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Phylogenetic relationships, diversification and expansion of chili peppers (*Capsicum*, Solanaceae)

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• **Background and Aims** *Capsicum* (Solanaceae), native to the tropical and temperate Americas, comprises the well-known sweet and hot chili peppers and several wild species. So far, only partial taxonomic and phylogenetic analyses have been done for the genus. Here, the phylogenetic relationships between nearly all taxa of *Capsicum* were explored to test the monophyly of the genus and to obtain a better knowledge of species relationships, diversification and expansion.
• **Methods** Thirty-four of approximately 35 *Capsicum* species were sampled. Maximum parsimony and Bayesian inference analyses were performed using two plastid markers (*matK* and *psbA-trnH*) and one single-copy nuclear gene (*waxy*). The evolutionary changes of nine key features were reconstructed following the parsimony ancestral states method. Ancestral areas were reconstructed through a Bayesian Markov chain Monte Carlo analysis.
• **Key Results** *Capsicum* forms a monophyletic clade, with *Lycianthes* as a sister group, following both phylogenetic approaches. Eleven well-supported clades (four of them monotypic) can be recognized within *Capsicum*, although some interspecific relationships need further analysis. A few features are useful to characterize different clades (e.g. fruit anatomy, chromosome base number), whereas some others are highly homoplastic (e.g. seed colour). The origin of *Capsicum* is postulated in an area along the Andes of western to north-western South America. The expansion of the genus has followed a clockwise direction around the Amazon basin, towards central and south-eastern Brazil, then back to western South America, and finally northwards to Central America.
• **Conclusions** New insights are provided regarding interspecific relationships, character evolution, and geographical origin and expansion of *Capsicum*. A clearly distinct early-diverging clade can be distinguished, centred in western–north-western South America. Subsequent rapid speciation has led to the origin of the remaining clades. The diversification of *Capsicum* has culminated in the origin of the main cultivated species in several regions of South to Central America.

Key words: *Capsicum*, chilli peppers, phylogeny, pungency, flowering features, dysploidy, geographical expansion, South America.

INTRODUCTION

Capsicum (Solanaceae), with approx. 35 species (Carrizo García *et al*., 2013), is native to tropical and temperate Americas and distributed from Mexico to Brazil, Paraguay and Central Argentina. The genus is of great economic importance because it includes the sweet and hot chili peppers, which are vegetables and spices cultivated and consumed worldwide. The economically most important species belong to the *Capsicum annuum* complex (*C. annuum*, *C. chinense* and *C. frutescens*); two other species (*C. baccatum* and *C. pubescens*) are cultivated predominantly in Latin America (Pickersgill, 1997).

Capsicum species are shrubs (annuals in cultivation and as weeds) that produce flowers with mostly stellate to rotate corollas (exceptionally also urceolate or campanulate) that show diverse patterns of pigmentation, and fleshy, mostly globose berries of different sizes and colours (Figs 1 and 2). At the morphological level, *Capsicum* exhibits an exceptional feature in Solanaceae, which is an entire cup-shaped calyx, mostly with five to ten teeth as nerve prolongations (Figs 1 and 2), shared only with *Lycianthes*. The two genera differ in two main characters: the anther opening by longitudinal slits and the presence of a nectary in *Capsicum*, in contrast to the anther opening by apical pores and the absence of a nectary in *Lycianthes*. Another remarkable feature of *Capsicum* is the occurrence of dysploidy, as the base chromosome number can be either $x = 12$ or 13 (Moscone *et al*., 2007). Probably the most singular character in *Capsicum* is fruit pungency due to the production of capsaicinoids, an exclusive group of alkaloids synthesized in the placenta of the fruits (Stewart *et al*., 2007) and also in the pericarp in 'super-hot' chili peppers (Bosland *et al*., 2015). However, non-pungent fruits are produced in some species (e.g. *C. rhomboideum*, *C. geminifolium*) and particular cultivars (Stewart *et al*., 2007).

Family-wide analyses of Solanaceae have recovered *Capsicum* and *Lycianthes* as sister taxa (Olmstead *et al*., 2008; Särkinen *et al*., 2013). The two genera are now regarded as the only members of tribe Capsiceae (Olmstead *et al*., 2008).

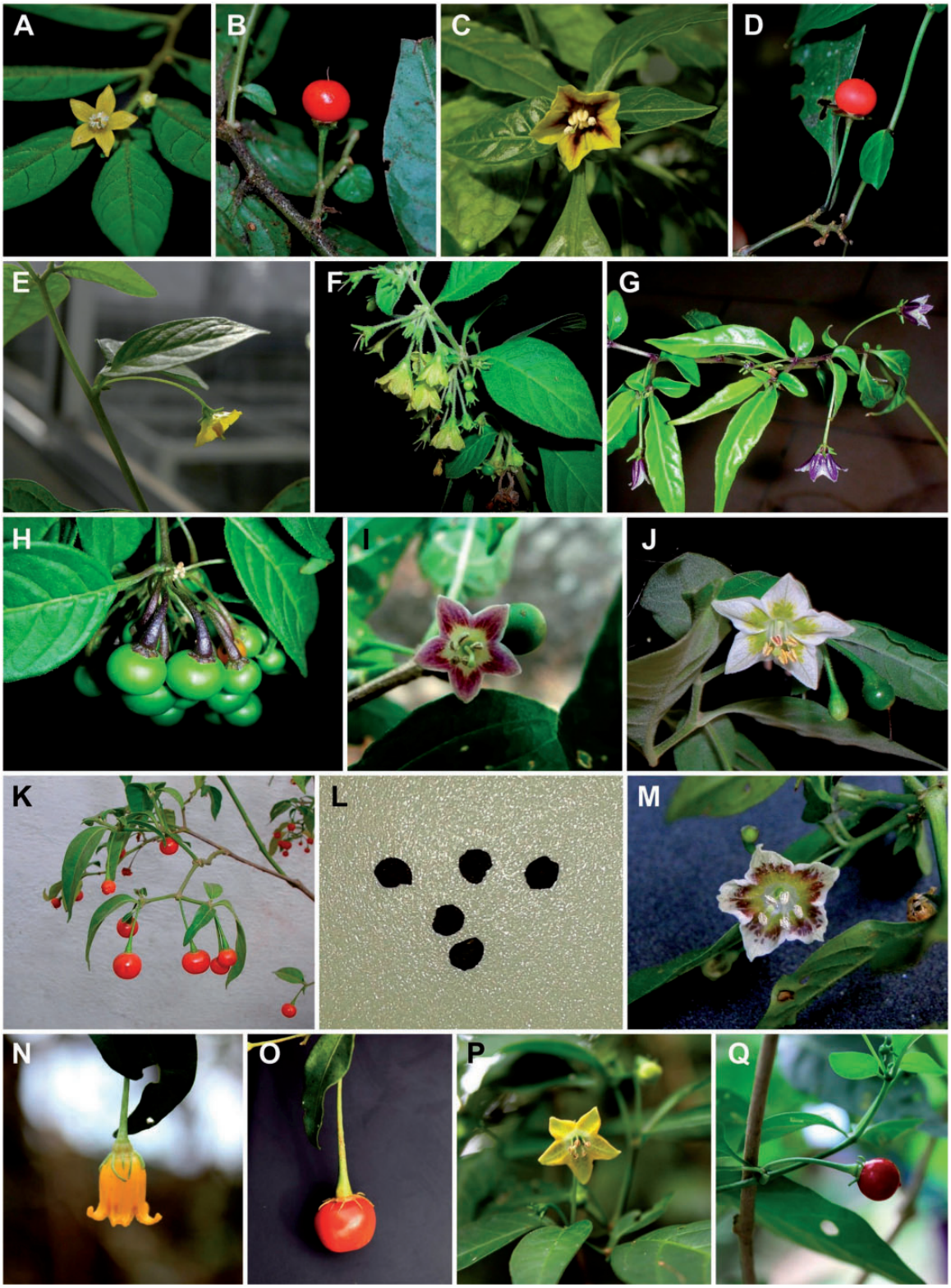

FIG. 1. Flowers and fruits of the Andean (A–G), Caatinga (H, I), Flexuosum (J–M) and Bolivian (N–Q) clades. (A, B) *Capsicum dimorphum* flower (A) and fruit (B). (C, D) *Capsicum geminifolium* flower (C) and fruit (D). (E, F) Different accessions of *C. rhomboideum* showing variations in corolla shape (rotate-campanulate in E vs. campanulate in F) and flower arrangement (solitary flowers in E vs. multi-flowered fascicles in F); pedicels always non-geniculate. (G) *Capsicum lanceolatum* flowering branch showing non-geniculate pedicels and white and violet corollas. (H) *Capsicum caatingae* fascicle of immature fruits (note the toothless calyx). (I) *Capsicum parvifolium* flower and immature fruit. (J–L) *Capsicum flexuosum* flower (J), fruiting branch, showing red pendant mature fruits (K) and blackish brown seeds (L). (M) *Capsicum* aff. *flexuosum* flower. (N, O) *Capsicum caballeroi* flower (N) and mature fruit (O) showing fully yellow corolla, non-geniculate pedicels and red pericarp. (P, Q) *Capsicum minutiflorum* flower (P) and mature dark red fruit (Q). Photos by G. Beltrán (A, B, D), C. Carrizo García (C, E, F, J–Q), M. Sterpetti (G, H) and G. Barboza (I).

