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Karyotype Diversification and Evolution in Diploid and Polyploid South American *Hypochaeris* (Asteraceae) Inferred from rDNA Localization and Genetic Fingerprint Data

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• *Background and Aims* Changes in chromosome structure and number play an important role in plant evolution. A system well-suited to studying different modes of chromosome evolution is the genus *Hypochaeris* (Asteraceae) with its centre of species' diversity in South America. All South American species uniformly have a chromosome base number of $x = 4$ combined with variation in rDNA number and distribution, and a high frequency of polyploidy. The aim of this paper is to assess directions and mechanisms of karyotype evolution in South American species by interpreting both newly obtained and previous data concerning rDNA localization in a phylogenetic context.
• *Methods* Eleven *Hypochaeris* species from 18 populations were studied using fluorescence *in situ* hybridization (FISH) with 35S and 5S rDNA probes. A phylogenetic framework was established from neighbour-net analysis of amplified fragment length polymorphism (AFLP) fingerprint data.
• *Key Results* A single 5S rDNA locus is invariably found on the short arm of chromosome 2. Using 35S rDNA loci, based on number (one or two) and localization (interstitial on the long arm of chromosome 2, but sometimes lacking, and terminal or interstitial on the short arm of chromosome 3, only very rarely lacking), seven karyotype groups can be distinguished; five of these include polyploids. Karyotype groups with more than one species do not form monophyletic groups.
• *Conclusions* Early evolution of *Hypochaeris* in South America was characterized by considerable karyotype differentiation resulting from independent derivations from an ancestral karyotype. There was marked diversification with respect to the position and evolution of the 35S rDNA locus on chromosome 3, probably involving inversions and/or transpositions, and on chromosome 2 (rarely 3) concerning inactivation and loss. Among these different karyotype assemblages, the apargioides group and its derivatives constitute by far the majority of species.

Key words: Asteraceae, fluorescence *in situ* hybridization, *Hypochaeris*, karyotype evolution, polyploidy, rDNA, South America.

INTRODUCTION

Karyotypic changes involving chromosome structure, such as inversions, translocations and deletions, and chromosome number by aneuploidy/dysploidy or polyploidy play an important role in plant evolution and speciation (Stebbins, 1971; Lim *et al.*, 2000; Levin, 2002; Lysak *et al.*, 2006; Schubert, 2007). The *in situ* hybridization techniques of genomic *in situ* hybridization (GISH) and, particularly, fluorescence *in situ* hybridization (FISH) allow the tracing of subtle chromosomal changes, and have been successfully applied in numerous plant groups (Zhang and Sang, 1998; Adams *et al.*, 2000; Lim *et al.*, 2000, 2007; Weiss-Schneeweiss *et al.*, 2003, 2007*a*; Clarkson *et al.*, 2005). In an evolutionary context, the markers most often used, especially in non-model organisms, are the 5S and 35S ribosomal genes due to their abundance as 'house-keeping genes' and their relatively conserved nature (Małuszyńska *et al.*, 1998). Interpreting changes in rDNA loci numbers and localizations in related species within a phylogenetic framework (usually derived from molecular data such as DNA sequences or AFLP fingerprints) can be a powerful approach towards obtaining a clearer understanding of mechanisms and directions of chromosomal changes and their impact on plant evolution (Clarkson *et al.*, 2005; Lim *et al.*, 2006; Weiss-Schneeweiss *et al.*, 2007*a*).

A particularly well-suited system to study different modes of chromosome evolution is provided by the genus *Hypochaeris* (Asteraceae, Lactuceae), which includes 50–60 species found in northern Africa, Europe, Asia and South America. Although the Old World group comprises only about 15 species, it shows considerable cytological diversity ($x = 3$, 4, 5, 6; summarized in Cerbah *et al.*, 1998), which agrees well with the current sectional classification based on morphological and molecular data (Samuel *et al.*, 2003, and references therein). By contrast, the more species-rich South American group of 40–50 species (depending on the taxonomic treatment; Lack, 1979; Tremetsberger *et al.*, 2006, and references therein) is

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uniform with respect to its chromosome base number ($x = 4$; summarized in Weiss-Schneeweiss *et al.*, 2007*b*). This agrees with its relatively young age and rapid diversification (Samuel *et al.*, 2003; Tremetsberger *et al.*, 2005). It has only recently been shown that the South American clade is not a member of section *Achyrophorus*, as previously suggested (Hoffmann, 1893), but is instead sister to the Moroccan *H. angustifolia*, a species with $x = 4$ and as yet not assigned to any section (Tremetsberger *et al.*, 2005). These findings have not only allowed inference of a putative origin of the New World species from northwestern Africa, but have also suggested a cytological link between $x = 5$ and $x = 4$ karyotypes (Tremetsberger *et al.*, 2005).

