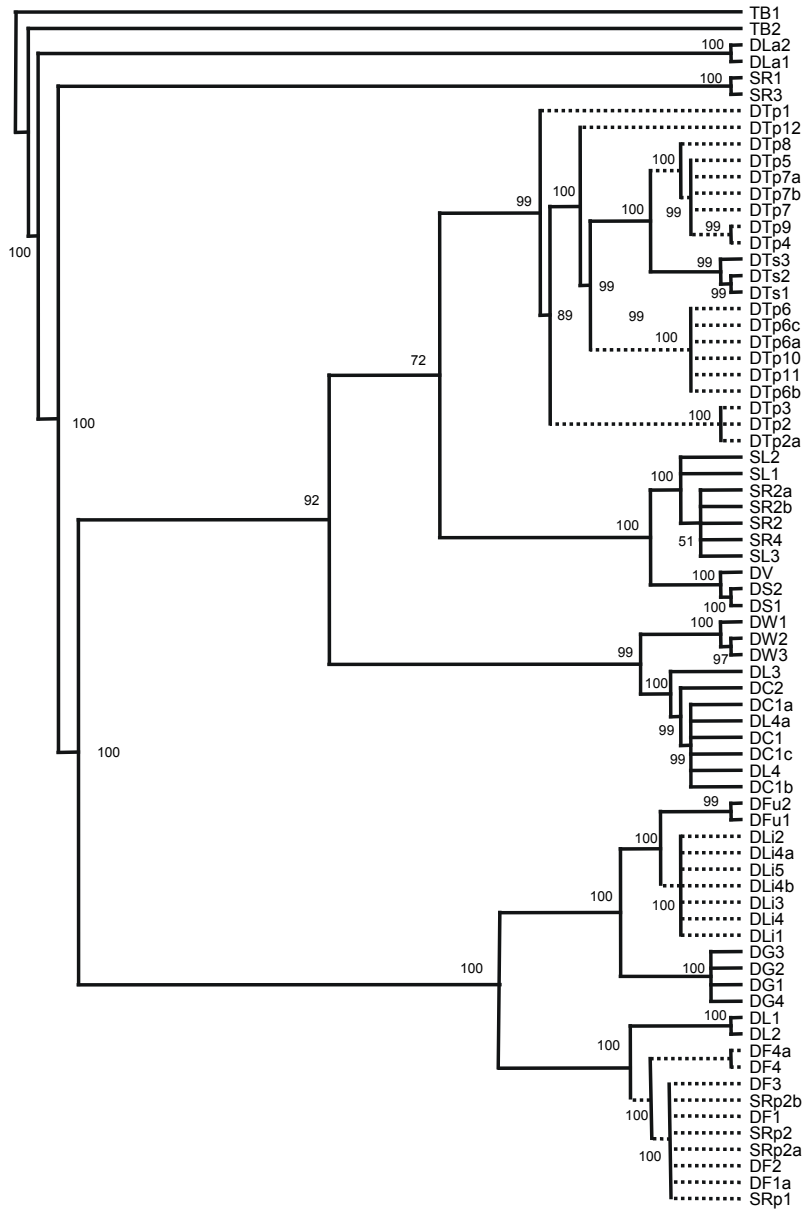

Fig. 2. Bayesian tree obtained from the combined sequence of COI, $tRNA_{leu}$, COII and *12S*, using the JC + gaps model of nucleotide substitution with no gap weight in MAC5. Numbers at the nodes represent the support values. Nodes with 50% or less support were collapsed. Black and dashed branches indicate sexual and parthenogenetic lineages, respectively.

ter for identification of these species. Although the sequence data were unable to completely resolve the relationships because of incongruence among different trees obtained with different models of substitution, our results still raise significant questions about the current taxonomy and relationships of Naryciinae species.

The position of *Siederia* haplotypes in the trees varied along with the model of nucleotide substitution implemented and how gaps were treated. Nevertheless, *Siederia* always formed two distinct groups which were never monophyletic, questioning the validity of classifying these bagworm moths in two different genera (*Dahlica* and *Siederia*).

Although the Finnish *Siederia* samples could be separated into two clearly different morphological groups on the basis of their wing scales (*S. rupicolella* and *S. listerella*), in our mtDNA analysis, all Finnish specimens of sexual *Siederia* formed a homogenous, well-supported group with the inclusion of two *S. listerella* and

