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Bemisia argentifolii is a race of *B. tabaci* (Hemiptera: Aleyrodidae): the molecular genetic differentiation of *B. tabaci* populations around the world

P.J. De Barro[1]*, J.W.H. Trueman[2] and D.R. Frohlich[3]

[1]CSIRO Entomology, 120 Meiers Road, Indooroopilly, QLD 4068, Australia: [2]School of Botany and Zoology, Australian National University, ACT 0200, Australia: [3]Department of Biology, University of St Thomas, 38000 Montrose Blvd, Houston, TX 77006, USA

Abstract

The phylogenetic relationships between genotypes of *Bemisia tabaci* were compared using ITS1 and CO1 nucleotide sequences. Phylogenetic and minimum spanning network analyses identified six major races, Asia, Bali, Australia, sub-Saharan Africa, Mediterranean/Asia Minor/Africa and New World as well as a large collection of genotypes from the Asia region with no strong association with any of the races. The term race is based on its usage in Mallet (2001). Mating incompatibility occurs between some races. There is insufficient data to raise races to species status, but the data supports the recognition of the six races and an unresolved core of ungrouped genotypes under the single *Bemisia tabaci* (Gennadius) species name. To clarify the identity of the race to which the *B. tabaci* under investigation is known, the following nomenclature is suggested, *B. tabaci* (Asia), *B. tabaci* (Bali), *B. tabaci* (Australia) *B. tabaci* (sub-Saharan Africa), *B. tabaci* (Mediterranean/Asia Minor/Africa) and *B. tabaci* (New World). Further, there is insufficient molecular or biological data to support the separation of *B. tabaci* and *B. argentifolii* Bellows & Perring and its use should be discontinued.

Introduction

Bemisia tabaci (Gennadius) (Hemiptera: Aleyrodidae) is a pest and virus vector of agricultural and ornamental crops in all tropical and subtropical regions. The purported species complex (*sensu* Brown *et al*., 1995a; Perring, 2001) first drew major attention in the mid-1980s when severe crop losses were observed in Israel and the southern USA (see De Barro, 1995 for review). Subsequently, major outbreaks and invasions have occurred on all continents except Antarctica. Prior to this *B. tabaci* had long been known to exhibit host plant related races or biotypes. Bird (1957) noted in Puerto Rico the existence of the narrowly specific 'Jatropha' and the more polyphagous 'Sida' races of *B. tabaci* while Burban *et al*. (1992) observed in Ivory Coast a biotype largely confined to cassava and a polyphagous biotype which did not include cassava as a host.

During the late-1980s, a particularly fecund race from the southern US, which demonstrated high levels of insecticide resistance, transmitted previously unknown begomoviruses and induced a physiological change in squash known as 'squash silverleafing' was found to exhibit a unique electromorph pattern for general esterases and became designated the B-biotype. The indigenous American *B. tabaci* exhibited a different esterase pattern and was named the A-biotype (Costa & Brown, 1991; Burban *et al*., 1992; Perring *et al*., 1992; Costa *et al*., 1993). Since then, over 20 different biotypes have been identified (Bedford *et al*., 1994; Brown *et al*., 1995b, 2000; Perring, 2001), the most widespread being the B-biotype which has spread around the world owing to its association with ornamentals and the world trade in this commodity (Beitia *et al*., 1997; De Barro *et al*., 1998; Abdullahi *et al*., 2003;

*Fax: +61 7 3214 2885
E-mail: paul.debarro@csiro.au

Table 1. Sequences used to construct the weighted parsimony tree for ITS1.