Whereas the European and Mediterranean *Hypochaeris* taxa are well known cytologically (Parker, 1971, 1976; Mugnier and Siljak-Yakovlev, 1987; Barghi *et al.*, 1989; Cerbah *et al.*, 1995, 1998; Hall and Parker, 1995), such detailed information is still lacking for many South American taxa (Weiss-Schneeweiss *et al.*, 2007*b*). Chromosome numbers in the New World *Hypochaeris* have been established for 39 species and two hybrids, with karyotypes and microphotographs available for 30 (Stebbins *et al.*, 1953; Siljak-Yakovlev *et al.*, 1994; Ruas *et al.*, 1995, 2005; Cerbah *et al.*, 1998; Weiss *et al.*, 2003; Weiss-Schneeweiss *et al.*, 2003, 2007*b*). Only 15, however, have been analysed so far with FISH (Cerbah *et al.*, 1998; Weiss-Schneeweiss *et al.*, 2003; Ruas *et al.*, 2005). All the South American taxa examined have very similar bimodal and asymmetrical karyotypes with two large and two small chromosome pairs. The application of molecular cytogenetic techniques, however, has allowed discrimination of seven distinct karyotypic groups, as well as generating hypotheses concerning their relationships (Cerbah *et al.*, 1998; Weiss-Schneeweiss *et al.*, 2003; Ruas *et al.*, 2005).

The aim of this paper is to assess directions and mechanisms of karyotype evolution in South American *Hypochaeris* species. To this end, we have investigated a further eleven species using FISH with 35S and 5S rDNA probes. These findings have been combined with the data already available for 15 other species (Weiss-Schneeweiss *et al.*, 2003; Ruas *et al.*, 2005), all set within a phylogenetic context established from AFLP fingerprint data. Specifically, we (1) test and refine the previously suggested karyotype groups; (2) compare the dynamics of change in chromosome structure and rDNA localization in diploids and tetraploids; and (3) identify the mechanisms involved in karyotype changes and interpret them in a phylogenetic context, allowing us to infer trends and directions of chromosome evolution in this speciating group.

MATERIALS AND METHODS

Plant material

Eleven species and 18 populations of South American *Hypochaeris* were analysed (for localities, voucher numbers and chromosome numbers see Table 1). Surface-sterilized seeds were germinated on wet filter paper in Petri dishes. Two to three days after germination, seedlings were pre-treated with 0·1 % colchicine for 2 h at room temperature and 2 h at 4 °C, fixed in ethanol:acetic acid (3:1) for at least 12 h at room temperature, and stored at −20 ºC.

Fluorescence in situ *hybridization*

Chromosomes were prepared by enzymatic digestion/squashing as described in Weiss-Schneeweiss *et al.* (2003). Fluorescence *in situ* hybridization (FISH) was carried out according to the methods of Schwarzacher and Heslop-Harrison (2000) and Weiss-Schneeweiss *et al.* (2003, 2007*a*) with minor modifications. Probes used for FISH were 35S (= 18S/25S) rDNA from *Arabidopsis thaliana* in plasmid pSK +, and 5S rDNA from *Beta vulgaris* in plasmid pBx1-2, labelled with biotin or digoxygenin (Roche, Vienna, Austria), respectively. Probes were labelled either directly by PCR (5S rDNA) or using a nick translation kit (35S rDNA; Roche, Vienna, Austria). Digoxygenin was detected with antidigoxygenin conjugated with FITC (1 μg mL^{-1}; Roche, Vienna, Austria) and biotin with ExtrAvidin conjugated with Cy3 (3 μg mL^{-1}; Sigma-Aldrich, Vienna, Austria). Analyses of preparations were made with an Axioplan2 epifluorescent microscope (Carl Zeiss, Vienna, Austria), images acquired with a CCD camera and files processed using Axiovision ver. 3·5 (Carl Zeiss, Vienna, Austria). For rDNA localization, a minimum of 30 well-spread metaphases and prometaphases was analysed in each species. Basic karyotype and idiogram construction followed Weiss-Schneeweiss *et al.* (2007*b*).

Phylogenetic analysis

AFLP data were generated following the protocols described previously (Tremetsberger *et al.*, 2006). In each species the products of the restriction–ligation reactions of three individuals, chosen to encompass the whole geographic distribution of the species based on available collections (see Supplementary Information, available online), were pooled together and used as a single sample in the preselective amplification. Following results of Tremetsberger *et al.* (2006), two samples were included for *H. palustris*, one from the Coastal Cordillera of Chile and one from the Andes. The Moroccan endemic *H. angustifolia* was used as the outgroup. To obtain better resolution than in the study of Tremetsberger *et al.* (2006), we added three additional AFLP primer combinations [*Mse*I-CAAG/*Eco*RI-ACT (Fam), *Mse*I-CAAG/*Eco*RI-ACC (Ned), *Mse*I-CTCG/*Eco*RI-AGG (Hex)] to the six already selected [*Mse*I-CAG/*Eco*RI-ACT (Fam), *Mse*I-CAG/*Eco*RI-AGC (Ned), *Mse*I-CTC/*Eco*RI-ACG (Hex), *Mse*I-CTGA/*Eco*RI-ACT (Fam), *Mse*I-CTCG/*Eco*RI-ATC (Ned), *Mse*I-CTTC/*Eco*RI-ACG (Hex)], giving a total of nine selective primer pairs (preselective *Mse*I-primers had two base pairs fewer). Genographer 1·6 (available from http://hordeum.oscs.montana.edu/genographer) was used for scoring band presence and absence. A total of 1415 unambiguously scorable bands, of which