Although the validity of the genus *Capsicum* has not been questioned, its relationship with *Lycianthes* is not yet well understood. *Lycianthes* species are grouped in a few major clades that either split in a polytomy, which includes a clade that contains all *Capsicum* species (Guzmán *et al*., 2009), or form successive sister branches of which only one is sister to the *Capsicum* clade (Olmstead *et al*., 2008; Särkinen *et al*., 2013). Therefore, *Lycianthes* is paraphyletic if both genera are recognized in their current circumscription.

Several morphological taxonomic studies have focused on *Capsicum*, but all of them have covered only part of the genus (e.g. Hunziker, 1950, 2001; Heiser and Smith, 1953; Eshbaugh, 1979; Hernández-Verdugo *et al*., 1999; Barboza and Bianchetti, 2005; Barboza *et al*., 2011). The recent molecular phylogenetic analyses were based on fewer than half of the species of the genus (Walsh and Hoot, 2001; Jarret and Dang, 2004; Guzmán *et al*., 2009; Sehr *et al*., 2013). These studies place *C. rhomboideum* [= *C. ciliatum*] as a sister to all other *Capsicum* species studied. At the opposite end, these studies characterize the *C. annuum* complex as the most derived clade. However, many interspecific relationships are unresolved and uncertainties remain due to insufficient species sampling.

Two plastid markers, *matK* and the *psbA-trnH* intergenic spacer, not yet used for *Capsicum*, and one single-copy nuclear gene, *waxy*, already applied (Walsh and Hoot, 2001; Jarret and Dang, 2004), are here used to explore the phylogenetic relationships among a considerably enlarged sample of *Capsicum* taxa. Our main objectives are to test the monophyly of *Capsicum* and to resolve its internal relationships. The resulting phylogenetic trees should help in recognizing natural species clades, exploring patterns of character evolution and reconstructing the eco-geographical expansion of the genus.

MATERIALS AND METHODS

Taxon sampling

Thirty-four of approx. 35 *Capsicum* species were analysed, including all seven varieties, as well as one undescribed species and three accessions with doubtful identification (Supplementary Data Appendix S1). Nine additional species belonging to six genera of tribes Capsiceae, Physalideae and Solaneae according to Olmstead *et al*. (2008) were included as outgroups (Appendix S1), although *Jaltomata bicolor* alone was designated as the outgroup in all analyses.

Multiple accessions were sampled for several taxa, particularly for those that are widespread and/or morphologically variable. However, only a single accession was included in the analyses when all the sequences obtained from different accessions were identical within a taxon (i.e. *C. annuum*, *C. chinense*, *C. frutescens*, *C. baccatum* vars. *baccatum*, *pendulum* and *umbilicatum*). More than one accession per species was included only when the samples had quite different origins (e.g. *C. tovarii*, *C. praetermissum*) and/or when there were differences in the sequences (e.g. *C. dimorphum*).

DNA extraction, amplification and sequencing

Total genomic DNA was isolated using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) or the CTAB method (Doyle and Doyle, 1987), modified for mini-columns, either from frozen leaves kept at –80 °C or silica gel-dried leaf material.

Two plastid DNA markers were analysed, the maturase K gene, including non-coding parts of the *trnK* introns (the whole region is subsequently indicated as '*matK*'), and the *psbA-trnH* intergenic spacer. Sequences between exons 2 and 10 of the single-copy nuclear gene *waxy* (GBSSI, granule-bound starch synthase) were also analysed (Fig. 3).

The regions of interest were amplified using 10 μL 1·1× ReddyMix PCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA), to which 0·2 μL each primer (25 mM) and 1 μL template DNA were added. In addition, different enhancers were used, that is 0·2 μL 0·2 % bovine serum albumin for the plastid markers and 0·4 μL dimethyl sulphoxide for *waxy*. The *matK* region was amplified in two overlapping fragments using already published primers, with or without modifications (Table 1). Standard primers were use for *psbA-trnH* (Table 1). The *waxy* region was amplified in two, three or four overlapping fragments (Fig. 3) using mostly original primers developed for this study (Table 1). PCR with specific conditions for the different markers and pairs of primers used are summarized in the Supplementary Data (Table S1).

After amplification, PCR products were cleaned up using Exonuclease I (Thermo Fisher Scientific) and Thermosensitive Alkaline Phosphatase (FastAP, Thermo Fisher Scientific) according to the manufacturer's instructions. Cycle sequencing was performed using the BigDye Terminator v3.1 Cycle Sequencing Kit Mix (Applied Biosystems, Foster City, CA, USA) using the same primers as those for PCR but with a concentration of 4 pM. Samples were sequenced on an A3730 DNA Analyzer (Thermo Fisher Scientific, Life Technologies).

Sequence editing and alignment

The raw sequences were edited and assembled in an AutoAssembler (Applied Biosystems). Multiple sequence alignments were performed using Muscle in MEGA5 (Tamura *et al*., 2011), with default parameters, and adjusted visually.

Phylogenetic analysis

Maximum parsimony (MP) analysis was conducted for the combined data set in PAUP* 4.0b10 (Swofford, 2003) using heuristic searches and tree bisection-reconnection (TBR) branch swapping with MulTrees in effect. A total of 1000 replicates of random taxon addition were performed, holding ten trees at each step. All character transformations were treated as equally likely and unordered. To assess support for clades (bootstrap support = BS), 1000 bootstrap pseudo-replicates were performed with equal weights using TBR branch swapping with ten trees held at each step and random taxon addition with 100 replicates. MP analyses were done using individual markers to evaluate their informativeness, but interpretations are based on the combined data set in all cases.

For Bayesian inference (BI) analysis, the best nucleotide substitution model for the combined data set was selected using the Akaike information criterion as implemented in JModelTest

FIG. 2. Flowers and fruits of the Longidentatum (A), Atlantic (B–I), Purple Corolla (J–K), Pubescens (L, M), Baccatum (N, O) and Annuum (P, Q) clades. (A) *Capsicum longidentatum* fruit. (B) *Capsicum cornutum* flower with stellate corolla and geniculate pedicel. (C) *Capsicum pereirae* flower with spotted stellate corolla. (D) *Capsicum friburgense* urceolate-campanulate corolla and pedicel geniculate. (E) *Capsicum mirabile* stellate corolla with dark red spots. (F) *Capsicum hunzikerianum* flower with spotted stellate corolla. (G, H) *Capsicum* sp. nov. (GEB & CCG 3637) stellate corolla with golden-green spots (G) and mature greenish-golden yellow fruit, without well-developed calyx teeth (H). Note the different patterns of spots in the corolla in C and E–G. (I) *Capsicum villosum* var. *muticum* immature fruit; note the absence of well-developed teeth. (J) *Capsicum cardenasii* pendant flower with shortly tubular corolla and non-geniculate flowering pedicel. (K) *Capsicum eximium* flower with stellate corolla. (L, M) *Capsicum pubescens* flower (L) and longitudinal section of a mature fruit showing large blackish brown seeds (M). (N) *Capsicum baccatum* var. *pendulum* flower showing the distinctive green spots in the corolla. (O) *Capsicum chacoense* flower showing immaculate white corolla and geniculate flowering pedicel. (P) *Capsicum annuum* var. *annuum* flowering and fruiting branch showing typical white corolla and entire calyx without well-developed teeth. (Q) *Capsicum chinense* flowering branch showing pendant flowers with non-geniculate flowering pedicels and entire calyx without well-developed teeth. Photos by G. Barboza (A, B, F), M. Sterpetti (C, D) and C. Carrizo García (E, G–Q).

v2.1.3 (Darriba *et al*., 2012). The best-fitting model was GTR+G. BI analysis was done in MrBayes 3.2.2 (Ronquist *et al*., 2012), with five million generations, using a Markov chain Monte Carlo (MCMC) search. The initial 25 % of trees were discarded as burn-in and the remaining trees were used for the construction of a majority-rule consensus tree, accompanied by posterior probability (PP) values.

Character evolution

The evolutionary changes of nine key features (morphological, anatomical and karyological; Supplementary Data Table S2), also relevant for species characterization, were reconstructed using Mesquite (Maddison and Maddison, 2014) following the parsimony ancestral states method ('Trace Character History' tool) on the strict consensus tree from the MP analysis. Delayed transformations were favoured (i.e. parallelisms over reversals) when different equally parsimonious resolutions were obtained. Information on the state of these characters was compiled from the literature or based on personal observations (including fruit tasting to determine pungency in many cases, done by G.E.B.) and coded in a character matrix (Table S2). No polymorphic character states were distinguished within a species/sample.

Ancestral areas reconstruction

A Bayesian MCMC analysis (BBM) was performed using RASP v3.2 (Yu *et al*., 2015) to reconstruct ancestral areas states. The distribution range of the species was based on herbarium collection data. Fourteen geographical areas were defined, based on country boundaries, administrative regions or ecoregions as defined by Olson *et al*. (2001): A, Peru; B, Ecuador; C, Colombia; D, Venezuela; E, Central America

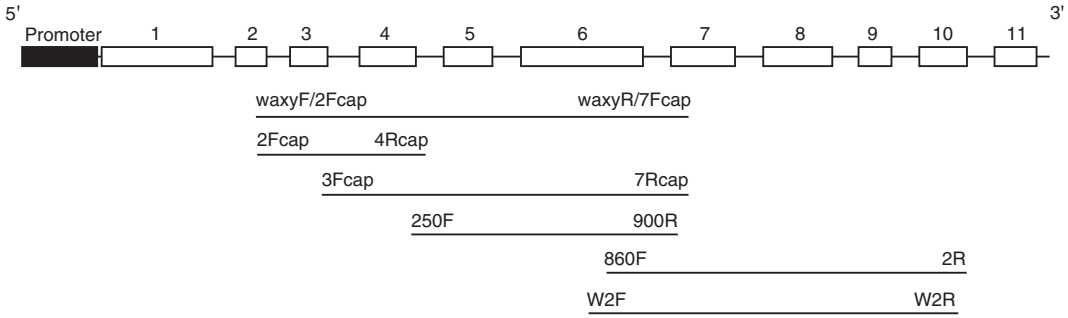

FIG. 3. Schematic structure of *waxy* and position of the primers used in this study (the horizontal lines represent the fragments amplified/sequenced).