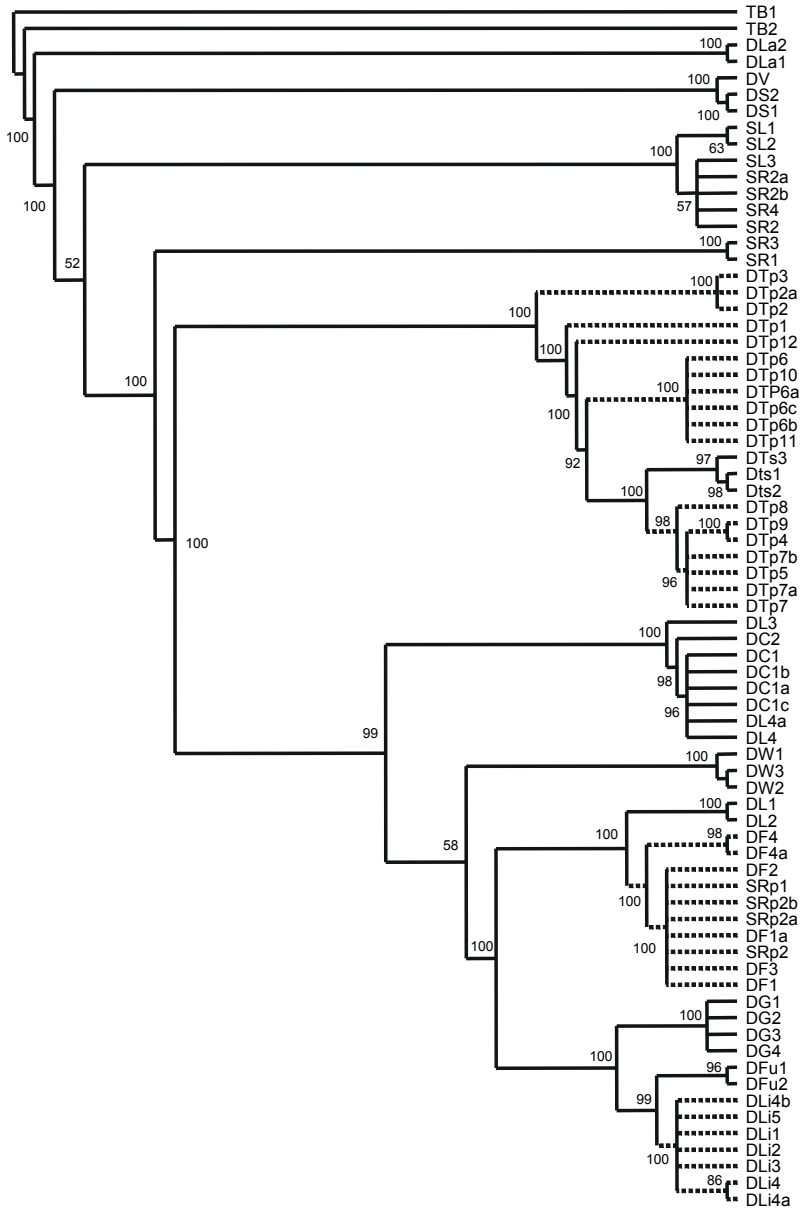

Fig. 3. Bayesian tree obtained from the combined sequence of COI, $tRNA_{leu}$, COII and *12S* using the F84 + gaps model of nucleotide substitution with no gap weight in MAC5. Numbers at the nodes represent the support values. Nodes with 50% or less support were collapsed. Black and dashed branches indicate sexual and parthenogenetic lineages, respectively.

one *S. rupicolella* from Sweden. Very different (10% divergence) were two haplotypes of *S. rupicolella* collected in Switzerland and Sweden. From these results it seems that all Finnish samples can be classified as *S. listerella* according to the mtDNA. Thus, contrary to the general understanding, *S. listerella* seems very common in Finland. Its wing scale morphology is more variable than previously thought and widely overlaps with that of *S. rupicolella,* making this morphological character not a diagnostic to separate these two species. *S. rupicolella* is a rare species in the Alps and in Scandinavia but clearly has wide distribution as previously reported (Hättenschwiler 1997).

D. fennicella was considered by Suomalainen (1980) a separate species, locally restricted and very rare. Many collectors instead considered parthenogenetic moths in other parts of Finland as the parthenogenetic form of *S. rupicolella*. In our analysis, all specimens identified as "parthenogenetic *S. rupicolella*" form a monophyletic