TABLE 1. *South American species of* Hypochaeris *analysed by FISH*

Taxa	2*n*	Location (voucher number)
H. alba Cabrera	8	Argentina, Prov. Corrientes, Mburucuyá, near Estancia Santa Teresa in National Park (*EU*, *KT 156*); Fig. 1A.
	8	Argentina, Prov. Corrientes, Bella Vista, Parque Cruz de los Milagros (*EU*, *KT 159*)
H. caespitosa Cabrera	16	Argentina, Prov. Córdoba, Cerro Los Gigantes (*EU*, *KT 148*); Fig. 1J.
H. elata (Wedd.) Griseb.	8	Bolivia, Depto. La Paz, approx. 3 km on dirt road toward Peñas from main motorway La Paz-Huarina (*TS*, *KT*, *RH 18506*); Fig. 1B.
H. hookeri Phil.	8	Argentina, Prov. Río Negro, 29 km E of junction from routes 23 + 237, on dirt road toward Pichileufu (*TS*, *EU*, *KT 18040*).
	8	Argentina, Prov. Río Negro, Estancia Rayhuao approx. 29 km S of Pilcaniyeu (*TS*, *EU*, *KT 18044*); Fig. 1C.
H. incana (Hook. & Arn.) Macloskie	8, 16	Argentina, Prov. Río Negro, 19 km S Río Ñirihuao near top of Cerro Buitrero (*TS*, *EU*, *KT 18022*); Fig. 1K.
	8	Chile, Región XII, Punta Arenas, in front of the airport (*PS*, *MS*, *AT 5640*); Fig. 1D.
H. parodii Cabrera	8	Argentina, Prov. Jujuy, Laguna Yala, down approx. 2 km from main laguna (*TS*, *EU*, *KT 18057*); Fig. 1E.
H. patagonica Cabrera	8	Argentina, Prov. Santa Cruz, Patagonia, Río Pintura, approx. 3 km NW of Cueva de las Manos (*FE*, *PS 6202*); Fig. 1F.
H. petiolaris (Hook. & Arn.) Griseb.	8	Argentina, Prov. Buenos Aires, Sierra de la Ventana (*EU*, *KT 122*); Fig. 1G.
H. pinnatifida (Speg.) Azevêdo-Gonçalves & Matzenbacher	8	Argentina, Prov. Buenos Aires, Sierra de Tandil, left slope of La Cascada (*KT*, *PSI 1003*); Fig. 1H.
	8	Argentina, Prov. Buenos Aires, Sierra de Tandil, ridge on the right side of La Cascada (*KT*, *PSI 1006*).
H. sessiliflora Kunth.	8	Ecuador, Prov. Cotopaxi, 5·5 km E of Pujilí (*TS*, *KT*, *HV*, *RH 18549*). Fig. 1I.
	8	Ecuador, Prov. Pichincha, 9·5 km S of San Juan on road to Volcán Atacazo, near the antennas (*TS*, *KT*, *HV*, *RH 18536*).
	8	Ecuador, Prov. Pichincha, 12·5 km S of San Juan on road to Volcán Atacazo (*TS*, *KT*, *HV*, *RH 18538*).
H. taraxacoides (Walp.) Benth. & Hook. f.	8, 16	Argentina, Prov. Jujuy, 31·4 km W of Humahuaca on road to El Aguilar (*TS*, *EU*, *KT 18089*); Fig. 1M.
	16	Bolivia, Depto. La Paz, approx. 47 km WNW of La Paz on road to Huarina (*TS*, *KT*, *RH 18508*); Fig. 1L.

Abbreviations of collectors: AT, A. Tribsch; EU, E. Urtubey; FE, F. Essl; HV, H. Valdebenito; KT, K. Tremetsberger; MS, M. Staudinger; PS, P. Schönswetter; PSI, P. Simón; RH, R. Hössinger; TS, T. Stuessy. Vouchers are deposited in CONC, LP, LPB, QUSF and WU.

1385 (98 %) were polymorphic, were revealed in the ingroup. This total increased to 1492, of which 1469 (98 %) were polymorphic, when *H. angustifolia* was also included. Phylogenetic relationships were inferred using the distance-based neighbour-net method (implemented in SplitsTree 4, available from www.splitstree.org; Huson and Bryant, 2006) based on Nei–Li distances (Nei and Li, 1979) calculated with TreeCon 1·3b (van der Peer and de Wachter, 1997). In contrast to commonly used tree-building methods, a network method allows the visualization of potentially conflicting signals, such as may be caused by homoplasy or hybridization (Huson and Bryant, 2006). Group support was assessed via a neighbour-joining bootstrap analysis in TreeCon 1·3b using 1000 pseudo-replicates and Nei–Li-distances. All clades with bootstrap support (hereafter abbreviated to BS) >50 are indicated, irrespective of whether these are supported by splits or not, thus allowing direct comparison of incongruences arising from methodological differences.

RESULTS

Fluorescence in situ *hybridization*

All newly analysed diploid accessions have the single 5S rDNA locus localized on the short arm of chromosome 2 (Fig. 1). By contrast, four different karyotypes can be distinguished based on the localization of 35S rDNA. Thus *Hypochaeris alba*, *H. elata*, *H. hookeri*, *H. incana* (2*x* cytotype), *H. parodii* and *H. pinnatifida* all possess two loci of 35S rDNA, one on the long arm of chromosome 2 and the other interstitial on the short arm close to the centromere of chromosome 3 (Fig. 1A–E, H). The karyotype of *H. sessiliflora* is similar but differs in the terminal position of the locus on the short arm of chromosome 3 (Fig. 1I). Only one locus is present in *H. patagonica* and *H. petiolaris*, and this is located on the short arm of chromosome 3 in a terminal or interstitial position, respectively (Fig. 1F, G).