TABLE 1. *List of primers used in the present study*

Markers - primers	Primer sequence (5′–3′)	Reference
matK		
−19Fmod*	TGTTTTRACYRTATYGCACTATGTAT	modified from Molvray *et al*. (2000)
matk50-Fdi	GTTTTGACTGTATCGCACTATGTATC	Demaio *et al*. (2011)
850R	TTTCCTTGATACCTAACATAATGCATG	Gruenstaeudl *et al*. (2009)
850R-sol	TTTCCTTGATACCTAACATAATG	modified from Gruenstaeudl *et al*. (2009)
700F	CAATCTTCTCACTTACGATCAACATC	Gruenstaeudl *et al*. (2009)
700F-sol	CCAATCTTTTCATTTACGATCAA	modified from Gruenstaeudl *et al*. (2009)
1710R	GCTTGCATTTTTCATTGCACACG	Samuel *et al*. (2005), Barfuss *et al*. (2005)
trnK-R2	TCGAACCCGGAACTHGTCGG	Wicke and Quandt (2009)
psbA-trnH		
psbA3'F	GTTATGCATGAACGTAATGCTC	Sang *et al*. (1997)
trnHR	CGCGCATGGTGGATTCACAATCC	Tate and Simpson (2003)
waxy		
waxyF	GTTATGACCAATACAAAGATGCCTGGG	this study
2Fcap	CCCCGTTATGACCAATACAAAGATGC	this study
3Fcap	TTCTTTCACTGCTATAAACGTGGGGTTGATCG	this study
250F	CTGSTTCAAAAATCTATGGC	this study
860F	CATAACATTGCCTACCAAGG	this study
W2F	TCTATATGAATGCGAAGG	this study
4Rcap	TGGACAATGAACTTAGGTTCAGCTTGTTGTGTC	this study
waxyR	AAATCGGCCTTGGTAGGCAATGTTATG	this study
7Rcap	CATACCCATCAATGAAATCAAAAGAACTC	this study
900R	GAAATCAAAAGAACTCCTG	this study
W2R	GGTCTCATTCAGTTRCAT	this study
2R	GTTCCATATCGCATAGCATG	Levin *et al*. (2006)

TABLE 2. *Summary statistics obtained from parsimony analyses of the three markers separately and of the combined data set*

Phylogenetic information	*matK*	*psbA-trnH*	*waxy*	All combined
No. of characters	1705	690	2103	4498
No. of constant characters	1578	618	1599	3795
No. of variable characters	127	72	504	703
No. of potentially parsimony-informative characters	42 (2·46 %)	46 (6·67 %)	301 (14·31 %)	389 (8·65 %)
Number of most parsimonious trees	5601	9770	18	2
Tree length (steps)	169	110	757	1051
Consistency Index	0·833	0·791	0·771	0·767
Retention Index	0·940	0·911	0·912	0·908

(including Mexico); F, south-eastern Brazil (Atlantic Forest ecoregion); G, central–eastern Brazil (Caatinga ecoregion); H, north-eastern Argentina and eastern Paraguay (Alto Paraná Atlantic forests ecoregion); I, central-western Paraguay; J, north-western and central Argentina; K, northern; north-eastern and south-eastern Bolivia (mostly lowlands); L, western and south-western Bolivia (mostly highlands); M, western Brazil; and N, Galapagos Islands. The analysis was done using the trees generated after the BI analysis as input file, with five million cycles, ten chains, sampling every 100 cycles, with a temperature setting of 0·1 and fixed JC+G as model. The maximum number of areas for all nodes was set to four. The root was not defined *a priori*.

RESULTS

Phylogenetic reconstruction

MP analysis of the combined data set produced two most parsimonious trees of 1051 steps [consistency index (CI) 0·767, retention index (RI) 0·908; Table 2]. Of 4498 total characters, 8·65 % were potentially parsimony-informative (PI, Table 2). Using the individual markers, the plastid markers produced large numbers of most parsimonious trees (Table 2); in each case, the strict consensus had low levels of resolution in *Capsicum* (not shown). Analysis of *waxy* sequences produced 18 most parsimonious trees (Table 2), with the strict consensus highly resolved at all levels (data not shown). *Waxy* and *matK* had the highest and lowest percentages of PI characters, respectively (Table 2).

In the MP consensus tree and the BI phylogram obtained by using the combined data set, *Capsicum* is resolved as monophyletic with strong branch support (91 % BS, 1 PP; Fig. 4). *Lycianthes* is resolved as the sister group to *Capsicum* (Fig. 4). If MP tree reconstruction is done using only *waxy* sequences, the Andean clade of *Capsicum* (described below) appears nested in *Lycianthes* (77 % BS; data not shown), whereas the rest of *Capsicum* forms a strongly supported monophyletic group (100 % BS). In contrast, using the plastid markers, separately or together, the Andean clade is clearly placed in *Capsicum*, which is resolved as a monophyletic group including all the species here recognized (77, 98 and 99 % BS using *psbA-trnH*, *matK* and both markers, respectively; data not shown).

Within *Capsicum*, the earliest diverging and strongly supported clade (98 % BS, 1 PP) is formed by species from the Andes of western–north-western South America and Central America (Andean clade, Fig. 4). *Capsicum dimorphum* is sister to the remaining species of the clade.

Two morphologically closely related species from Brazilian Caatinga, *C. parvifolium* and *C. caatingae*, are resolved together, here labelled as members of the Caatinga clade (Fig. 4). This clade is strongly supported as sister group to the remaining *Capsicum* species (100 % BS, 1 PP; Fig. 4).

At this point of the trees some weakly supported incongruences can be observed between the MP and BI reconstructions (70 % BS, 0·92 PP; Fig. 4), which concern the placement of the group Flexuosum + Bolivian clades (A), the relatively isolated species *C. longidentatum* (B) and the Atlantic Forest clade (C). In the MP tree their sequence is (A((B,C)(remaining *Capsicum*))), whereas the BI tree places them (C(A(B, remaining *Capsicum*))) (Fig. 4). The species included in each clade are the same in the MP and BI trees. Group (A) is formed by two strongly supported clades that include clearly distinct species. The Flexuosum clade (100 % BS, 1 PP; Fig. 4) comprises *C. flexuosum* and the accession called *C.* aff. *flexuosum*. Second is the Bolivian clade, also with high support in both analyses (88 % BS, 0·99 PP; Fig. 4), which includes four well-separated Bolivian species (*C. coccineum*, *C. caballeroi*, *C. minutiflorum* and *C. ceratocalyx*). Group (B) includes only the relatively isolated *C. longidentatum*, an endemic of the Brazilian Caatinga. It appears as an unsupported sister group to the Atlantic Forest clade in the MP tree (43 % BS; Fig. 4), but in the BI tree as sister to the more advanced Purple Corolla to Annuum clades, though with weak support (0·88 PP; Fig. 4). Group (C) corresponds to the species-rich Atlantic Forest clade, including nine species and two accessions of uncertain taxonomic status, centred in south-eastern Brazil (primarily Rio de Janeiro and São Paulo). According to the slightly differing MP and BI trees, its internal subclades are strongly to weakly supported, leaving interspecific relationships not fully resolved (Fig. 4).

Higher up in the trees, a large superclade containing all the cultivated chilies and a few other species is well distinguished (99 % BS, 1 PP; Fig. 4). Two successive strongly supported clades split at the base, one formed by *C. eximium*, *C. eshbaughii* and *C. cardenasii* (98 % BS, 1 PP; Fig. 4), identified as the Purple Corolla clade, the other is the Pubescens clade with all *C. pubescens* accessions (100 % BS, 1 PP; Fig. 4). *Capsicum tovarii* comes out next as an independent monotypic clade, sister to the remaining species, although with low to medium support (56 % BS, 0·95 PP; Fig. 4).