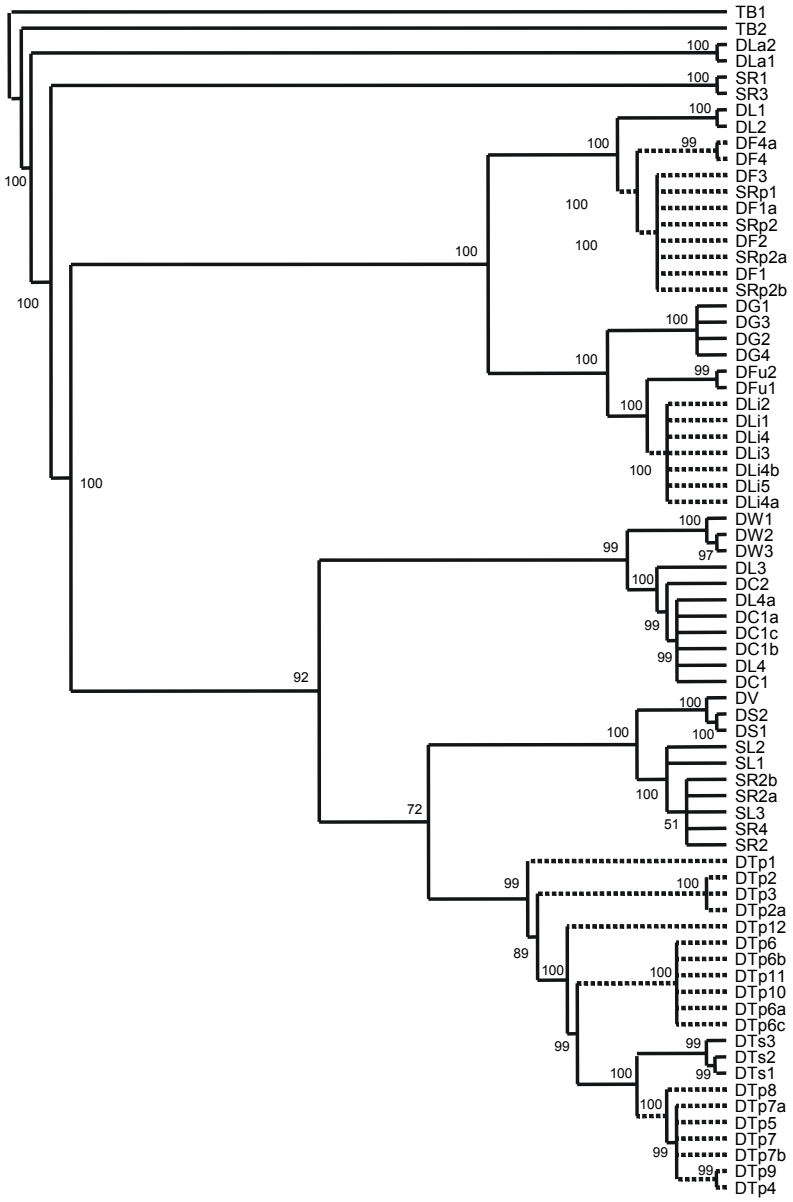

Fig. 4. Bayesian tree obtained from the combined sequence of COI, $tRNA_{leu}$, COII and *12S*, using the JC + gaps model of nucleotide substitution with gap weight in MAC5. Numbers at the nodes represent the support values. Nodes with 50% or less support were collapsed. Black and dashed branches indicate sexual and parthenogenetic lineages, respectively.

group with *D. fennicella* sharing the most common haplotype. The question thus arises: are these two asexual bagworm moths the same species? Most likely they are. In consequence, morphology, cover material and size of larvae sack in *D. fennicella* are more variable than earlier thought. Habitat requirements also vary considerably since this species has earlier been found only on rocks and stones (Suomalainen 1980). We found most of our samples of *D. fennicella* and "asexual *S. rupicolella*" on tree trunks. We found these two asexual moths (from now on called *D. fennicella*) in six of eight randomly chosen potentially suitable habitat patches. In most of these, *D. fennicella* was very numerous (being the most abundant species of *Dahlica* or *Siederia*). This species is often found in warm and half-open habitats such as parks, scattered plantations of forest or wooded garden areas, where the ground layers of vegetation are not too dense. Most of the habitats are close to lake shores (Kumpulainen 2004). However, there is