Whereas all tetraploid species analysed show the expected pattern of the 5S rDNA locus being present on the short arms of all four copies of chromosome 2 (Fig. 1J–M), the 35S rDNA loci number deviates from this strictly additive pattern. The putatively autotetraploid cytotype of *H. incana* has 35S rDNA signals on only two copies of chromosome 2 (Fig. 1K). Although *H. taraxacoides* has diploid and tetraploid cytotypes, only tetraploids were included in the FISH analysis due to under-representation of diploids in the population seed samples. All plants in the uniformly tetraploid *H. taraxacoides* population *18508* have four equally strong 35S rDNA signals at both the locus on the long arm of chromosome 2 and at a

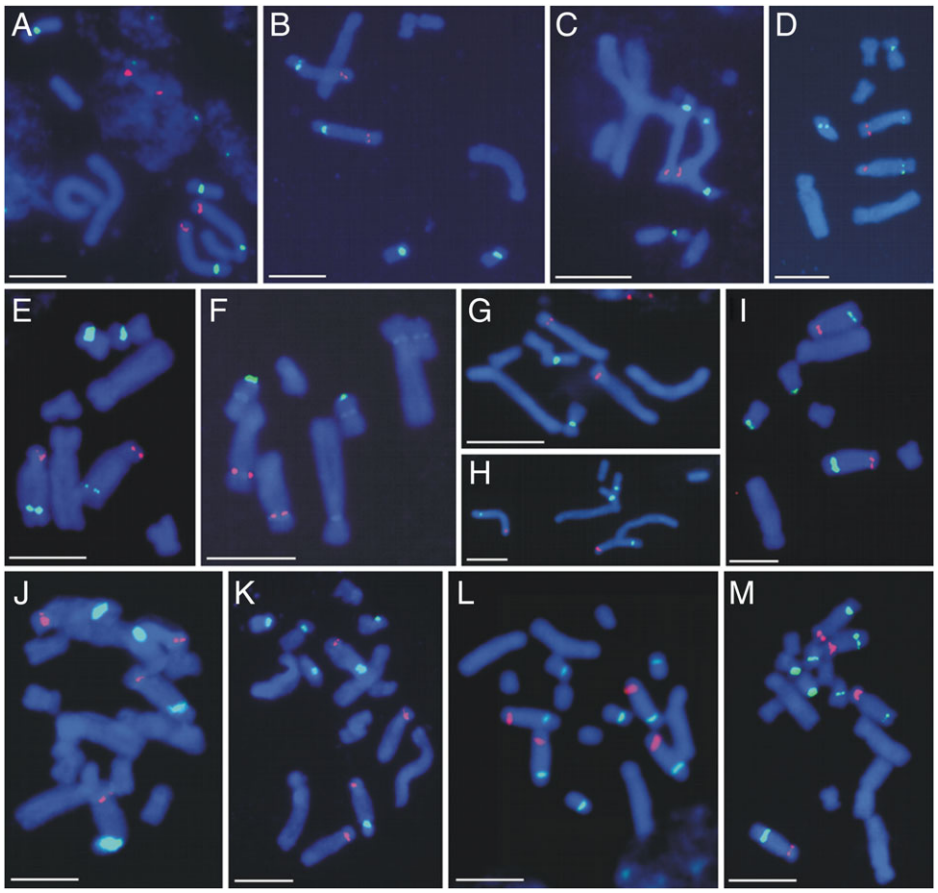

FIG. 1. Localization of 5S and 35S rDNA in chromosomes of diploid (A–I) and tetraploid (J–M) *Hypochaeris* taxa. (A) *H. alba 156*; (B) *H. elata 18506*; (C) *H. hookeri 18044*; (D) *H. incana 5640*; (E) *H. parodii 18057*; (F) *H. patagonica 6202*; (G) *H. petiolaris 122*; (H) *H. pinnatifida 1003*; (I) *H. sessiliflora 18549*; (J) *H. caespitosa 148*; (K) *H. incana 18022*; (L) *H. taraxacoides 18508*; and (M) *H. taraxacoides 18089*. Numbers refer to vouchers (see Table 1). Scale bar = 5 μm.

second locus localized interstitially on the short arm of chromosome 3 (Fig. 1L). By contrast, two individuals from the tetraploid population *18089* revealed a heteromorphic locus on chromosome 3, the signals being much stronger in two of the four homologues (Fig. 1M). In addition, a single individual from this population had three typical acrocentric plus one metacentric chromosome 1 and had lost the secondary constrictions from two of the four copies of chromosome 2.

Hypochaeris caespitosa possesses only a single locus of 35S rDNA, which, in contrast to all other species, whether diploid or tetraploid, is found on chromosome 2 (Fig. 1J).

Phylogenetic relationships

Here we use the term 'clade' for the phylogenetically defined groups delimited by Tremetsberger *et al.* (2006), to distinguish them from the groups based on karyotype features (Weiss-Schneeweiss *et al.*, 2007*b*; this study).