Nearly all of the cultivated species and their close allies are grouped in a well-supported major clade (91 % BS, 1 PP; Fig. 4). On the one hand, *C. praetermissum*, *C. baccatum* and

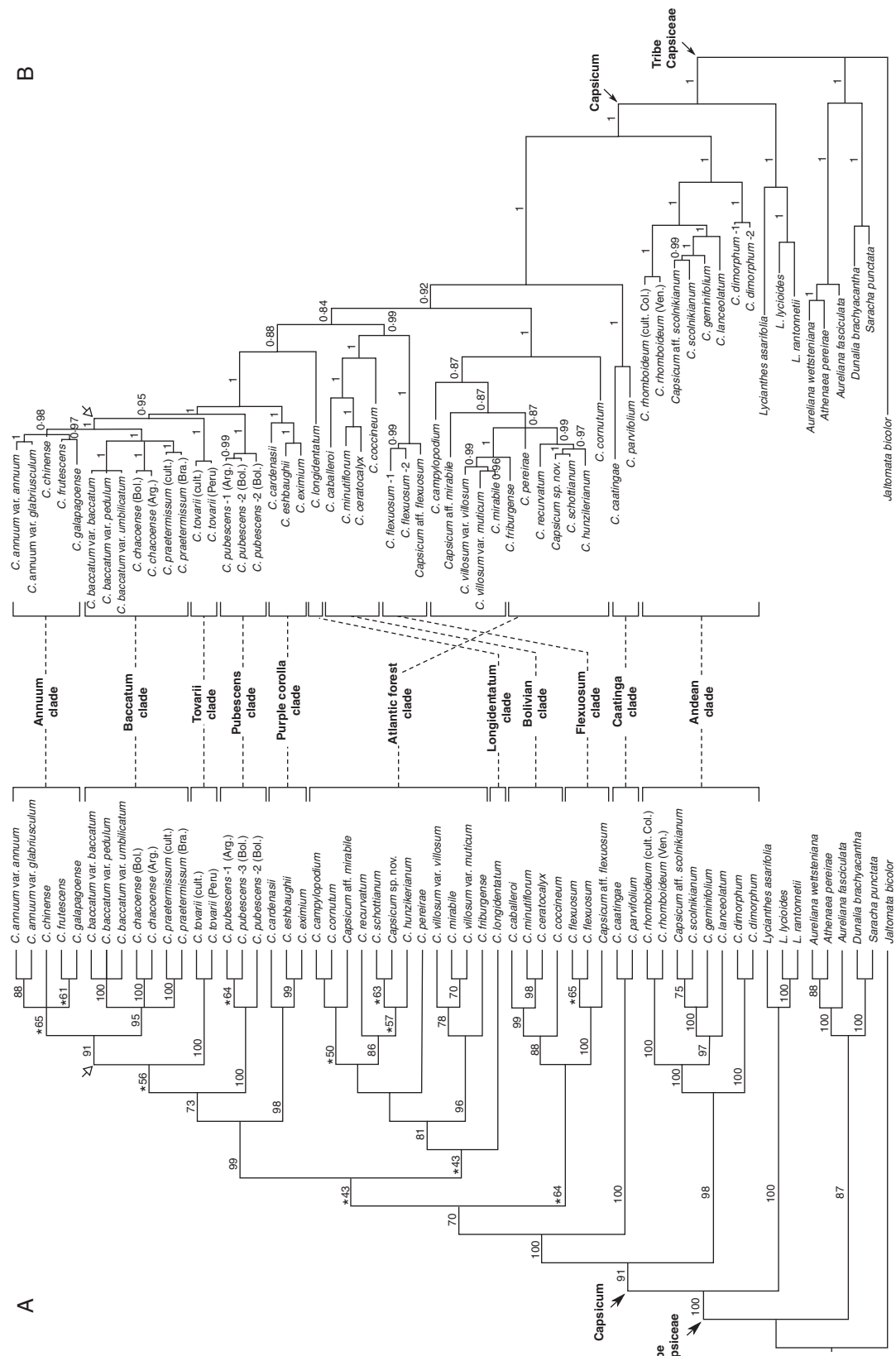

FIG. 4. Phylogenetic reconstruction of *Capsicum* (combined data set of three markers, *matK*, *psbA-trnH* spacer and *waxy*) and species grouping proposed. (A) Strict consensus of two most parsimonious trees obtained from maximum-parsimony analysis. Bootstrap support values, mostly above 50 %, are given on each branch. (B) Phylogram obtained from Bayesian inference analysis. Posterior probabilities above 0·8 are given for each branch. Names of taxa and accessions correspond to those listed in the Table S1. Empty arrows point to the clades including most cultivated chillies. Nodes with low BS (<70 %) are marked with an asterisk.

C. chacoense form a well-supported clade, here called the Baccatum clade (95 % BS, 1 PP). The different samples/varieties of each of these three species form strongly supported monophyletic groups, whereas their relationships to one another remain unresolved (Fig. 4). On the other hand, the three species of the *C. annuum* complex together with the wild species *C. galapagoense* form the Annuum clade, which is not strongly supported (65 % BS, 0·98 PP; Fig. 4). The interspecific relationships in this clade are not fully resolved (Fig. 4).

Based on the phylogenetic reconstructions obtained and the best supported clades recovered, a provisional scheme of species grouping for *Capsicum* is given in Table 3.

Character evolution

The evolution of the characters selected (Table S2) traced over the MP strict consensus tree (Fig. 5) reveals a few features useful for the characterization of different clades (e.g. fruit colour, giant cells in the mesocarp). In contrast, some other characters display high degrees of homoplasy (e.g. toothless calyx, seed colour).

Fruit pungency is clearly a derived phytochemical character in *Capsicum* and seems to have originated after the divergence of the Andean clade (Fig. 5). Occasional reversions to the ancestral non-pungent state are registered in *C. longidentatum*, some cultivars of *C. annuum* (sweet chili peppers) and *C. chinense*, and in some populations of *C. chacoense*, *C. baccatum*, *C. eximium* and *C. flexuosum* (Fig. 5).

The most frequent fruit colour in *Capsicum* is red (Fig. 5), and different shades of red can be observed across the genus (Figs 1B, D, K, O, Q and 2M). This condition is shared with several species of the outgroup genera, including all species of *Lycianthes*, the sister group to *Capsicum* (Fig. 5). Thus, red fruits may be considered a plesiomorphic state, and greenish-golden yellow fruits are derived within *Capsicum* (Fig. 2H). Greenish-golden yellow fruits could have evolved twice or three times, and distinguish the Caatinga clade, *C. longidentatum* and the Atlantic Forest clade (Fig. 5).

The development of giant cells in the mesocarp, generally obvious in cross-section (Fig. 6A vs. Fig. 6B, C), is a derived anatomical feature in *Capsicum*, absent in the Andean clade and with a single reversion in *C. baccatum* var. *umbilicatum* (Fig. 5).

The colour of the seeds varies across the genus, either (pale) creamy ochre or blackish brown. The creamy ochre seed colour is a plesiomorphic character state shared by most *Capsicum* species and the outgroup (Fig. 5). Blackish brown seeds can be considered a homoplastic derived state that characterizes the entire Atlantic Forest clade and may also help to distinguish single species, such as *C. flexuosum*, *C. pubescens* or *C. dimorphum* (Figs 1L, 2M and 5).

The pedicels of the flowers can have a geniculate apex, formed by a 90° angle (Fig. 2B, O). This contrasts with entirely straight pedicels, which keeps the flowers usually in a pendant position (Figs 1E–G, J, N and 2J, Q). Straight, non-geniculate pedicels appear as the plesiomorphic state, present in most outgroup species and in the earliest diverging clades of *Capsicum* (Fig. 5). Geniculate flowering pedicels have evolved several times in *Capsicum*, either in single species or in small clades, and are also present in *Lycianthes asarifolia* (Fig. 5). Reversals to the plesiomorphic state are also observed (e.g. in *C. pereirae* from the Atlantic Forest clade, or in *C. chinense* from the Annuum clade; Figs 2Q and 5).

TABLE 3. *Provisional scheme of a possible grouping of 35* Capsicum *species (and several accessions still without legitimate names) into 11 informal clades (four monotypic) according to their position in the strict consensus tree*

Clade	Species/accessions	
1. Andean	*C. dimorphum**	*C. rhomboideum*
	C. geminifolium	*C. scolnikianum**
	C. hookerianum[1]*	*Capsicum* aff. *scolnikianum*
	C. lanceolatum	
2. Caatinga	*C. caatingae*	*C. parvifolium*
3. Flexuosum	*C. flexuosum*	*Capsicum* aff. *flexuosum*[2]*
4. Bolivian	*C. caballeroi**	*C. coccineum **
	*C. ceratocalyx**	*C. minutiflorum **
5. Longidentatum	*C. longidentatum*	
6. Atlantic Forest	*C. campylopodium*	*C. recurvatum*
	C. cornutum	*C. schottianum*
	C. friburgense	*C. villosum* var. *villosum*[3]
	*C. hunzikerianum**	*C. villosum* var. *muticum*[3]*
	C. mirabile	*Capsicum* aff. *mirabile*[2]*
	C. pereirae	*Capsicum* sp. nov. (GEB & CCG 3637)*
7. Purple Corolla	*C. cardenasii*	*C. eximium*
	*C. eshbaughii**	
8. Pubescens	*C. pubescens*	
9. Tovarii	*C. tovarii*	
10. Baccatum	*C. baccatum*	*C. praetermissum*
	C. chacoense	
11. Annuum	*C. annuum*	*C. frutescens*
	C. chinense	*C. galapagoense*

[1]Molecular data not available, species not included in the present analyses.
[2]Further studies are needed to determine taxonomic rank.
[3]Taxa that should be potentially recognized at species level.
*Taxa without karyological data.

The presence of a non-lobed calyx, with tooth-like prolongations slightly below the calyx rim, is a distinctive character of tribe Capsiceae (*Capsicum* and *Lycianthes*), but there are also species, from both genera, in which the calyx teeth are not well developed or lacking altogether. The toothless calyx is a derived feature observed in particular species (e.g. *C. campylopodium*, *C. flexuosum*, *C. caatingae*) and characteristic of the Annuum clade (Fig. 5). Furthermore, the calyx gross morphology can differ between clades that share character states (Fig. 1H, K vs. Fig. 2H, I, Q).