Country	Race	GenBank accession number
Lipaleyrodes atriplex (Froggatt)*	Outgroup	AF216042
Egypt	Mediterranean	AF216015–17
Nigeria	Mediterranean	AF216042–45, AJ315797, AJ315818
Spain	Mediterranean	AJ315795
Sudan	Mediterranean	AF216055
Togo	Mediterranean	AJ509016
Indonesia	Bali	New
Australia	Australia	AF215984–88, New
Indonesia	Australia	New
Benin	Sub-Saharan Africa	AF215997–6000, AJ509016
Central African Republic	Sub-Saharan Africa	AJ315815
Congo	Sub-Saharan Africa	AJ315808, AJ315816, AJ315810
Ghana	Sub-Saharan Africa	AJ315812
Guinea	Sub-Saharan Africa	AJ315813
India	Sub-Saharan Africa	AJ315806–7
Ivory Coast	Sub-Saharan Africa	AJ315814, AJ315805
Kenya	Sub-Saharan Africa	AJ315808
Nigeria	Sub-Saharan Africa	AJ315819, AJ509017, AJ509021
Spain	Sub-Saharan Africa	AF216050–51, AJ315820
Uganda	Sub-Saharan Africa	AJ315811, New
Australia	B group, Mediterranean/Asia Minor/Africa	AF215989–91
Brazil	B group, Mediterranean/Asia Minor/Africa	AF216008–10
Congo	B group, Mediterranean/Asia Minor/Africa	AJ315800
Iran	Unresolved, Mediterranean/Asia Minor/Africa	AJ315799
Iran	B group, Mediterranean/Asia Minor/Africa	AF216025–27
Israel	B group, Mediterranean/Asia Minor/Africa	AF216028–33
Morocco	Unresolved, Mediterranean/Asia Minor/Africa	AJ315802
Philippines	B group, Mediterranean/Asia Minor/Africa	New
Sudan	B group, Mediterranean/Asia Minor/Africa	AF216056–57
USA	B group, Mediterranean/Asia Minor/Africa	AF216069–72
Yemen	B group, Mediterranean/Asia Minor/Africa	AF216073–75
Indonesia	Unresolved, Asia	New
Malaysia	Asia2, Asia	New
North India	Unresolved, Asia	AF216022–24
Pakistan	Asia2, Asia	New
Sri Lanka	Asia2, Asia	New
Sri Lanka	Asia2, Asia	New
Thailand	Asia2, Asia	New
Hainan	Asia1, Asia	AF216018
Korea	Asia1, Asia	AF216034
Thailand	Asia1, Asia	New
Turkey	Asia1, Asia	AF216062–65
Bangladesh	Unresolved	AF215994–96
China	Unresolved	AF509593–96
Federated States of Micronesia	Unresolved	New
Nauru	Unresolved	AF216035–37
Nepal	Unresolved	AF216039–41
Pakistan	Unresolved	AF216046–49
Sri Lanka	Unresolved	AF216052–54
South India	Unresolved	AF216019–21, AJ315803–4
Taiwan	Unresolved	AF216058–61
Vietnam	Unresolved	New
Colombia	New World	AF216011
Costa Rica	New World	AF216012–14
Puerto Rico	New World	New
USA	New World	AF216066–68

* Previously named as *Bemisia* nr *tabaci* in De Barro *et al.* (2000), accession numbers commencing with AF2, De Barro *et al.* (2000), accession numbers commencing with AF5, Wu *et al.* (2003), accession numbers commencing with AJ, Abdullahi *et al.* (2003).

Wu *et al.*, 2003; Perring, 2001 for review). Genetic differentiation between the various biotypes has been further supported by numerous studies involving random amplified polymorphic DNA–polymerase chain reaction (RAPD–PCR) (e.g. Gawel & Bartlett, 1993; De Barro *et al.*, 1997; Guirao *et al.*, 1997).

The subsequent discovery of genetic and biological differences between the A and B biotypes led to the controversial raising of the *B. tabaci* B-biotype to separate species status with the coining of the name *B. argentifolii* Bellows & Perring (Bartlett & Gawel, 1993; Perring *et al.*, 1993; Bellows *et al.*, 1994; Campbell *et al.*, 1994). The primary basis

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Table 2. Subset of collections for which CO1 and ITS1 sequences were obtained.

Country	COI GenBank accession number	ITS1 GenBank accession number
Australia	New	AF215984–AF215987
Lipaleyrodes atriplex	New	AF213988
Benin 1–3	AF110693, AF110692, AF085680	AF215997–AF216000
Costa Rica	AF110700	AF216012
Egypt	New	AF216015–AF216017
Hainan	New	AF216018
North India	New	AF216022, AF216023
South India	AF110704	AF216019–AF216021
Israel 1–2	AF110701, AF164667	AF216028–AF216030
Malaysia	New	New
Nauru	New	AF216035–AF216037
Nepal	AF342779	AF216039–AF216041
Puerto Rico 1–2	AF110705, New	New
Spain	AF342775	AJ315795
Spain Ipomoea	New	AF216050, AF216051
Sudan 1–2	AF110706, AF110707	AF216055
Taiwan	New	AF216058–AF2160610
Thailand1	New	New
Thailand2	New	New
Turkey	New	AF216062–AF216065
USA A 1–2	AF110694, AF110695	AF216066–AF216068
USA B	AF110697	AF216069–AF216072
Yemen 1–3	AF110709, AF110712, AF110711	AF216073–AF216075

for the split was the absence of dorsal setae, width of the tracheal folds and width of wax extrusions from the thoracic tracheal fold. This study was a particularly poor piece of taxonomy as individuals representing the full range of *B. tabaci* races were not included in the study. To determine whether these morphological characters were able to separate the different *B. tabaci*, Rosell *et al*. (1997) compared individuals representing the North American A and B biotypes as well as individuals from Israel, India, Benin and Yemen. All morphological characters including those used in Bellows *et al*. (1994) were unable to separate individuals reliably according to their designated biotype. Attempts to use adult characters have similarly failed to consistently and persistently separate biotypes (Calvert *et al*., 2001). This failure is unsurprising. Russell (1957) recognized this plasticity and used it to synonymize some 23 different species into the single taxon, *B. tabaci* Gennadius. Mound (1963) further demonstrated this by rearing individuals from a single female *B. tabaci* on a range of different host plant species thereby showing the plasticity of morphological characters.