Phylogenetic relationships of about two-thirds of South American *Hypochaeris* species are illustrated in Fig. 2. Although each species is well separated (as illustrated by heavily weighted splits), the basal relationships of clades, usually including a few species only, are poorly resolved with many contradicting, yet often only weakly weighted, splits in the centre of the network. Most of the nodes supported by BS > 50 in a neighbour-joining analysis are also supported by splits in the network. The few clades not supported by any split usually have weak bootstrap support (BS < 65), with the exception of a clade including, among others, *H. petiolaris*, *H. pampasica*, *H. parodii* and *H. alba* (BS 87; see Fig. 2).

If we take *H. angustifolia* as the outgroup species (Tremetsberger *et al.*, 2005), then *H. patagonica* and *H. chondrilloides* (*chondrilloides* clade, BS 90) are sister to the remainder of the South American taxa (BS 91). The *apargioides* clade (e.g. *H. apargioides*, *H. gayana*, *H. spathulata*; BS 100), the *pampasica* clade (e.g. *H. pampasica*; BS 76), the *microcephala* clade (e.g. *H. microcephala*, *H. chillensis*, *H. alba*; BS 95) with *H. variegata* and *H. petiolaris* together form a major clade with very strong bootstrap support (BS 98). The relationships of *H. lutea* (syn. *H. rosengurttii*), *H. scorzonerae* and *H. caespitosa* to each other, and to remaining species, are essentially unresolved.

Two more clades can be discriminated: one including *H. elata*, *H. sessiliflora* and *H. taraxacoides* (if we exclude *H. elata* this corresponds to the *sessiliflora* clade; BS 99) and the second consisting of *H. acaulis*,

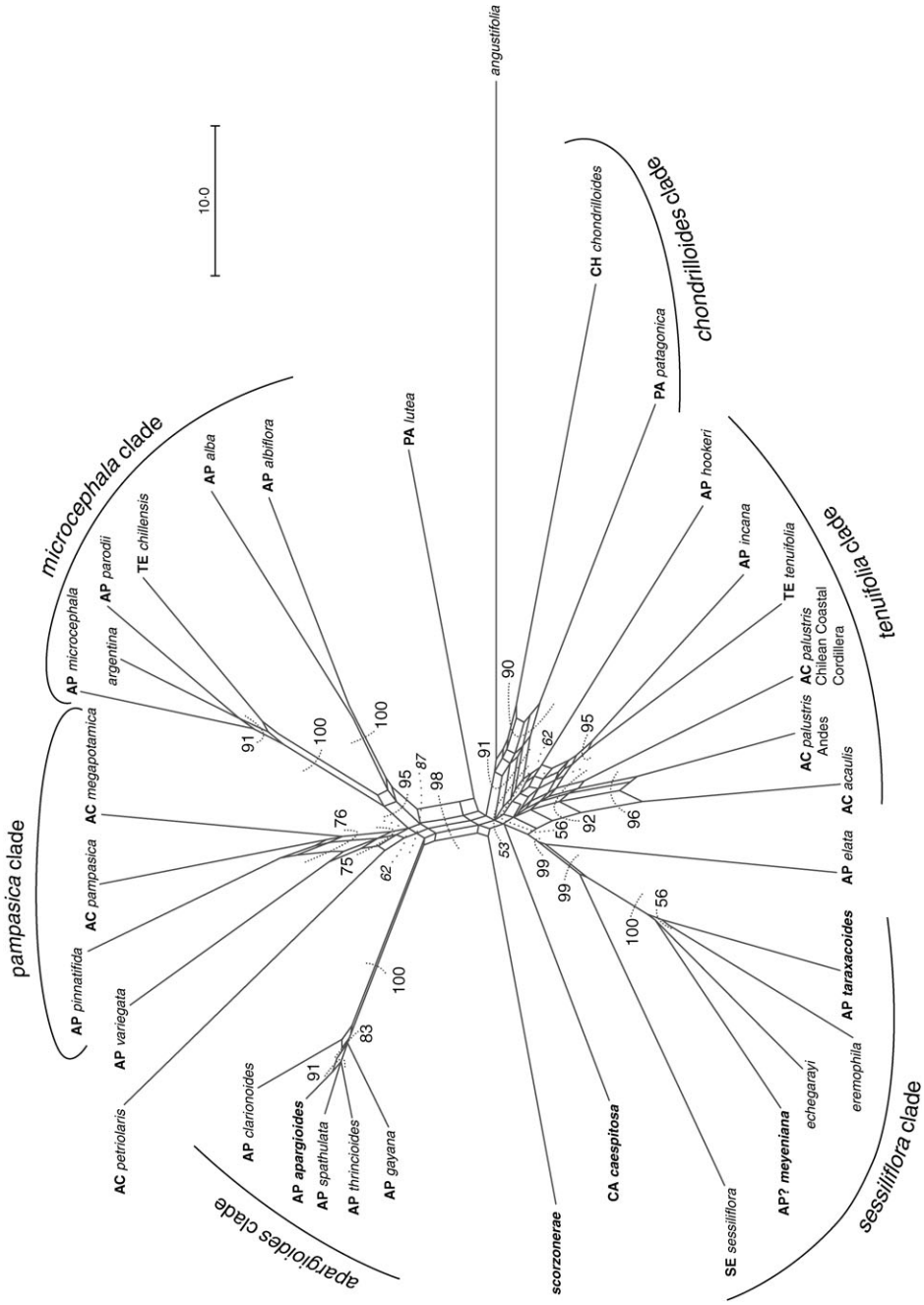

FIG. 2. Neighbour-net diagram derived from AFLP data. Splits with weight <0·5 (approx. two-thirds of splits) have been removed to aid legibility. Clades found in a neighbour-joining analysis are indicated by dotted lines with their bootstrap support values; those clades that are not supported by a split are indicated by widely spaced dotted lines and italicised bootstrap support values. The two letters preceding the species name indicate the karyotype groups (where known): AC, *acaulis* group; AP, *apargioides* group; CA, *caespitosa* group; CH, *chondrilloides* group; PA, *patagonica* group; SE, *sessiliflora* group; and TE, *tenuifolia* group. Species including polyploid cytotypes are indicated in bold. The circumscription of phylogenetically defined clades (Tremetsberger *et al.*, 2006) is indicated. Scale bar represents a 10 % distance.