Two base chromosome numbers have been recorded in *Capsicum*, $x = 12$ versus $x = 13$, and are always constant within a species. The record of base chromosome number is incomplete for the in- and outgroup species included in our analysis. However, $x = 12$ has been registered in several outgroup species (*Saracha punctata*, *Dunalia brachyacantha*, *Lycianthes lycioides* and *L. rantonnetii*), and therefore the base chromosome number $x = 12$ can be traced as the ancestral state in *Capsicum* (Fig. 5). The base chromosome number $x = 13$ has evolved twice and independently in the genus, in the Andean and in the Atlantic Forest clades (Fig. 5). No case of polyploidy has been recorded in wild *Capsicum* species.

Anther opening by longitudinal slits and the presence of a nectary (not mapped on Fig. 5) are plesiomorphic

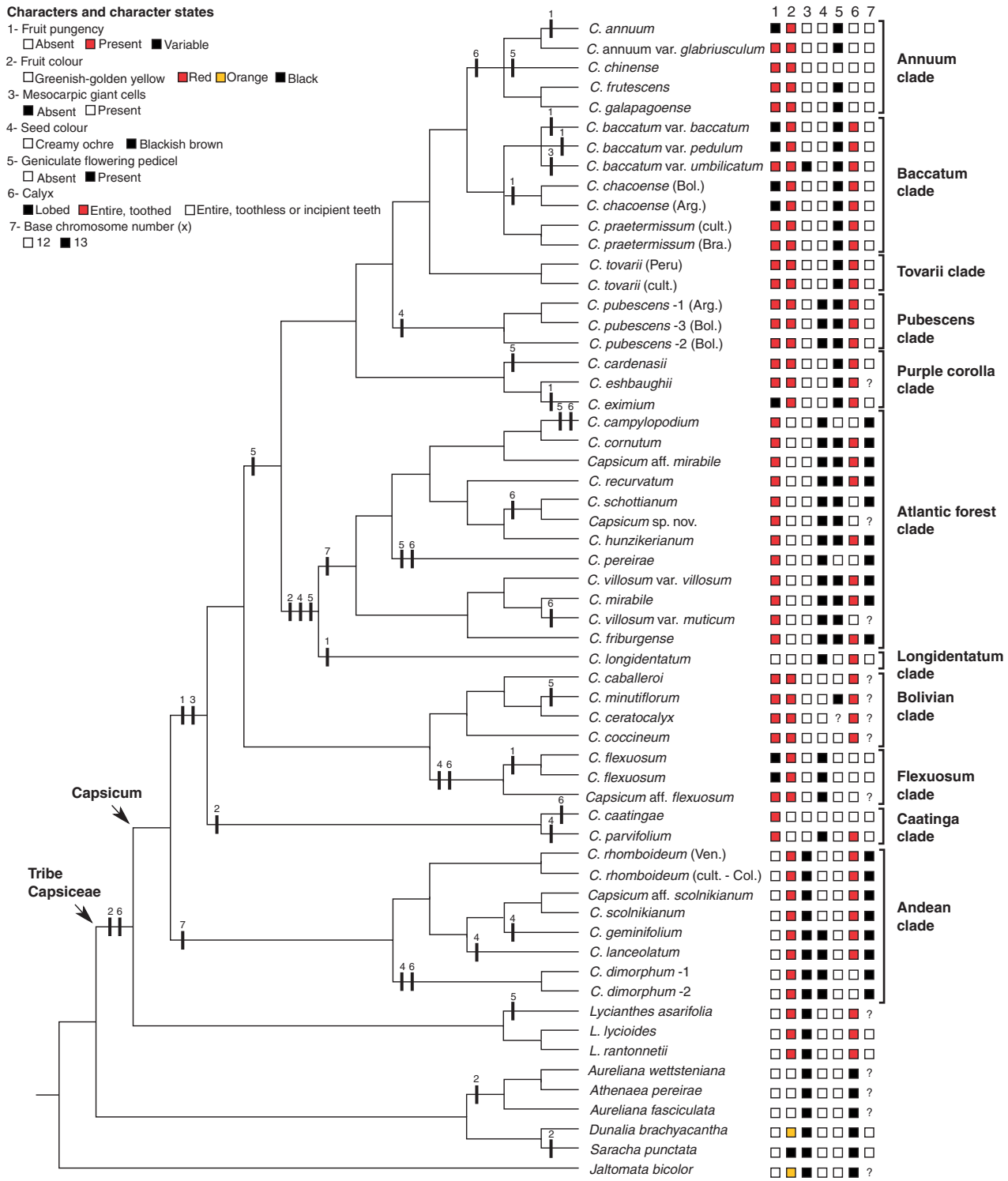

FIG. 5. Evolution of key characters in *Capsicum* using parsimony as reconstruction method. Character state changes are shown by black rectangles with the number of the character (see Table S2; characters 8 and 9 do not vary in *Capsicum*, not shown in the figure) on a maximum-parsimony consensus tree.

character states uniform in *Capsicum* and shared with most species of the outgroup, excluding *Lycianthes*. In fact, these features contrast with the poricidal anther opening and the lack of a nectary observed in *Lycianthes*, typical synapomorphies for this genus.

Reconstruction of ancestral areas

The Bayesian MCMC analysis suggests the origin of *Capsicum* in a broad area including Peru, Ecuador and Colombia along the Andes in western–north-western South America, with Peru having the highest percentage (Fig. 7). The

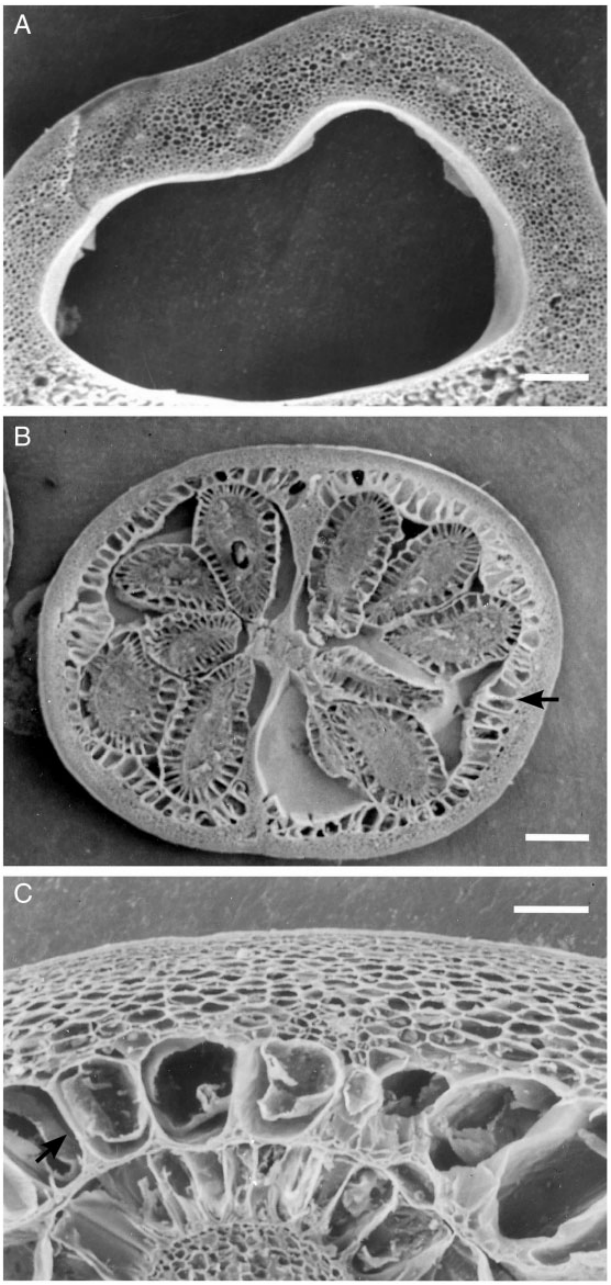

FIG. 6. Fruit pericarp anatomy in *Capsicum* based on scanning electron microscopy in transverse section. (A) Pericarp without giant cells in *C. rhomboideum*. (B, C) Pericarp with giant cells in the innermost layer of the mesocarp (arrow, B) and detail of giant cells (arrow, C) in *C. flexuosum*. Scale bars: A, B, 500 µm; C, 200 µm.

same area has been recovered as the place of origin for the Andean Clade (Fig. 7). The rest of the genus apparently has followed a clockwise expansion and diversification around the subcontinent, avoiding the Amazonian lowlands, starting with the Caatinga Clade from Colombia and central–eastern Brazil (Fig. 7). Then, several clades and lineages apparently have appeared across south-eastern Brazil, Paraguay, northern Argentina, Bolivia and Peru (i.e. the Longidentatum, Flexuosum, Atlantic Forest and Bolivian clades; Fig. 7). This expansion seems to have been followed on the one side by backward developments towards Paraguay, north-eastern–central Argentina and south-eastern Brazil (Baccatum clade; Fig. 7), and on the other side by spreading out northwards to north-western South America and in Central America, including Mexico, and the Galapagos Islands (Annuum clade and further expansion of the Andean and Baccatum clades; Fig. 7).

A particularly important centre of the diversification cycle and the origin of cultivated species is apparently Bolivia, followed by Peru (Fig. 7A). Independently from the differentiation of the Bolivian clade, a common ancestor of all cultivated species and their allies may have evolved in Bolivia, producing at least three lineages, i.e. the Purple Corolla, the Baccatum and the Pubescens clades (Fig. 7A). Another apparently important centre of diversification is in the Andes of western–north-western South America, not only in relation to the origin of *Capsicum* but also as regards later processes of speciation and/or northward species dispersal. This mainly concerns the more recently evolved Annuum clade (Fig. 7A), which probably had several speciation centres (Fig. 7B).