The real relationships between global races of *B. tabaci* have not been easy to elucidate. Effects of morphological plasticity and differences in host plant preferences aside; the situation is further complicated by mating differences between biotypes. *Bemisia tabaci* are haplodiploid and so unfertilized eggs, or unmated females, produce only male progeny. A range of studies has shown that while some inter-biotype matings are incapable of producing female

Table 3. The outcomes of mating experiments performed using individuals of *Bemisia tabaci* belonging to the major races, subraces or unresolved clusters. All combinations included reciprocal mating combinations.

Cross	Combination	Outcome	Reference
A × B	New World × Mediterranean/Asia Minor/Africa	Males only	Liu *et al*. (1992); Costa *et al*. (1993); Bedford *et al*. (1994)
B × K	Mediterranean/Asia Minor/Africa × unresolved Asian cluster	Males only	Bedford *et al*. (1994)
B × M	Mediterranean/Asia Minor/Africa × Asia, Asia1	Males only	Bedford *et al*. (1994)
B × D*	Mediterranean/Asia Minor/Africa × New World	Males only	Bedford *et al*. (1994)
K × M	Unresolved Asian cluster × Asia, Asia1	Males only	Bedford *et al*. (1994)
K × D	Unresolved Asian cluster × New World	Males only	Bedford *et al*. (1994)
M × D	Asia, Asia1 × New World	Males only	Bedford *et al*. (1994)
B × L	Mediterranean/Asia Minor/Africa, B group × Mediterranean/Asia Minor/Africa, Mediterranean	Males and females	Byrne *et al*. (1995)
B × Q	Mediterranean/Asia Minor/Africa, B group × Mediterranean/Asia Minor/Africa, Mediterranean	Males and females	Ronda *et al*. (1999); Moya *et al*. (2001)
An × B	Australia × Mediterranean/Asia Minor/Africa	Males, sterile females	De Barro & Hart (2000)
Uganda** × southern India	Sub-Saharan Africa × unresolved Asia	Males only	Maruthi *et al*. (2001)

* The D biotype originated in Nicaragua and given that all other biotypes from this region belong to the New World race, it seems reasonable to conclude the D biotype does likewise.
** All sub-Saharan African *B. tabaci* derived from cassava have so far been found to belong to the sub-Saharan Africa race, so it is probable that the individuals used in this study were also of this race. In a similar vein, all southern Indian *B. tabaci* from the Bangalore region have been shown to belong to the unresolved cluster of Asian *B. tabaci*.

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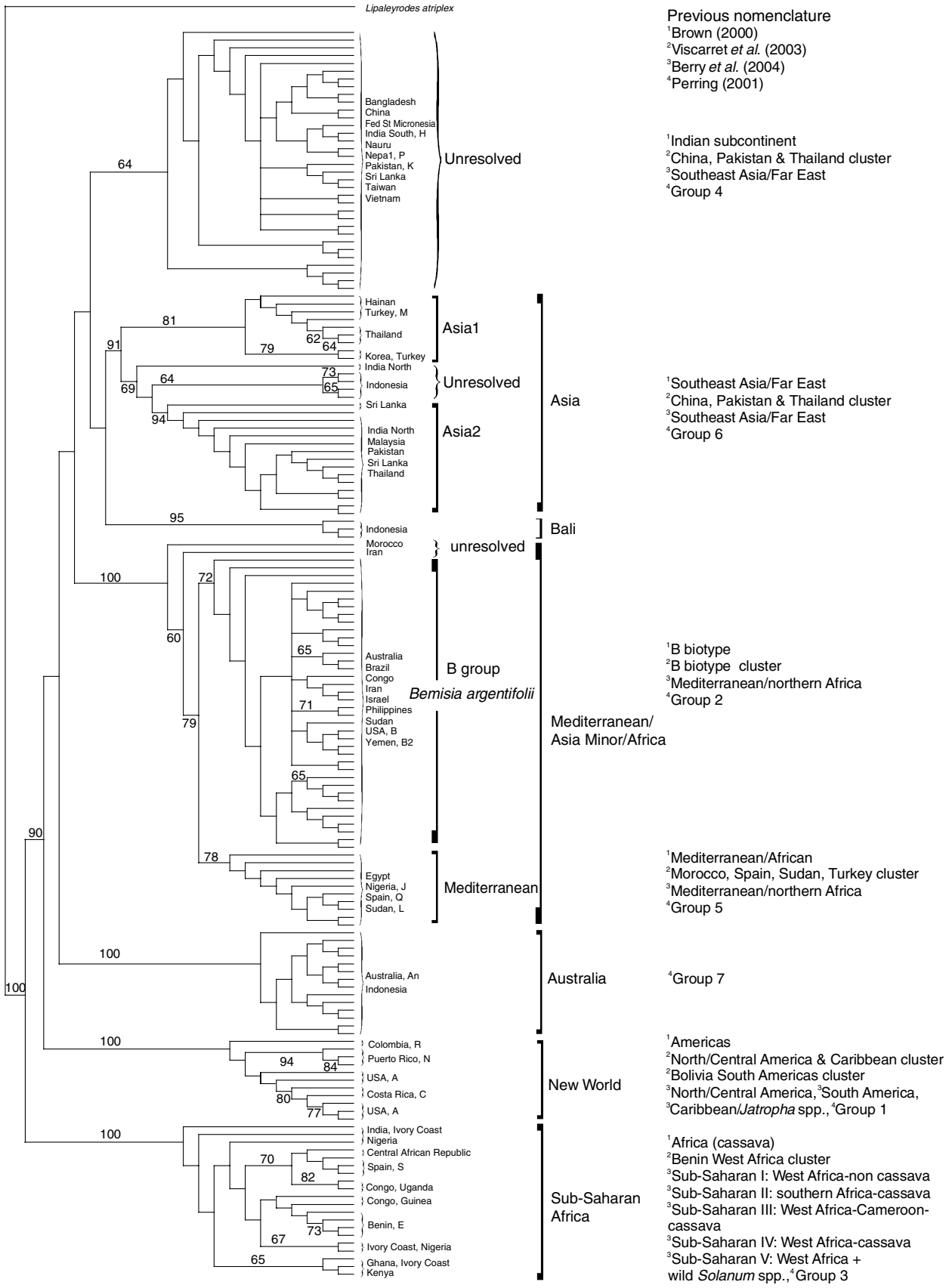