H. palustris, *H. tenuifolia*, *H. incana* and *H. hookeri* (all of them in the *tenuifolia* clade; BS 56).

DISCUSSION

Karyotype groups in South American Hypochaeris

As a result of this study of 18 populations from eleven species the number of cytogenetically FISH-characterized South American *Hypochaeris* is now 25, about half of all the New World species. The 5S rDNA studies have been uninformative, with a single locus in the short arm of chromosome 2 in all species. By contrast, analyses of basic chromosome morphology and 35S rDNA localization have enabled the definition of seven karyotypic groups (Weiss-Schneeweiss *et al.*, 2007*b*; Fig. 3). These groups have been named here after one of their constituent species (Weiss-Schneeweiss *et al.*, 2007*b*). This classification replaces the designations A, B, etc., introduced previously by Weiss-Schneeweiss *et al.* (2003), in order to avoid confusion in case new groups are found, and so as not to suggest any evolutionary directionality in their relationships.

(1) The *apargioides* group (formerly group B) is the largest and includes 17 species (table 3 in Weiss-Schneeweiss *et al.*, 2007*b*). The species *H. alba*, *H. elata*, *H. hookeri*, *H. incana*, *H. parodii* and *H. pinnatifida* have been analysed for the first time. The species of the *apargioides* group have two interstitial 35S rDNA loci, one on the long arm of chromosome 2 and one on the short arm of chromosome 3, both forming secondary constrictions (Fig. 3). Interestingly, tetraploid cytotypes of *H. incana* and *H. taraxacoides* often show deviations from a strictly additive pattern (see below 'Evolution of polyploids').
(2) The species of the *tenuifolia* group (formerly group C) (usually) lack the secondary constriction on chromosome 2, but have the corresponding 35S rDNA locus intact. This is most likely due to locus inactivation.
(3) The *sessiliflora* group so far consists only of *H. sessiflora*. Its karyotype differs from the *apargioides* group by the terminal location of the 35S rDNA locus on chromosome 3. This species was erroneously assigned to the *apargioides* group by Weiss *et al.* (2003) due to the failure to detect the terminal satellite on chromosome 3 in Feulgen-stained preparations.
(4) The sole member of the *chondrilloides* group (Weiss-Schneeweiss *et al.*, 2007*b*) is *H. chondrilloides*, which has a telocentric rather than submetacentric chromosome 3. However, the presence of a satellite on this chromosome, detected in Feulgen-stained chromosomes, still requires confirmation via FISH analysis.

Two other small karyotypic groups have only a single 35S rDNA locus per genome, located on the short arm of chromosome 3 in both.

(5) This is terminal in the *patagonica* group (formerly group A), including *H. patagonica* and *H. lutea* (Ruas *et al.*, 2005; Weiss-Schneeweiss *et al.*, 2007*b*),
(6) or interstial in the *acaulis* group (formerly group D) including, among others, *H. petiolaris*.
(7) The last distinct karyotype is characterized by having a single locus of 35S rDNA on chromosome 2 instead of chromosome 3. This has been found only in the tetraploid genome of *H. caespitosa* (*caespitosa* group).

The overall karyotypic similarity of South American *Hypochaeris* species suggests that large structural rearrangements have been of minor importance in the evolution of this group. However, analysis of F_1 hybrids or application of a wide range of dispersed markers is necessary to establish this. It is also not possible to assess the evolutionary significance of spontaneous changes at the individual level, such as the centric shift in chromosome 1 of *H. apargioides* (Weiss-Schneeweiss *et al.*, 2007*b*; H. Weiss-Schneeweiss, unpubl. res.). More detailed studies are clearly necessary.

Evolution in polyploids

Polyploidy is relatively frequent amongst South American *Hypochaeris* species, occurring in different karyotypic groups and in different phylogenetic clades (Figs 2 and 3), suggesting that polyploidization is an active process (Weiss-Schneeweiss *et al.*, 2007*b*). Polyploid cytotypes are so far known only at the tetraploid level. They have been reported in *H. caespitosa* (*caespitosa* group), *H. chondrilloides* (*chondrilloides* group), *H. sessiliflora* (*sessiliflora* group), *H. apargioides*, *H. incana*, *H. taraxacoides*, *H. meyeniana* (all in *apargioides* group), *H. tenuifolia* (*tenuifolia* group) and in the unassigned species *H. scorzonerae* (Weiss *et al.*, 2003). A summary of previous chromosome number reports is given by Weiss *et al.* (2003), Baeza *et al.* (2006) and Weiss-Schneeweiss *et al.* (2007*b*). Tetraploid cytotypes of *Hypochaeris* often co-occur with conspecific diploids within the same population (Weiss-Schneeweiss *et al.*, 2007*b*), suggesting an autopolyploid origin.