DISCUSSION

Monophyly of Capsicum

The monophyly of *Capsicum* is well supported, despite its heterogeneity with respect to several morphological, anatomical, karyological and phytochemical characters. This has been demonstrated by the DNA–analytical studies on representative *Capsicum* species done so far (Walsh and Hoot, 2001; Olmstead *et al*., 2008; Guzmán *et al*., 2009; Sehr *et al*., 2013) and also in the present contribution. The close relationship between *Capsicum* and *Lycianthes* is strongly supported by the present data and is in line with other recent DNA-based phylogenetic analyses (e.g. Olmstead *et al*., 1999, 2008; Martins and Barkman, 2005; Olmstead and Bohs, 2007; Guzmán *et al*., 2009; Särkinen *et al*., 2013). Therefore, it is fully justified to regard *Capsicum* and *Lycianthes* as the only members of tribe Capsiceae (Olmstead *et al*., 2008; Särkinen *et al*., 2013). This contrasts with the classification proposed by Hunziker (2001), based on homoplastic morphological features, in which *Capsicum* was reunited with *Aureliana* Sendtn., *Athenaea* Sendtn., *Dunalia* Kunth, *Withania* Pauq. and other genera into the large subtribe Capsicinae of tribe Solaneae. This proposal cannot be maintained in view of present evidence. Indeed, all genera of Capsicinae *sensu* Hunziker (2001) except for *Capsicum* are now segregated into different clades of tribe Physalideae (Olmstead *et al*., 2008).

The phylogenetic relationship between *Capsicum* and the much more species-rich *Lycianthes* (approx. 150 species according to Hunziker, 2001) is not yet well understood. If *Capsicum* is recognized as a distinct genus, *Lycianthes* is paraphyletic (Olmstead *et al*., 2008; Guzmán *et al*., 2009; Särkinen *et al*., 2013). The intricate relationship between these two genera is also evident from the MP analysis of *waxy* sequences alone (data not shown).

Major lines of Capsicum *phylogeny and comments on individual clades*

Most of the phylogenetic reconstructions for *Capsicum* presented here are well resolved and strongly supported. This

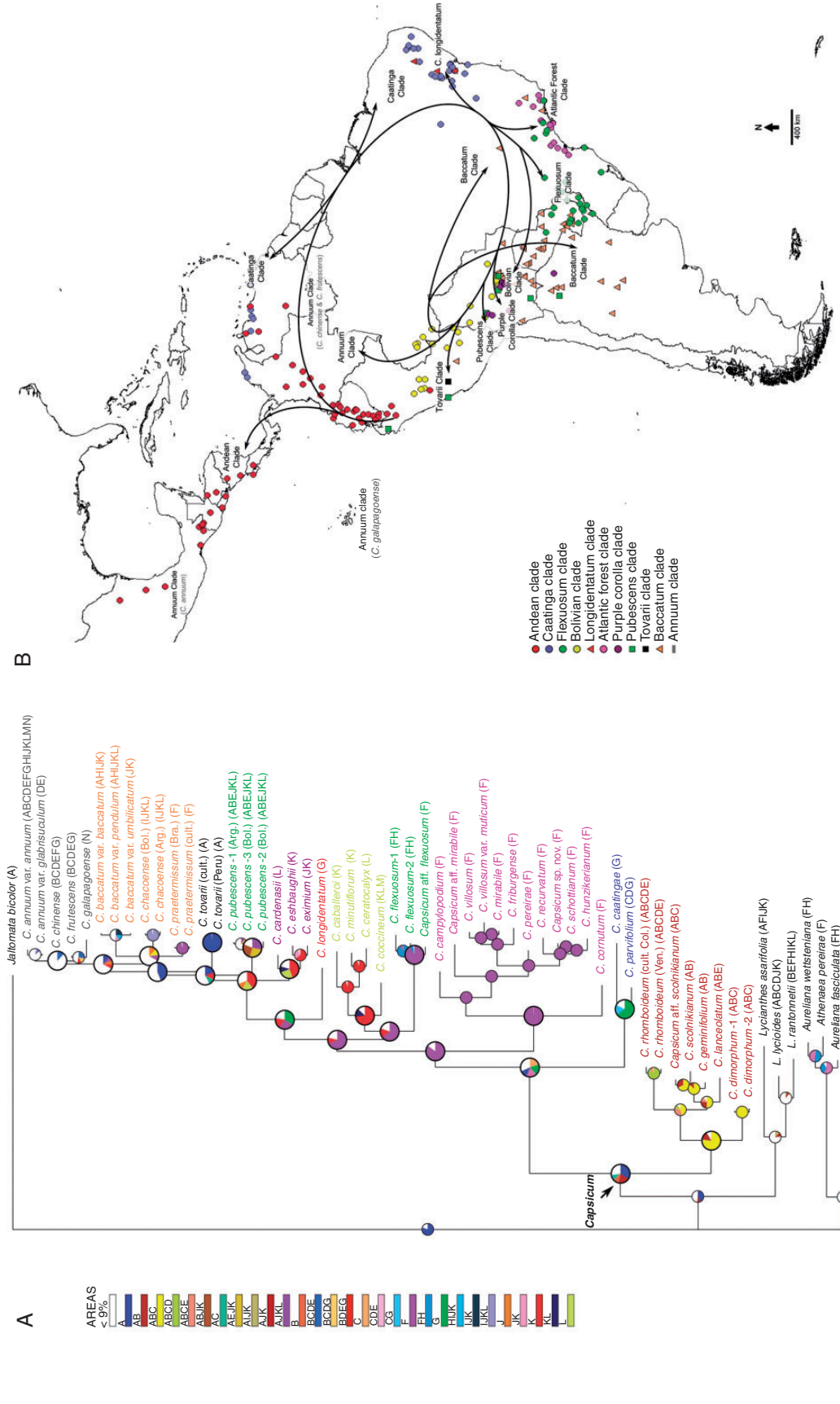

FIG. 7. Hypothesis of *Capsicum* expansion. (A) Ancestral areas reconstructed by Bayesian MCMC analysis. Pie charts are larger for the main nodes to make them more evident. Area assignment for each species is shown after taxon name. Colour codes reflect the major clades based on the phylogenetic results (grey scale for the Annuum Clade). References: A, Peru; B, Ecuador; C, Colombia; D, Venezuela; E, Central America (including Mexico); F, south-eastern Brazil (Atlantic Forest ecoregion); G, central–eastern Brazil (Caatinga ecoregion); H, north-eastern Argentina and eastern Paraguay (Alto Paraná Atlantic forests ecoregion); I, central–western Paraguay; J, north-western and central Argentina; K, northern, north-eastern and south-eastern Bolivia (mostly lowlands); L, western and south-western Bolivia (mostly highlands); M, western Brazil; and N, Galapagos Islands. (B) Schematic expansion of the species. The arrows represent clades and monotypic lineages going across and/or pointing to the areas inhabited by their species. Markings in different colours/shapes indicate selected population localities. In order not to over-complicate the presentation, the taxa of the Annuum Clade are mentioned in their appropriate place but without markings and partly without arrows.

allows us to propose a provisional informal classification of *Capsicum* into 11 clades of which four consist of one isolated species only (Table 3).

The **Andean clade** consists of species native to the Andes of western to north-western South America and Central America (Table 3, Fig. 7A), including *C. rhomboideum*, *C. lanceolatum* and *C. geminifolium*, species found to be sister to the rest of the genus in previous studies (Walsh and Hoot, 2001; Jarret and Dang, 2004; Guzmán *et al.*, 2009; Särkinen *et al.*, 2013). *Capsicum dimorphum*, *C. scolnikianum* and a population apparently related to the latter species are added here to the group. *Capsicum hookerianum*, not sampled in this study, is apparently also a member of the Andean clade (Table 3), following the comments of Hunziker (1961, p. 215) regarding this species. Actually, the close affinities between the species now included in the Andean clade were already recognized long ago by the same author (Hunziker, 1961). Generally these species are characterized by non-geniculate flowering pedicels, primarily rotate to campanulate, yellow to ochre corollas (Fig. 1A, C, E, F), except in *C. lanceolatum* (Fig. 1G), orange–red or red fruits (Fig. 1B, D), mostly blackish brown seeds, the absence of fruit pungency and by the base chromosome number $x = 13$ (Moscone *et al.*, 2007). Of particular importance is the lack of giant cells in the inner mesocarp of these species (Figs 5 and 6A). The deviations of the Andean clade species from other representatives of the genus are so extensive that most of them were originally described as members of other genera, e.g. *Witheringia*, *Acnistus* or *Brachistus* (Barboza, 2011).