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offspring, others are (this is expanded upon later, but for those interested see table 3).

Frohlich *et al*. (1999) used two mitochondrial markers (cytochrome oxidase I or COI, 16S ribosomal DNA) to reconstruct a phylogeography of ten collections from the New World, India, the Middle East and North Africa, and concluded that the B-biotype was an introduction into the New World from desert/sahel-like regions in or around Israel and Yemen. The same study also argued for treating *B. tabaci* as a highly cryptic group of sibling species, or a species complex (Frohlich *et al*., 1999). De Barro *et al*. (2000) independently undertook a similar study using ribosomal intergenic transcribed spacer 1 (ITS1), and analysed 31 collections from Australia, Africa, Asia, the Mediterranean, North America and the Middle East. Their study revealed strong geographic patterns and since then a number of further studies using either AFLPs, CO1 or ITS1 have re-affirmed these results (Brown, 2000; Cervera *et al*., 2000; Perring, 2001; Legg *et al*., 2002; Abdullahi *et al*., 2003; Viscarret *et al*., 2003; Wu *et al*., 2003; Berry *et al*., 2004). Various names for the different groups have been proposed, but there has been little consistency in nomenclature or attempts to unify the different datasets.

The intent of the present study was to estimate a global phylogeny using both ITS rDNA and CO1 data from the same locations or individuals and overlay this with a review of the biological interactions between the different groups. Using these, the existence of six distinct races is demonstrated as is the synonymy of *Bemisia argentifolii* and *B. tabaci*. Throughout, the term race is used to define the major groups and subgroups of *B. tabaci* so as to avoid the confusion raised by the use and misuse of terms such as population, biotype and clade. Our usage is that of Mallet (2001) and is taken to mean a genetically definable group of individuals where the issue of formal taxonomic assignment is in question.

Materials and methods

Phylogenetic analyses – full ITS1 analysis

To provide a framework within which to understand the *B. tabaci* complex, 113 sequences from the ITS1 datasets of De Barro *et al*. (2000), Abdullahi *et al*. (2003) and Wu *et al*. (2003) were combined with 48 new sequences (table 1). A further addition is the incorporation of biotype designations. The tree was constructed using both parsimony and likelihood as described in De Barro *et al*. (2000). Three parsimony models and the HKY85 model under likelihood analysis yielded the same structure and relationships among the different individuals. Here the parsimony model with transversions and transitions weighted 2:1 is presented.

Phylogenetic analysis using matched CO1 and ITS1

To attempt to estimate a global phylogeny, ITS1 and CO1 data from the same locations were selected from a subset of locations representing the major races and subraces (table 2). Sequences were obtained from Genbank where the origin of both ITS1 and CO1 sequences could be confirmed as being from the same location. If this could not be done, a new sequence was obtained using the methods described in Frohlich *et al*. (1999) or De Barro *et al*. (2000). Parsimony analysis using the same approach as for ITS1 was then applied to the dataset.

Minimum spanning networks

In order to get an overall impression of the interconnectedness of the sequences without forcing a tree onto them, the CO1 and ITS1 sequences were examined in a minimum spanning networks framework using the program MINSPNET (Schneider *et al*., 2000). This provides a list of branches or connections among sequences from which to construct an unrooted digraph based on clustering by similarity, and a second list of alternative connections by which that digraph can be expanded into a network. Similar sequences are clustered together and successively dissimilar sequences further away, without implying a hierarchy.