The process of diploidization in newly formed polyploids does not follow a clear pattern in *Hypochaeris* (Adams and Wendel, 2005; Clarkson *et al.*, 2005; Comai, 2005; Ma and Gustafson, 2005). The number of 5S rDNA increases additively with tetraploidy, as shown in other species' groups (Adams *et al.*, 2000; de Melo and Guerra, 2005; Weiss-Schneeweiss *et al.*, 2007*a*; but see also Mishima *et al.*, 2002; Clarkson *et al.*, 2005). The 35S rDNA locus number by contrast deviates from a strictly additive pattern on chromosome 2 of *H. incana* and chromosome 3 of some individuals of *H. taraxacoides*, and the locus is completely lost from chromosome 3 of *H. caespitosa*. Epigenetically regulated inactivation, asymmetrical amplification/reduction of loci via unequal recombination and eventual loss have been reported in other polyploids (e.g. Vaughan *et al.*, 1993; Chen and Pikaard, 1997; Dadejová *et al.*, 2007) and are likely also to be operating in

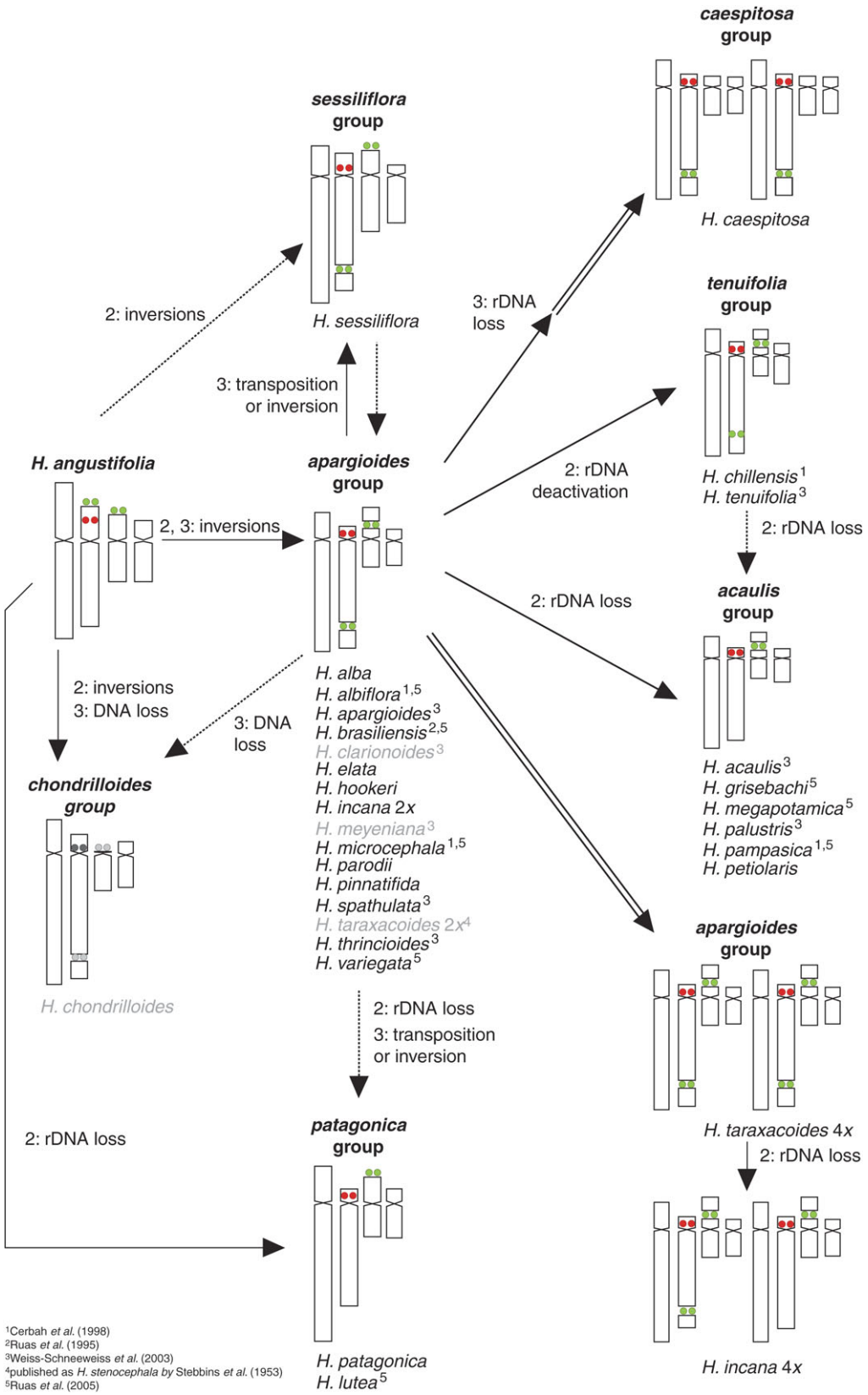

FIG. 3. Evolutionary links between the different karyotype groups of South American *Hypochaeris*. The evolutionary direction is indicated by arrows; in the case of more than one pathway, the less likely alternatives (see text for details) are indicated by dashed arrows; double-lined arrows indicate polyploidization events. Next to the arrows the most likely chromosomal mechanisms, and the number of the chromosome involved, are indicated (see also the text). Red and green dots indicate positions of 5S and 35S rDNA loci, respectively (in *H. chondrilloides* no FISH data are available; positions of the two rDNA types are inferred from the basic karyotype and indicated by black and grey dots, respectively). Taxa for which no FISH data are available are indicated in grey.