The close affinity between the two species that form the small **Caatinga clade**, *C. caatingae* and *C. parvifolium* (Table 3), was discussed and supported by morphological and karyological data by Barboza *et al.* (2011). They described *C. caatingae* and distinguished it from *C. parvifolium* by its multi-flowered fascicles (Fig. 1H vs. Fig. 1I), toothless calyx (Fig. 1H) and ochre seeds. Both species grow in the xerophytic Caatinga ecoregion of central–eastern Brazil, although *C. parvifolium* also extends into northernmost Venezuela and Colombia. *Capsicum longidentatum* is also native to the Caatinga ecoregion, but its close affinity with *C. caatingae*, suggested by Barboza *et al.* (2011), is not supported by the current results. Instead, *C. longidentatum* is resolved as an isolated species and forms the monotypic **Longidentatum clade** of uncertain placement. The species has greenish-golden yellow fruits (Fig. 2A), comparable to those of the Atlantic Forest clade, but differs by the secondary loss of fruit pungency and by $x = 12$ (Barboza *et al.*, 2011). Besides, it is the only *Capsicum* species with branched hairs on young stems, leaves, pedicels and calyces (Barboza *et al.*, 2011). All three species centred in the Caatinga ecoregion have the base chromosome number $x = 12$.

The poorly resolved and differing topologies between the MP strict consensus tree and the BI phylogram (Fig.4) between the Caatinga and the Purple Corolla clades may signal a period of rapid speciation, leading to several, quite well-defined lineages.

Capsicum flexuosum has been affiliated with the *C. annuum* and *C. baccatum* complexes (Buso *et al.*, 2002), but a rather isolated position has also been suggested (Moscone *et al.*, 2007) and its affinities were not resolved in the molecular phylogenetic analysis of Guzmán *et al.* (2009). The present results suggest a closer, although not strong affinity of *C. flexuosum* with the Bolivian clade and justify the recognition of the monotypic **Flexuosum clade**. Typical members of *C. flexuosum* show distinctive pendant flowers with toothless calyces, spotted white corollas and small red berries with black seeds (Fig. 1J–L). A peculiar sample from a single locality, here referred to as *C.* aff. *flexuosum*, differs mainly by its corolla shape and pigmentation pattern (Fig. 1M; Carrizo García *et al.*, 2013). As the present results do not allow a reliable specific separation from typical *C. flexuosum*, it may be considered provisionally as a local variation. *Capsicum flexuosum* is characterized by the base chromosome number $x = 12$ (Pozzobon *et al.*, 2006; Moscone *et al.*, 2007), but there is no record for the *C.* aff. *flexuosum* population.

The species of the **Bolivian clade** are poorly known; three of them were included in the phylogenetic study by Guzmán *et al.* (2009), but not fully resolved and therefore not assigned to any particular group. The clade has dominantly yellow corollas (Fig. 1N, P), but otherwise its four species are diverse with respect to floral structure (Fig. 1N, P), inflorescences and fruit features (Fig. 1O, Q). No chromosome data are yet available for these species. All interspecific relationships in the Bolivian clade are strongly supported. Our results show that *C. caballeroi* and *C. ceratocalyx* are not related to domesticated species growing in the same area (such as *C. pubescens* and *C. baccatum*), supporting the findings of Nee *et al.* (2006). Although the Bolivian clade appears genetically close to *C. flexuosum*, there are no observable synapomorphies.

The species of the **Atlantic Forest clade** are shrubs or small trees characterized by stellate white corollas with different spot patterns (except in *C. friburgense*; Fig. 2C–G), greenish-golden yellow fruits (Fig. 2H), scarcely pungent at maturity, blackish brown seeds and the base chromosome number $x = 13$ (Pozzobon *et al.*, 2006; Moscone *et al.*, 2007). The phylogenetic coherence of this clade is demonstrated by our DNA sequence data and also by the random amplified polymorphic DNA analysis of Buso *et al.* (2003). The Atlantic Forest clade evidently is not directly related to the Andean clade, also with $x = 13$, but has originated independently from ancestors with $x = 12$. According to recent studies (Barboza and Bianchetti, 2005; Moscone *et al.*, 2007; Barboza *et al.*, 2011) and our own results, the Atlantic Forest clade is apparently in a phase of rapid speciation as many of its internal branches have weak or no support. All the taxa/accessions in this clade are endemic to the Brazilian Atlantic Forest, particularly to the coastal rainforests. Most species occupy closely adjacent or even overlapping and relatively small areas (e.g. *C. friburgense*: Barboza and Bianchetti, 2005; Carrizo García *et al.*, 2013). There are several taxonomic issues still to be addressed in this clade. *Capsicum* aff. *mirabile* shares some morphological features with *C. mirabile* (Carrizo García *et al.*, 2013), but is not closely related to it. Like *Capsicum* sp. nov. (GEB & CCG 3637), it seems rather isolated and may deserve specific rank. For *C. campylopodium*, a karyologically and genetically isolated position has already been suggested (Moscone *et al.*, 2007; Särkinen *et al.*, 2013). Finally, *C. villosum* var. *villosum* and var. *muticum* deserve attention, because the latter (Hunziker, 1971; without calyx teeth, Fig. 2I) is genetically closer to *C. mirabile* than to the typical variety. Thus, they should not be regarded as the same species. Even if some interspecific relationships are well supported in

the Atlantic Forest clade, further studies are needed to better understand its interspecific relationships. Chromosome studies may help to clarify controversial situations, as the group is heterogeneous with regard to karyotypes (Moscone *et al*., 2007) and relevant information is still lacking for some taxa/accessions (Table 3).

The traditional 'purple flowered group', i.e. *C. pubescens*, *C. eximium*, *C. cardenasii* and *C. tovarii* (McLeod *et al*., 1982; Eshbaugh, 1982, 1993; Eshbaugh *et al*., 1983), was in part sustained on evidence from interspecific crosses (Eshbaugh, 1979, 1982; McLeod *et al*., 1979, 1983; Choong, 1998; Tong and Bosland, 1999; Onus and Pickersgill, 2004). However, *C. tovarii* was eventually excluded from the group based on the same sort of experiences (Tong and Bosland, 1999; Onus and Pickersgill, 2004). There is conflicting evidence regarding the affinities between *C. pubescens*, *C. eximium* and *C. cardenasii*. The three species occur in Bolivia, with *C. cardenasii* being endemic, *C. eximium* reaching north-western Argentina (Eshbaugh, 1982; Hunziker, 1998; Barboza, 2011) and *C. pubescens* being cultivated from north-western Argentina to Mexico (Bosland, 1996; C. Carrizo García, pers. observ.). Different species groupings have been proposed based on molecular data (Eshbaugh, 1982; Choong, 1998; Walsh and Hoot, 2001; Ryzhova and Kochieva, 2004; Ince *et al*., 2010; Ibiza *et al*., 2012) and it was even suggested that *C. eximium* and *C. cardenasii* form a single, morphologically variable species, to be included in the *C. pubescens* complex (Ibiza *et al*., 2012). Our results disagree with that idea, because *C. eximium* and *C. cardenasii* belong to a different clade from *C. pubescens* and they are easily distinguishable taxa (cf. Fig. 2J vs. Fig. 2K). Moreover, *C. eximium* exhibits a different chromosome banding pattern with less heterochromatin than *C. cardenasii*, which in turn also differs from *C. pubescens* and *C. tovarii* (Moscone *et al*., 2007).

In retrospect and according to our present data (Fig.4), the 'purple flowered group' is better split into three clades. The close affinities between *C. eximium*, *C. eshbaughii* (originally *C. eximium* var. *tomentosum*) and *C. cardenasii* justify the recognition of the **Purple Corolla clade**. The cultivated *C. pubescens*, characterized by strongly violet corollas (Fig. 2L), large blackish brown seeds (Fig. 2M) and dense pubescence on the entire plant, stands as the monotypic **Pubescens clade**. *Capsicum tovarii* is an endemic of the Mantaro river basin in Peru (Eshbaugh *et al*., 1983). Its relationships have always been controversial, with closer affinity suspected with either the purple-flowered group or the white-flowered taxa (Jensen *et al*., 1979; McLeod *et al*., 1979; Tong and Bosland, 1999; Moscone *et al*., 2007; Ince *et al*., 2010; Ibiza *et al*., 2012). Choong (1998) could not resolve its relationships with the Annuum, Baccatum and Pubescens complexes. Our results settle this controversial situation by suggesting an isolated position for *C. tovarii* in the genus as the monotypic **Tovarii clade**, as the sister group to the following mostly white-flowering clades.

The three preceding (Purple Corolla, Pubescens and Tovarii) and the two following (Baccatum and Annuum) clades form a well-supported terminal superclade of *Capsicum* (Fig. 4). The species included in the current Baccatum and Annuum clades have been traditionally distinguished as the white-flowered species (Jensen *et al*., 1979; McLeod *et al*., 1982, 1983; Ince *et al*., 2010; Fig. 2N–Q), usually divided into two groups that roughly match these two clades here recognized. The three species forming the **Baccatum clade** have wide and partially overlapping distribution ranges: *C. praetermissum* (once considered a variety of *C. baccatum*: Hunziker, 1971, 2001) grows in south-eastern Brazil, *C. chacoense* is found from Bolivia to central Argentina and Paraguay, and *C. baccatum* occurs from Bolivia to northern Argentina and south-eastern Brazil, reaching Colombia in the north. The three varieties of *C. baccatum* could not be separated using the present molecular data. Indeed, wild and cultivated forms are usually separated by quantitative features, such as fruit size and shape (Eshbaugh, 1970; G. E. Barboza and C. Carrizo García, pers. observ.). *Capsicum baccatum* var. *umbilicatum* stands out due to the lack of giant cells in the mesocarp (Hunziker and Barboza, 1998). The affinities of *C. chacoense* have been controversial so far, and it has been regarded as being closer to *C. baccatum* (McLeod *et al*., 1983; Pickersgill, 1991; Choong, 1998; Walsh and Hoot, 2001; Ince *et al*., 2010; Ibiza *et al*., 2012), the *C. annuum* complex (Tam *et al*., 2009; Ince *et al*., 2010), to *C. eximium* (Guzmán *et al*., 2009) or the *C. pubescens* complex (Ince *et al*., 2010). Our results show that *C. chacoense* is strongly nested in the Baccatum clade and that no closer affinities to other species can be demonstrated. More informative data are needed to solve the interspecific relationships in this clade.