Mating studies

The literature was searched for studies that tested experimentally the capacity for different biotypes of *B. tabaci* to interbreed (table 3). The data were then re-examined in the light of the phylogenetic analysis by identifying the races to which the individuals used in the study belonged.

Results

Phylogenetic analyses – full ITS1 analysis

Six major branches (fig. 1) are supported by a bootstrap (bs) value >80, with four having values of 99 or 100. Several of these contain smaller groupings with similarly moderate to high bs scores. We have named the six as follows, Asia (bs 91), Bali (bs 95), Australia (bs 100), sub-Saharan Africa (bs 100), Mediterranean/Asia Minor/Africa (bs 100) and New World (bs 100). In addition, sub-Saharan Africa is separated from all the others by a node with a bs score of 90. However, all remaining relationships among races are equivocal.

Within Mediterranean/Asia Minor/Africa there are two subraces, B group (72) and Mediterranean (78). Based on these scores, support for each of the two individually is tenuous, while support for the two together as a race is equally tenuous at 79. Two individuals, one from Morocco and one from Iran, remain unplaced, i.e. they belong to Mediterranean/Asia Minor/Africa, but cannot be assigned to either subrace. When these are incorporated within Mediterranean/Asia Minor/Africa, the bs score increases to 100. Similarly for the Asia race, there are two well supported subraces Asia1 (81) and Asia2 (94), but five individuals, four from Indonesia and one from North India, do not fit into either.

Fig. 1. Strict consensus of trees from weighted parsimony with bootstrap scores >60%. Biotypes identified within each race or subrace are indicated as are the major races. These were obtained from Bedford *et al*. (1994), Brown *et al*. (1995b, 2000) and Perring *et al*. (2001). The letters for the corresponding biotypes form a suffix for some of the individuals sequenced. These sequences were obtained from individuals in the type colonies kept at John Innes Institute. [1]Brown (2000), [2]Viscarret *et al*. (2003), [3]Berry *et al*. (2004), [4]Perring (2001).

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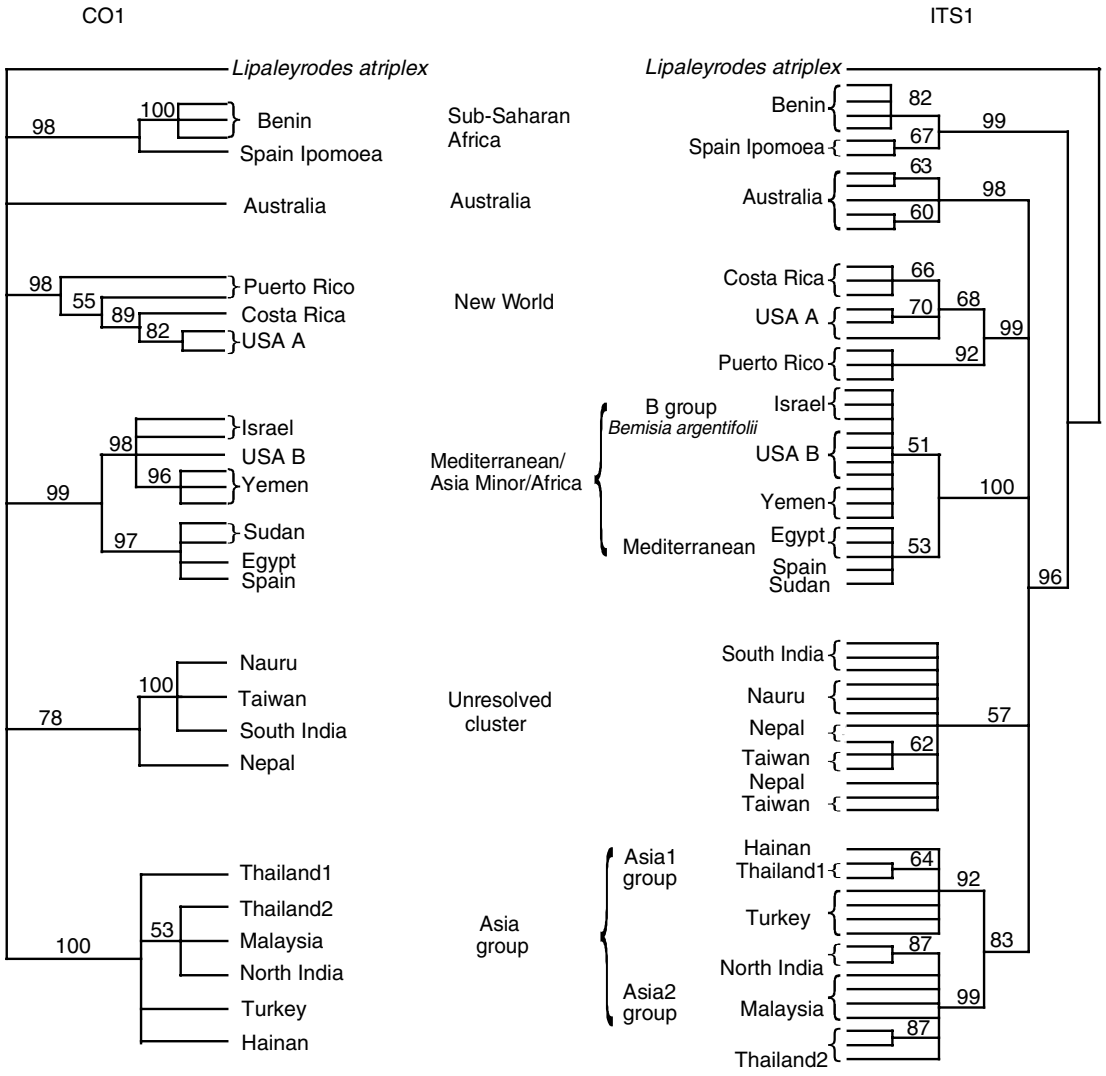