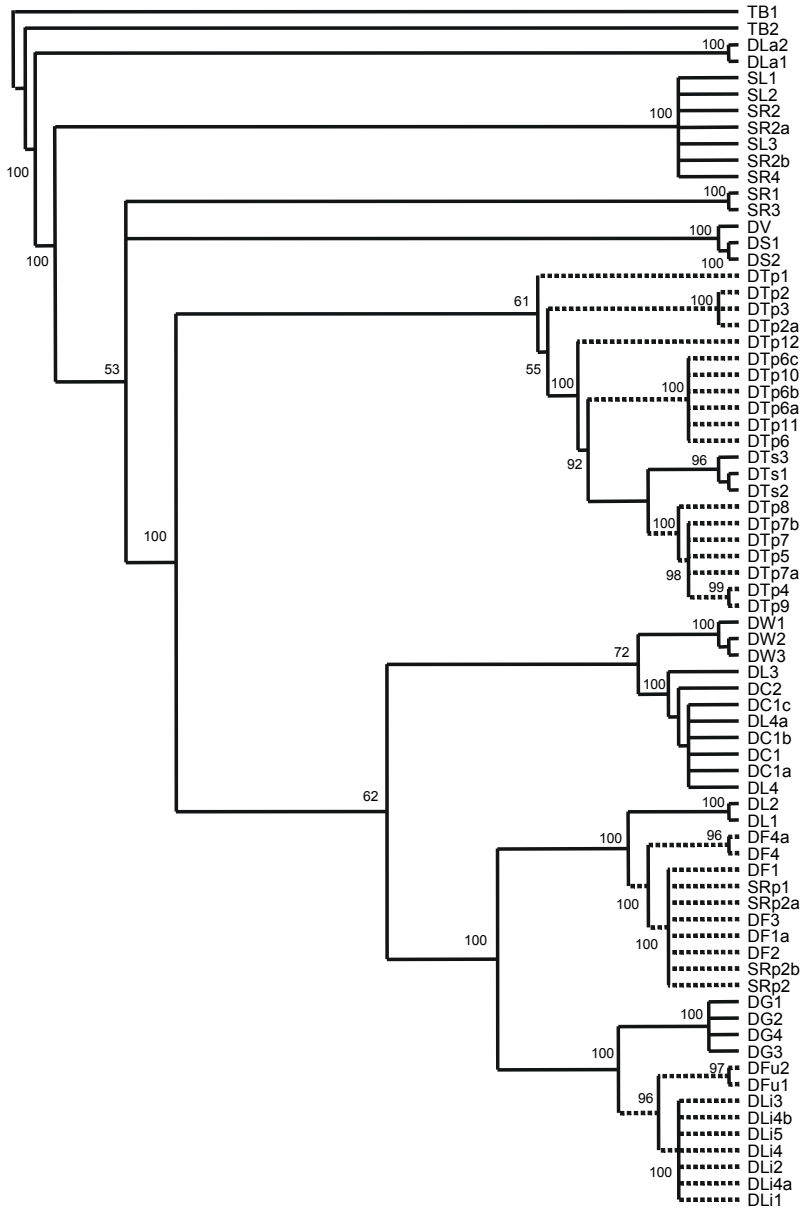

Fig. 5. Bayesian tree obtained from the combined sequence of COI, $tRNA_{leu}$, COII and *12S* using the F84 + gaps model of nucleotide substitution with no gap weight in MAC5. Numbers at the nodes represent the support values. Nodes with 50% or less support were collapsed. Black and dashed branches indicate sexual and parthenogenetic lineages, respectively.

considerable overlap in habitat preferences of many *Dahlica* and *Siederia* species and, thus, habitat alone cannot be used for species determination, except between *D. fennicella* and *D. lichenella*, which are not known to co-occur.

It is, however, possible that "asexual *S. rupicolella*" is a morphologically differentiated clone of *D. fennicella* that has separated from ancestral *D. fennicella* so recently that the genetic differences are too slight to be detected in our data. Finally, asexuality in both species could be due to hybridisation involving the same maternal species. In many animals (including insects), asexual reproduction has been associated with interspecific hybridisation. Hybridisation implies reticulation of lineages and the possibility of introgression of genetic markers resulting in incongruence between gene trees and species trees (Normark & Lanteri 1998). Interspecific hybridization, however, is unlikely to be at the point of origin of parthenogenetic species in the *Dahlica*/*Siederia* group. Asexual *Dahlica*

Fig. 6. Bayesian tree obtained from the combined sequence of COI, $tRNA_{leu}$, COII and *12S*, using the JC + γ model of nucleotide substitution and gap treated as missing data in MrBayes ver. 3.0. Numbers at the nodes represent the support values. Nodes with 50% or less support were collapsed. Black and dashed branches indicate sexual and parthenogenetic lineages, respectively.

triquetrella (and most likely all other species) reproduce by automictic parthenogenesis (Narbel 1950). In this type of parthenogenesis, the early stages of meiosis in the egg are similar to those in the fertilized eggs of sexual species. The zygoid phase is restored by the fusion of the two central azygoid nuclei. This results in the formation of the so called "Richtung-Kopulations-Kerns" (RKK). The fusion always leads to heterogamy and production of females. RRK also forms in the sexual form of *D. triquetrella*, but does not develop and the zygoid phase is restored with the fusion of the sperm nucleus (Seiler 1967).

From our results *D. lichenella* is a separate, obligatorily parthenogenetic species. There is very little genetic variation among the Finnish, Swiss and Canadian samples. In Finland, according to Suomalainen (1980), larvae of this species can be found on stones but we observed numerous larvae also on walls of buildings and on tree trunks (T. Kumpulainen, K. Kulmala & J. Mappes pers. obs.). In coastal Finland, *D. lichenella* seems to