The analysis of the **Annuum clade** shows *C. galapagoense* nested among the closely related *C. frutescens*, *C. chinense* and *C. annuum*, as already shown by Choong (1998), Walsh and Hoot (2001) and Ince *et al*. (2010). Whereas *C. annuum* var. *glabriusculum* is known as a weed throughout Central and northern South America up to southern North America, *C. annuum* var. *annuum*, *C. frutescens* and *C. chinense* are only known from cultivation. Although the close affinities between *C. annuum*, *C. frutescens* and *C. chinense* have been recognized previously (e.g. Jensen *et al*., 1979; McLeod *et al*., 1983; Prince *et al*., 1995; Baral and Bosland, 2004), different results have been found regarding their interspecific relationships. For instance, closer affinities have been found between *C. annuum* and *C. frutescens* (Toquica *et al*., 2003, Castañón-Najera *et al*., 2008) or between *C. frutescens* and *C. chinense* (Moscone *et al*., 2007; Sanatombi *et al*., 2010; Thul *et al*., 2012), but most studies on these species analysed germplasm restricted to particular regions where they are cultivated, possibly biasing the conclusions. In our results, interspecific relationships are not strongly resolved in the Annuum clade and thus give further evidence for its high genetic uniformity.

Comments on character evolution

Among the characters analysed (Table S2), the different states recorded for geniculate flowering pedicels, calyx teeth and seed colour are useful for identifying species or small clades in combination or with other characters (Fig. 5). Nevertheless, these characters mostly fail in efforts to characterize major clades.

With regard to fruit colour, the state 'greenish-golden yellow' is derived but limited to the Caatinga, Atlantic Forest and Longidentatum clades. All species with greenish-golden yellow fruits are native to Brazil, although they belong to different clades.

The lack of mesocarpic giant cells is typical for the Andean clade and therefore such giant cells are a clearly apomorphic character in all other members of *Capsicum* (Hunziker, 2001: 233–242), except in the cultivated *C. baccatum* var. *umbilicatum*.

Fruit pungency is a peculiar attribute of most *Capsicum* species. Pungency appears as an apomorphic character in the genus, which apparently originated after the split between the Andean clade and the rest of *Capsicum*, with a single specific reversal in *C. longidentatum*. In a recent study it was hypothesized that pungency could have 'originated by unequal duplication of existing genes and owing to changes in gene expression in fruits after speciation' (Kim *et al*., 2014). The genes involved in the biosynthesis of capsaicinoids, the determinants of *Capsicum* pungency, have been partially identified, but there is still much unknown about the evolution and regulation of the capsaicinoid pathway (Kim *et al*., 2014). A few species are polymorphic for the production of capsaicinoids (i.e. pungent and non-pungent genotypes or populations), such as *C. chacoense*, *C. baccatum*, *C. eximium* (Tewksbury *et al*., 2006) and *C. flexuosum* (Garcés-Claver *et al*., 2007), apart from *C. annuum*, with its well-known hot and sweet chili peppers, and rare cases of *C. chinense* (e.g. Stewart *et al*., 2007).

Capsicum is also remarkable because of the occurrence of dysploidy (Moscone *et al*., 2007), an infrequent phenomenon in Solanaceae (e.g. Nicotiana; Clarkson *et al*., 2004). With the exception of rare induced tetraploid cultivars (e.g. Ishikawa *et al*., 2001; Kulkarni and Borse, 2010), the genus is diploid throughout, predominantly with the base chromosome number $x = 12$ (concise data unknown only for a few species/accessions; Table 3). However, coupled with chromosome mutations and two independent dysploid changes from $x = 12 \rightarrow 13$, two different and well-separated groups have originated in *Capsicum*, the Andean and the Atlantic Forest clades, both with $x = 13$. Chromosome counts for species of the outgroup genera of *Capsicum* are scattered and only the base number $x = 12$ has been registered so far (Moscone, 1992; Acosta *et al*., 2005; Chiarini *et al*., 2010). Therefore, the most parsimonious explanation for the occurrence of dysploidy recorded in the two, not closely related clades of *Capsicum* is an independent origin of $x = 13$ linked to the divergence of the Andean and the Atlantic Forest clades. This interpretation is supported by the genetic distance, the karyotype differences (Moscone *et al*., 2007) and the geographical disjunction between the two clades.

Hypothesis on the eco-geographical expansion of Capsicum

The evolutionary diversification of most larger clades of neotropical land plants has been greatly affected by major geological events during the Late Tertiary (since approx. 23 Ma): the continuous uplifting of the Andes (since the Oligocene/Miocene), extensive marine introgressions in north-western South America (Pebas system; before and during the Miocene), and the relatively late opening of the Amazon lowlands towards the Atlantic since the Miocene/Pliocene (Antonelli *et al*., 2009; Hoorn *et al*., 2010; Antonelli and Sanmartin, 2011; Condamine *et al*., 2012). In contrast, the current species diversity apparently has originated during the Quaternary (since approx. 2·5 Ma), under the influence of climatic oscillations (Turchetto-Zolet *et al*., 2013, and references therein). In line with these hypotheses, a recent time-calibrated phylogenetic tree of Solanaceae estimated the split between *Capsicum* and *Solanum* clades and between *Capsicum* and *Lycianthes* at approx. 19 and approx. 13 Ma, respectively (Särkinen *et al*., 2013), i.e. early to mid Miocene. By then, the ancestors of *Capsicum* may have come into existence in the present region of Peru, Ecuador and Colombia (Fig. 7), an area of great importance for Neotropical plant evolution during the Oligocene/Miocene (Antonelli *et al*., 2009). This result differs from earlier proposals, which suggested Bolivia (McLeod *et al*., 1982; Moscone *et al*., 2007) or a continuous belt from south-eastern Brazil to the Andes (Bianchetti, 1996; Pozzobon *et al*., 2006) as ancestral areas for the origin of *Capsicum*. Regarding current species diversity in *Capsicum*, Särkinen *et al*. (2013) recovered a period around 1–3 Ma, i.e. the Quaternary, during which major speciation events may have occurred.

The separation of the Andean clade was dated at approx. 10 Ma (Särkinen *et al*., 2013), i.e. mid Miocene (approx. 12–10 Ma), when the Pebas Lake system may have acted as an isolating factor in the divergence between the Andean clade in the northern Andes and the remaining genus, represented today by members of the Caatinga clade, distributed along the northern part of the Guyana Shield and strongly disjunct in central to eastern Brazil (Fig. 7).

Rapid speciation events may have occurred later from east to west, resulting in the origin of several *Capsicum* clades. Extensive areas have been reconstructed for the common ancestors of the Purple Corolla, Pubescens, Tovarii, Baccatum and Annuum clades, which include north-western Argentina, Bolivia and Peru (Fig. 7). Actually, the place of domestication of *C. pubescens* was hypothesized to be either in mid-elevation regions of Bolivia (McLeod *et al*., 1982; Eshbaugh, 1993) or in northern Bolivia and southern Peru (Moscone *et al*., 2007), whereas southern Bolivia was already suggested as the centre of diversity for *C. baccatum* (Pickersgill, 1969; McLeod *et al*., 1982; Eshbaugh, 1993; Moscone *et al*., 2007).

At least since approx. 6 Ma (early Pliocene), migrations from South America northwards may have increased (Bacon *et al*., 2015). This may have allowed the dispersal and new speciation events of the Andean clade of *Capsicum* towards Central America, as well as later processes of speciation and/or species dispersal from northern South America. The latter concerns the more recent evolution of the Annuum clade and particularly its economically most important species, *C. annuum*, the centre of origin of which is apparently in Mexico (Pickersgill *et al*., 1979; Loaiza-Figueroa *et al*., 1989; Eshbaugh, 1993). The anagenetic origin of *C. galapagoense* after long-distance dispersal to the Galapagos Islands would deserve a particular analysis.

CONCLUSIONS

Eleven well-supported clades (four monotypic) can be recognized in *Capsicum* (Table 3). Their stepwise diversification and expansion can be reconstructed in a clockwise direction from western–north-western South America over a gap in the Amazonian lowlands to central and south-eastern Brazil, then back to central and western South America, and finally northwards to Central America (Fig. 7B). The morphological and

genetic distinctness of the Andean clade stands out in *Capsicum*. Rapid speciation has occurred (and may be still ongoing) in the rest of the genus. This has led to the origin of the high number of currently recognized *Capsicum* species, grouped into the clades recognized here, that can be characterized by a set of particular features. The diversification of the genus has culminated in the origin of the Annuum clade, in several regions of Central and South America, which has spread across the continent, due to the weediness and the domestication, as the well-known cultivated chilies.

SUPPLEMENTARY DATA

Supplementary data are available online at www.aob.oxfordjournals.org and consist of the following. Table S1: PCR conditions for the three markers sequenced, according to the pairs of primers used. Table S2: selected characters and their states used to analyse character evolution and the matrix of codified character states. Appendix S1: Taxa studied: species and varieties, their provenances and voucher specimens and GenBank accession numbers for each marker analysed.

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