Fig. 2. Two consensus trees summarizing multiple weighted parsimony trees from the matched CO1 and ITS1 datasets.

Phylogenetic analysis using matched CO1 and ITS1

Figure 2 shows two consensus trees summarizing multiple weighted parsimony trees from the matched CO1 and ITS1 datasets. Identical groupings occur except that the Asia1 and Asia2 subraces are not apparent from the CO1 data and the B group and Mediterranean subraces are scarcely distinguishable using this subset of the ITS1 data. The cluster we describe as unresolved has moderate support from CO1, but not from ITS1. Although there is no major disagreement between the CO1 and ITS1 trees an attempt at combined analysis was not made because some geographic localities showed multiple ITS sequences, multiple CO1 sequences, or both. The resulting tree from combining the data would of necessity be consistent with the separate trees, but any further resolution would be greatly affected by which ITS sequence was matched against which CO1 sequence, a situation that could not be achieved unambiguously.

Minimum spanning networks

The minimum spanning networks (figs 3, 4) identified the same races and subraces identified in both the individual CO1 and ITS1 trees (fig. 2). Overall the analyses identify six well-supported major clades, Asia, Bali, Australia, sub-Saharan Africa, Mediterranean/Asia Minor/Africa and New World, and a cluster of unassigned individuals from Asia.

Mating studies

Table 3 summarizes published data generated from mating studies and represents a small cross-section of known genotypes. Crosses between individuals representing the major races resulted in males only, as did matings between some of the unresolved Asian cluster and one of the six defined races, indicating reproductive incompatibility. Matings between individuals belonging to subraces within the same major race resulted in fertile female progeny.

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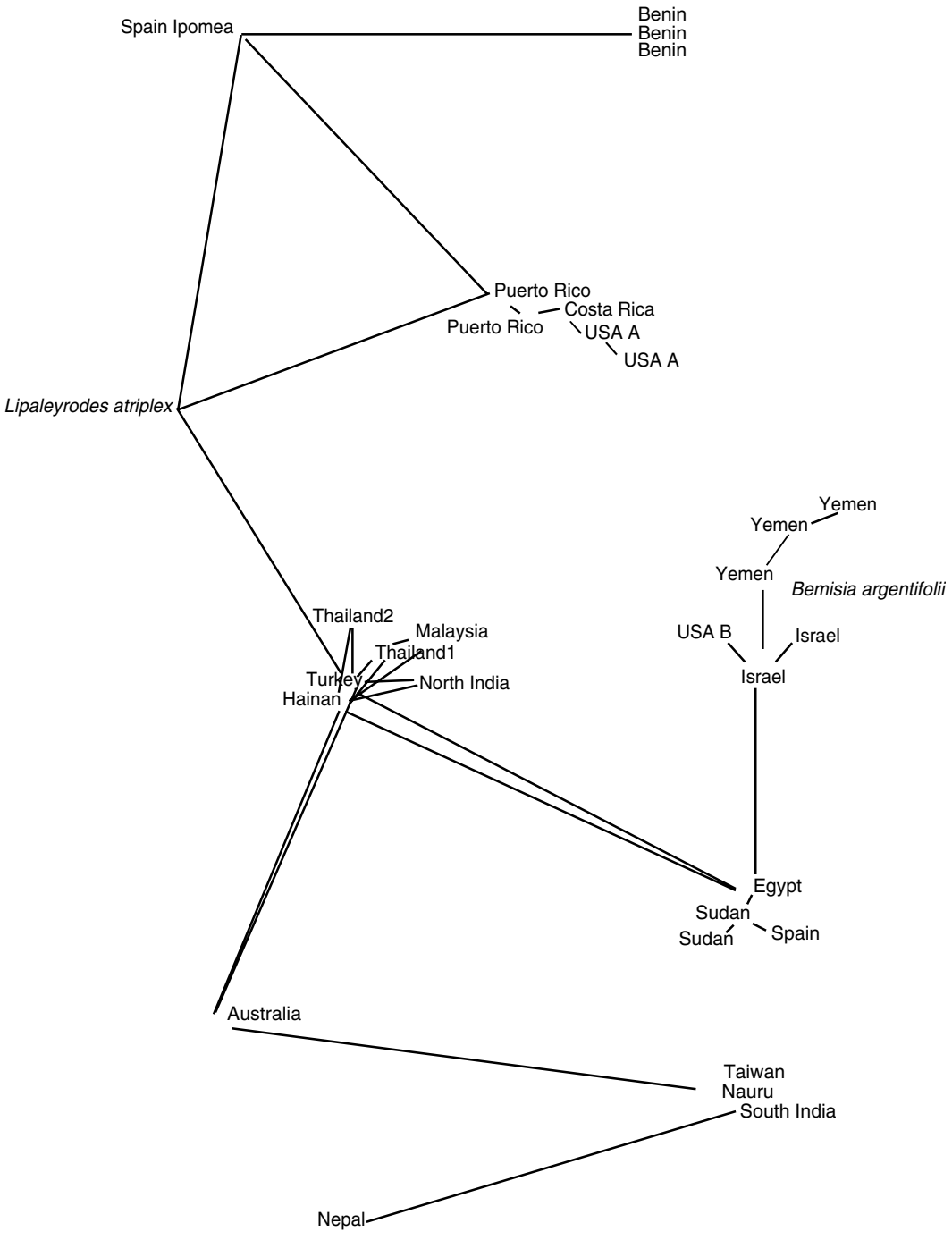