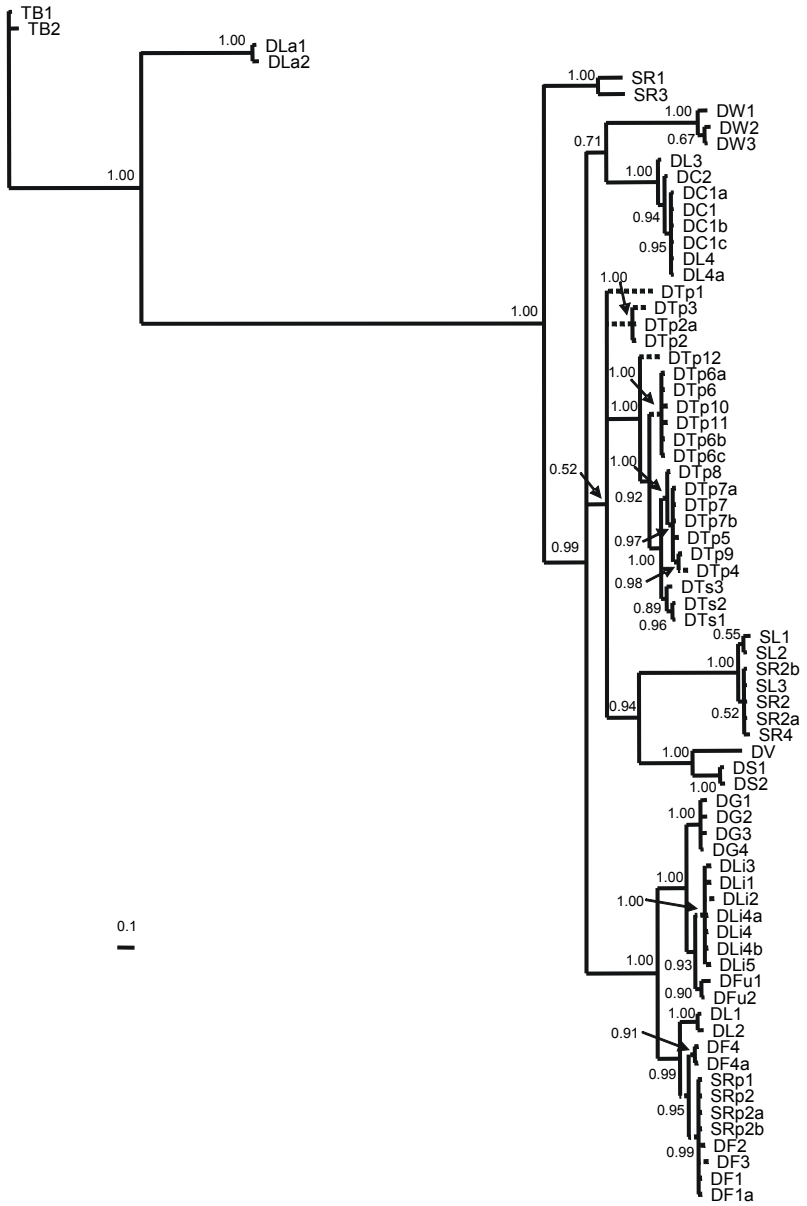

Fig. 7. Bayesian tree obtained from the combined sequence of COI, $tRNA_{leu}$, COII and *12S*, using the HKY85 + γ model of nucleotide substitution and gap treated as missing data in MrBayes ver. 3.0. Numbers at the nodes represent the support values. Nodes with 50% or less support were collapsed. Black and dashed branches indicate sexual and parthenogenetic lineages, respectively.

prefer habitats with humid and temperate microclimates such as sea- and lakeshore areas (T. Kumpulainen & A. Grapputo pers. obs.). Even in the absence of any reliable morphological or ecological characteristics that would separate *D. lichenella* from *D. fennicella*, since the differences in the species distribution are surely helpful for species identification (*see* above).

Moths classified morphologically as *D. lazuri* did not form a monophyletic group. In our analysis four specimens considered "*D. lazuri*" according to the wing scale morphology, clustered with *D. charlottae*. Two other "*D. lazuri"* specimens, in contrast, clustered together as the sister group of the asexual *D. fennicella*. These specimens could not be separated by male wing scale morphology from those that grouped together with *D. charlottae*. Thus, some other character is needed for determining the species based on their morphology. *D. lazuri* and *D. lichenella* (f. *fumosella*) did not cluster together in any trees, indicating that *D. lazuri* is a separate

species from the sexual morph of *D. lichenella* (Arnscheid 1985, Hermann 1994, Hättenschwiler 1997). Sexual *D. lichenella* f. *fumosella* samples from Switzerland formed the sister species of the asexual form *D. lichenella* confirming the previously established relationships.

Intraspecific variation among *D. triquetrella* was higher than that observed among the other species and similar to the interspecific variation observed among *Dahlica* species (Table 2). Despite the high genetic distances among haplotypes, *D. triquetrella* formed a monophyletic group when gaps were treated as 5th state. However the phylogenetic relationships among *D. triquetrella* and other *Dahlica* species were unresolved when gaps were treated as missing data. All *D. triquetrella* haplotypes have an insertion of three bases (*see* Materials and methods) that no other haplotypes from other species have. Gaps contain historical information suitable for phylogenetic analysis and such information is not recovered by those methods that omit gaps from their calculations (Giribet & Wheeler 1999). This is clearly shown in *D. triquetrella*. From the phylogenetic analysis using gaps, results show that asexual *D. triquetrella* in Finland are more similar to sexual and asexual *D. triquetrella* found in Austria than to asexual samples from Switzerland. High variation in the species has also been observed in the head morphology of female pupae and in the morphology of males (Hättenschwiler 1997). It is, however, not known if there is a direct association between genetic variation and such morphological variation. Seiler (1961, 1963) reported that different populations of parthenogenetic *D. triquetrella* show different patterns of egg development. Differences in life-history traits between *D. triquetrella* populations of distant geographical areas led Seiler to conclude that parthenogenesis may have evolved independently several times in different populations (Lokki *et al*. 1975). Our phylogenetic analyses (with gaps) and the position of sexual *D. triquetrella* with respect to the other parthenogenetic samples in the trees support Seiler's view. Otherwise, we have to assume that sexual reproduction has reverted from asexuality.