Fig. 3. Minimum span network, matched CO1.

Discussion

The problem surrounding the taxonomy of *B. tabaci* is a new version of an old problem, that is, how to treat a group that shows no conclusive morphological differences, but a range of biological factors that clearly distinguish some races? The association of races with particular geographic regions suggests isolation is the key mechanism driving the range of genetic diversity observed in *B. tabaci*. This is further supported by the failure of matings between representatives of the different races to produce fertile female offspring. The lack of evidence for gene flow and the geographic separation of race, point towards allopatric divergence as the process that best explains the observed relationship between different *B. tabaci* races. In contrast, evidence for sympatric divergence is weak and there is

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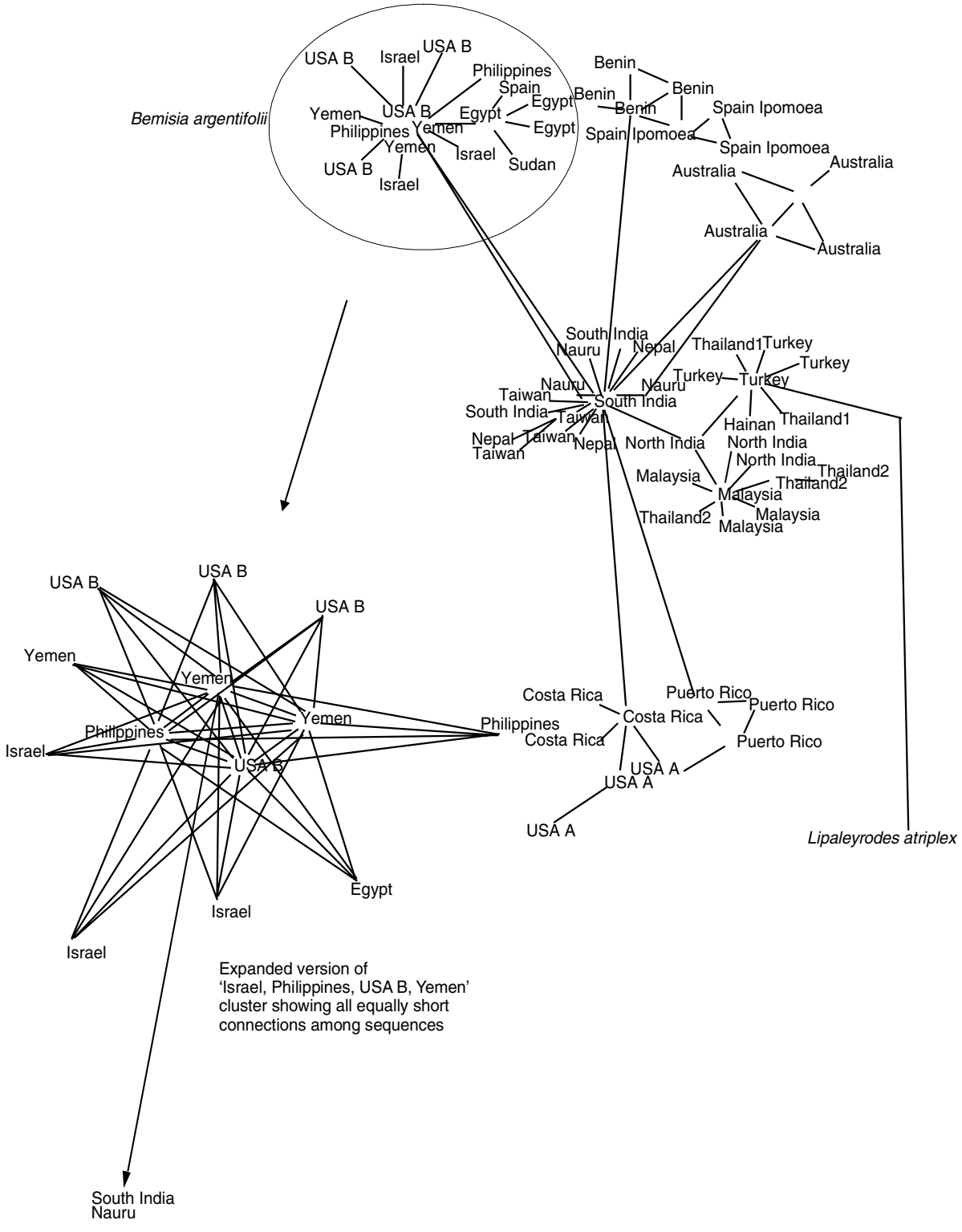

Fig. 4. Minimum span network, matched ITS1.

certainly no compelling basis for assuming segregation based on differential host utilization. There are only two examples of *B. tabaci* genotypes that are restricted to a small number of hosts, the sub-Saharan Africa cassava race (Burban *et al*., 1992; Abdullahi *et al*., 2003) and the N-biotype (New World race) on *Jatropha* (Bird, 1957). In the case of

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the former this can only be a new association as cassava is exotic to this region having been introduced in the late 16th century, unless it was introduced along with the crop plant which seems unlikely as these genotypes are very different from the genotypes found in the New World. In contrast, the overwhelming picture of *B. tabaci* is one of an ability to utilize multiple hosts, with many of these being common to most if not all genotypes (De Barro, 1995).

A major question is whether geographic isolation has been sufficient to push *B. tabaci* races to the level of individual species status. Based on sequence data alone there is no evidence for this. It is possible that wider differences, indicative of biological separation and thus of species status, exist in other parts of the genome. However, the molecular data available to date identifies only small breaks amongst the sampled sequences such that these samples can minimally be formed into clusters. The relationships among the major races are obscure and some sequences fall between the major races. If new sequences were available, would they fall within an existing race or would they tend to break down the separation between clusters? At this point it is not known.

Similarly, although mating studies to date show each major race to be a separate entity, gene flow through the ungrouped forms cannot yet be ruled out. Let us take the biological species concept of Mayr (1942, 1963) as a working definition of species. It has yet to be shown that the many ungrouped forms, only a few of which, it is certain, are included in our present sample, do not provide a bridge for gene flow among the major races.

There is a reasonable expectation that individuals from the B group and Mediterranean subraces will successfully interbreed in the laboratory, as will individuals of the Asia1 and Asia2 subraces. However, the question of whether they do so in the field is open to doubt as the only study examining this, Moya *et al*. (2001), has shown no evidence for in-field hybridization. There are no data on whether individuals from the Asia1 and Asia2 subraces can successfully interbreed.

The molecular evidence fails to show any clear pattern of relationships among these races and leaves many individuals unplaced or only weakly placed; while, although mating studies have shown that some combinations of biotypes are infertile, key experiments involving gene flow through the ungrouped forms have not been tried. Without this information the case for raising the six major races to species status is too weak and as a consequence they should remain as races.

It remains to decide what to do with *B. argentifolii*, which, as indicated fig. 1, is a member of the Mediterranean/Asia Minor/Africa race. Gennadius described *B. tabaci* from a whitefly on tobacco in Greece in 1889. Given the strong geographic pattern of distribution of each of the *B. tabaci* races, it is probable that Gennadius described an individual belonging to the race known as Mediterranean/Asia Minor/Africa. This in effect makes *B. argentifolii* a junior synonym of *B. tabaci* and as such this name becomes redundant and its use should be discontinued.

In conclusion, the data argue for the recognition of six races and a number of ungrouped genotypes under the single *Bemisia tabaci* (Gennadius) species name. In cases where the identity of the race to which the *B. tabaci* under investigation is known, the following nomenclature should be used, *B. tabaci* (Asia), *B. tabaci* (Bali), *B. tabaci* (Australia), *B. tabaci* (sub-Saharan Africa), *B. tabaci* (Mediterranean/Asia Minor/Africa) and *B. tabaci* (New World), to provide a guide to their overall relationship.

There is insufficient data to go the further step of raising these races to species status. Molecular distances among the races are trivial although in some cases the ability to interbreed has been lost. More data are needed on those crosses that remain viable and whether genes may spread throughout the complex by a stepping-stone process, through intermediate races, either within our unresolved grouping or which have as yet not been identified. Molecular data on as-yet unsampled individuals may show whether the six races are strongly separate from each other or are connected by intermediate forms. This may shed light on relationships among the five non-sub-Saharan Africa races and the poorly resolved cluster (which itself may or may not be monophyletic). At present these races are, in effect and because bootstrap support for them is lacking, sunk into a large polytomy at the base of the tree.

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