In general, parthenogenesis is very rare among Lepidoptera and often regarded as a secondary reproductive strategy with low offspring fitness as compared with that of sexually produced offspring. In psychid moths instead, parthenogenetic reproduction seems to have evolved several times as suggested by the alternation of parthenogenetic and sexual species along the phylogenetic trees. According to the mtDNA data, the parthenogenetic species *D. fennicella*, *D. triquetrella* and *D. lichenella* evolved independently from different sexual ancestors. Parthenogenetic *Dahlica* species have a reproductive output equal to that of their sexual relatives, and *Wolbachia* is not known to affect the reproductive strategy (Kumpulainen *et al*. 2004). *Wolbachia* is a feminizing bacterium which can cause parthenogenesis in many insect species (Stouthamer *et al*. 1999). Multiple evolutionary events of parthenogensis suggest that asexual reproduction is favoured in this group. Recently, it has been hypothesized that loss of sex, including facultative or cyclic parthenogenesis, should be associated with weak sexual selection when not accompanied by male parental contributions (West-Eberhard 2005). Psychidae moths fit this picture well since there is little opportunity for sexual selection by direct female choice, since the wingless females mate only once, with the first male to attempt copulation (personal observation). Overlapping sexual characteristics, like complex genitalia in *Siederia* and *Dahlica* also support the hypothesis of weak sexual selection. Moreover, sexual populations of this group are very small, genetically isolated and highly inbred, and males are severely sperm-limited. Although about 50% of males mate more than once, females that mate with already mated males lose 30%–100% of viable offspring (Kumpulainen 2004).

The closest sexual species to asexual *D. fennicella* and *D. lichenella* are quite rare as compared with the asexual relatives. Many theoretical models assume that the advantage of sex is primarily due to its ability, through recombination, to generate greater genetic diversity than asexuality and to spread good genes rapidly through a population by unlinking them from bad genotypes, and thereby enhancing adaptation in a changing environment (Hamilton 1980, Kondrashov 1993). Recently, we found lower genotype diversity in asexual *D. fennicella* as compared with that of the sexual *S. rupicolella* and *D. charlottae*. Nevertheless, clonal diversity

was very high in all asexual populations which may allow them to successfully compete with sexual species (A. Grapputo, T. Kumpulainen & J. Mappes unpubl. data). It is reasonable then to ask how sexual reproduction can be maintained in this group given the obvious disadvantages of it (cost of males, cost of mating and severe inbreeding depression). The most tempting current hypothesis for the maintenance of sex is that parasitoids and/or diseases can favour sexual reproduction due to their higher genetic diversity. Indeed, we found very strong evidence that sexuals cope better in environments with high parasitoid prevalence while asexual reproduction dominates in areas with very low parasitoid prevalence (Kumpulainen *et al*. 2004). Although clonal diversity of *D. fennicella* is extremely high and populations of sexual *S. rupicolella* and *D. charlottae* are inbred, sexual species still show higher genotype diversity than asexual *D. fennicella* which may explain why sexual reproduction still exists in this group.

In conclusion, wing scale morphology misled the identification of many specimens of *Dahlica lazuri* and sexual *Siederia*. Our study indicates that intraspecific variation for this character is higher than previously suggested making it a poor diagnostic. In cases of parthenogenetic species, ecological and distributional data are helpful for species determination in Finland. However, because of the high number of different species in central Europe, it is questionable if ecological or distributional data is generally useful. This study also raised many new questions and revealed novel problems. Firstly, our results from mtDNA markers question the existence of two separate genera. Secondly, the high genetic differentiation for certain species and the incongruence between morphological characters and mtDNA sequences suggest that several samples from different populations should be analysed in any attempt to elucidate the relationships among psychid moths. Multiple evolutionary events of parthenogenesis support the current understanding that asexuality is favourable strategy in Psychidae moths.

Acknowledgements

We are grateful to P. Hättenschwiler, E. Hauser and G. Palmqvist for providing us with samples from Switzerland, Austria, Sweden and Canada, and to A.-M. Koskela and T. Koskela for their help in collecting material in the archipelago of Rauma. We also thank K.E. Knott, S. Mills, J. Itämies, J. Kullberg, M. Björklund, P. Mutikainen, an anonymous referee and all the people at "the round table meeting" of the Evolutionary Ecology research unit, for their valuable comments on a previous version of the manuscript. We thank Kari Kulmala for assistance in field and laboratory work. This study was financed by the Academy of Finland (to JM, project numbers 768945, 779874) and the Centre of Excellence in Evolutionary Ecology.

References

Akaike, H. 1974: A new look at the statistical model identification. — *IEEE Trans. Automat. Contr.* 19: 716–723.

Alfaro, M. E., Zoller, S. & Lutzoni, F. 2003: Bayes or bootstrap? A simulation study comparing the performance of Bayesian Markov Chain Monte Carlo sampling and bootstrapping in assessing phylogenetic confidence. — *Mol. Biol. Evol.* 20: 255–266.

Arnscheid, W. R. 1985: Ein Beitrag zur Systematik der europäischen Arten der Gattung Solenobia Duponchel, 1842 (Lepid., Psychidae, Teleporiinae). — *Nachrichten des entomologischen Vereins Apollo* 4: 1–56.

Avise, J. C. 1994: *Molecular markers, natural history and evolution.* — Chapman & Hall, New York.

Bell, G. 1982: *The masterpiece of nature: the evolution and genetics of sexuality.* — University of California Press, Berkeley.

Brooks, D. R. & McLennan, D. A. 1991: *Phylogeny, ecology, and behavior: a research program in comparative biology.* — The University of Chicago Press, Chicago.

Brower, A. V. Z. 1994: Rapid morphological radiation and convergence among races of the butterfly *Heliconius erato* inferred from patterns of mitochondrial DNA evolution. — *PNAS* 91: 6491–6495.

Brower, V. Z. 1997: Cladistic analysis of *Heliconius* butterflies and relatives (Nymphalidae: Heliconiiti): a revised phylogenetic position for *Eueides* based on sequences from mtDNA and a nuclear gene. — *Proceedings: Biological Sciences* 264: 969–977.

Giribet, G. & Wheeler, W. C. 1999: On gaps. — *Mol. Phylogenet. Evol.* 13: 132–143.

Haddrath, O. & Baker, A. J. 2001: Complete mitochondrial DNA genome sequences of extinct birds: ratite phylogenetics and the vicariance biogeography hypothesis. — *Proc. R. Soc. Lond. B* 268: 939–945.

Hamilton, W. D. 1980: Sex versus non-sex versus parasite. — *Oikos* 35: 282–290.

Hasegawa, M., Kishino, H. & Yano, T. A. 1985: Dating of the human ape splitting by a molecular clock of mitochondrial-DNA. — *J. Mol. Evol.* 22: 160–174.

Hättenschwiler, P. 1985: Psychidae. — In: Heath, J. & Emmet, A. M. (eds.), *The moths and butterflies of Great Britain and Ireland 2*: 128–151. Harley Books, Colchester, UK.

Hättenschwiler, P. 1997: Die Sackträger der Schweiz (Lepidoptera, Psychidae). — *Schmetterlinge und ihre Lebens-*

räume Arten–Gefährdung–Schutz, Band 2: 165–308. Pro Natura, Basel, Switzerland.

Hermann, R. 1994: Psychidae: Naryciinae. — In: Ebert, G., Esche, T. & Hermann, R. (eds.), *Die Schmetterlinge Baden-Württembergs, Band 3, Nachtfalter*: 356–402. Ulmer Verlag, Stuttgart.

Huelsenbeck, J. P. & Ronquist, F. 2001: MRBAYES: Bayesian inference of phylogenetic trees. — *Bioinformatics* 17: 754–755.

Huelsenbeck, J. P. & Crandall, K. A. 1997: Phylogeny estimation and hypothesis testing using maximum likelihood. — *Ann. Rev. Ecol. Syst.* 28: 437–466.

Jobb, G., von Haeseler, A. & Strimmer, K. 2004: TREEFINDER: a powerful graphical analysis environment for molecular phylogenetics. — *BMC Evol. Biol.* 4: 18.

Jukes, T. H. & Cantor, C. R. 1969: Evolution of protein molecules. — In: Munro, H. N. (ed.), *Mammaliam protein metabolism*: 21–132. Academic Press, New York.

Kishino, H. & Hasegawa, M. 1989: Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. — *J. Mol. Evol.* 29: 170–179.

Kondrashov, A. S. 1993: Classification of hypotheses on the advantage of amphimixis. — *J. Hered.* 84: 372–387.

Kruse, J. J. & Sperling, F. A. H. 2002: Phylogeny of Nearctic species of the Xylosteana group of *Archips* Hübner (Lepidoptera: Tortricidae) based on combined analysis of morphological and mitochondrial DNA data sets. — *Ann. Entomol. Soc. Am.* 95: 288–301.

Kullberg, J., Albrecht, A., Kaila, A. & Varis, V. 2002: Checklist of Finnish Lepidoptera — Suomen perhosten luettelo. — *Sahlbergia* 6: 45–190.

Kumpulainen, T. 2004: The evolution and maintenance of reproductive strategies in bag worm moths (Lepidoptera: Psychidae). — *Jyväskylä Studies in Biological and Environmental Science* 132: 1–42.

Kumpulainen, T., Grapputo, A. & Mappes, J. 2004: Parasites and sexual reproduction in psychid moths. — *Evolution* 58: 1511–1520.

Lanave, C., Preparata, G., Saccone, C. & Serio, G. 1984: A new method for calculating evolutionary substitution rates. — *J. Mol. Evol.* 20: 86–93.

Lokki, J., Suomalainen, E., Saura, A. & Lankinen, P. 1975: Genetic polymorphism and evolution in parthenogenetic animals. II. Diploid and polyploid *Solenobia triquetrella* (Lepidoptera: Psychidae). — *Genetics* 79: 513–525.

Narbel, M. 1950: La cytologie de la parthénogénèse chez *Solenobia* sp. (Lepidopteres Psychides). — *Chromosoma* 4: 56–90.

Normark, B. & Lanteri, A. A. 1998: Incongruence between morphological and mitochondrial-DNA characters suggests hybrid origins of parthenogenetic weevil lineages (Genus *Aramigus*). — *Syst. Biol.* 47: 475–494.

O'Brien, S. J. 1987: The ancestry of the giant panda. — *Sci. Am.* 257: 102–107.

Pearce, J. M., Fields, R. L. & Scribner, K. T. 1997: Nest materials as a source of genetic data for avian ecological studies. — *J. Field Ornithol.* 68: 471–481.

Posada, D. & Crandall, K. A. 1998: Modeltest: testing the model of DNA substitution. — *Bioinformatics* 14: 817–818.

Sauter, W. 1956: Morphologie und Systematik der schweizerischen *Solenobia*-Arten (Lep. Psychidae). — *Rev. Suisse Zool.* 63: 451–550.

Sauter, W. & Hättenschwiler, P. 1991: Zum System der palaearktischen Pyschiden (Lep. Psychidae) 1. Teil: Liste der palaearktischen Arten. — *Nota Lepid.* 14: 69–89.

Seiler, J. 1961: Untersuchungen über die Entstehung der Parthenogenese bei *Solenobia triquetrella* F.R. (Lepidoptera, Psychidae) III. Die geographische Verbreitung der drei Rassen von *Solenobia triquetrella* (bisexuell, dipolid und tetraploid parthenogenetisch) in der Schweiz und in den angrenzenden Ländern und die Beziehung zur Eiszeit. Bemerkungen über die Entstehung der Pathenogenese. — *Zeitschrift für Vererbungslehre* 92: 261–316.

Seiler, J. 1963: Untersuchungen über die Entstehung der Parthenogenese bei *Solenobia triquetrella* F.R. (Lepidoptera, Psychidae) IV. Wie besamen begattete dipolid und tetraploid parthenogenetische Weibchen von *S. triquetrella* ihre Eier? Schicksal der Richtunggskörper im unbesamten und besamten Ei. Vergleich der Ergebnisse mit F1 Aufzuchten und beziehngen zur Genese der Parthenogenese. — *Zeitschrift für Vererbungslehre* 94: 29–66.

Seiler, J. 1967: Untersuchungen über die Entstehung der Parthenogenese bei *Solenobia triquetrella* F.R. (Lepidoptera, Psychidae) VII. Versuch einer experimentellen Analyse der Genetick der Pathenogenese. — *Mol. Gen. Genet.* 99: 274–310.

Simmons, M. P., Pickett, K. M. & Miya, M. 2004: How meaningful are Bayesian support values? — *Mol. Biol. Evol.* 21: 188–199.

Simon, C., Frati, F., Beckenbach, A., Crespi, B., Liu, H. & Flook, P. 1994: Evolution, weighting, and phylogenetic utility of mitochondrial gene-sequences and a compilation of conserved polymerase chain reaction primers. — *Ann. Entomol. Soc. Am.* 87: 651–701.

Stouthamer, R., Breeuwer, J. A. & Hurst, G. D. 1999: *Wolbachia pipientis*: microbial manipulator of arthropod reproduction. — *Annu. Rev. Microbiol.* 53: 71–102.

Suomalainen, E. 1962: Significance of parthenogenesis in the evolution of insects. — *Annu. Rev. Entomol.* 7: 349–365.

Suomalainen, E. 1970: Über die *Solenobia*-Arten Finnlands (Lepidoptera: Psychidae). — *Ann. Entomol. Fenn.* 36: 139–142.

Suomalainen, E. 1980: The Solenobiinae species of Finland (Lepidoptera: Psychidae), with a description of a new species. — *Ent. Scand.* 11: 458–466.

Tamura, K. & Nei, M. 1993: Estimation of the number of nucleotide substitutions in the control region of mitochondrial-DNA in humans and chimpanzees. — *Mol. Biol. Evol.* 10: 512–526.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F. & Higgins, D. G. 1997: The ClustalX windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. — *Nucl. Acids. Res.* 24: 4876–4882.

West-Eberhard, M. J. 2003: *Developmental plasticity and evolution*. — Oxford University Press, New York.

West-Eberhard, M. J. 2005: The maintenance of sex as a trap due to sexual selection. — *Q. Rev. Biol.* 80. [In press].