Hypochaeris. The occurrence of the same patterns of locus asymmetry (*H. spathulata* and *H. variegata* of the *apargiodes* group: Ruas *et al.*, 2005; H. Weiss-Schneeweiss, unpubl. res.), or inactivation and loss (*tenuifolia* and *acaulis* groups; Weiss-Schneeweiss *et al.*, 2003) on the diploid as well as on the tetraploid level, suggests that 35S rDNA diploidization may be triggered by factors other than polyploidization itself.

Despite its relatively high frequency, there is no convincing evidence for a causal contribution of polyploidization and accompanying microstructural changes to the rapid morphological and genetic diversification of South American *Hypochaeris* species. The frequency of polyploidy in this group, however, was greatly underestimated until only a few years ago. Further studies across as wide a range of species as possible are needed to assess fully the role of polyploidy in differentiation and eventual speciation of this group.

Mechanisms and directions of karyotype evolution

The following hypotheses depend on the phylogenetic hypothesis derived from the AFLP data. The use of AFLP data for phylogenetic purposes at the specific level is not uncontroversial (see discussion in Tremetsberger *et al.*, 2006, and Dixon *et al.*, 2008), yet in our case none of the employed sequence markers (nuclear ITS and plastid *matK* and *rps16* intron: Samuel *et al.*, 2003; Tremetsberger *et al.*, 2005) was able to resolve relationships within this group.

The restriction of conflicting signals to the centre of the network constructed using AFLP data (Fig. 2) suggests a high level of homoplasy at the deepest nodes, which results in a poorly resolved backbone. This renders the current phylogenetic hypothesis conservative, in the sense that relationships will not be resolved at all rather than resolved wrongly. In the absence of detailed chromosome maps or analysis of meiotic configurations in hybrids, which would allow definition of the patterns of chromosome rearrangement (e.g. Hall and Parker, 1995), we present here a hypothesis of karyotype evolution following the parsimony principle (Fig. 3). Inevitably, more complex evolutionary patterns cannot be excluded.

The majority of South American *Hypochaeris* species analysed belong to the *apargioides* karyotypic group, which occurs in all phylogenetic clades except the *chondrilloides* clade (Fig. 2). The high frequency of this karyotype together with the presence of the maximal number of 35S rDNA loci (two per haploid genome) found in the South American species, and also characteristic of the Old World *H. angustifolia* (Cerbah *et al.*, 1998; Weiss-Schneeweiss *et al.*, 2003; Ruas *et al.*, 2005; Tremetsberger *et al.*, 2005), suggest this karyotype as the ancestral one. If the karyotype seen today in *H. angustifolia*, which is sister to the New World species (Tremetsberger *et al.*, 2005), is the ancestral form of all the South American species, a number of mechanisms proposed for other groups may be invoked to derive the *apargioides* group, including inversions, translocations, rDNA transposition or rDNA dispersion (Jackson, 1973; Schubert and Wobus, 1985; Leitch and Heslop-Harrison, 1992; Dubcovsky and Dvořák, 1995; Weiss and Małuszyńska, 2000; Shishido *et al.*, 2000; Raskina *et al.*, 2004; Vaio *et al.*, 2005; Datson and Murray, 2006). Although some circumstantial evidence for or against any of these mechanisms may be put forward, for instance an enrichment in *copia*-type retroelements of the short arm of chromosome 3 and 4 (Ruas *et al.*, 2008) in support of retroelement-mediated rDNA transposition, the available data do not allow any inferences to be made beyond mere speculation.

Previously, a karyotype corresponding to that now known in the *sessiliflora* group was suggested as being ancestral to all South American taxa (Weiss-Schneeweiss *et al.*, 2003). This hypothesis was suggested by the (sub)terminal position of the 35S rDNA locus on the short arm of chromosome 3 characteristic of many Mediterranean and Eurasian *Hypochaeris* species, including *H. angustifolia* (Weiss-Schneeweiss *et al.*, 2003), and was in accordance with the idea that a nucleolar organizer region (NOR) occurring at a (sub)terminal position on a short chromosome arm would be the primitive character state (Lima de Faria, 1976). However, phylogenetic studies show that *H. sessiliflora* is derived and groups with *H. elata* and other species in the *sessiliflora* clade (Fig. 2). Since these species all belong to the *apargioides* group, phylogeny thus supports a derived state for the *sessiliflora* group. The mechanisms resulting in this reversal to a terminal position of the 35S rDNA locus position on chromosome 3, the ancestral condition seen in *H. angustifolia*, require elucidation but presumably involve paracentric inversion, transposition or minor locus expansion/major locus reduction.

The unclear relationships of *H. patagonica* and *H. lutea* at the base of the South American radiation perhaps indicate that the *patagonica* group karyotype might be derived from the ancestral *H. angustifolia*-like karyotype by a single-step loss of the locus on chromosome 2. This might have happened independently in *H. patagonica* (*chondrilloides* clade) and in *H. lutea*, which is not assigned to any phylogenetic group yet.

The phylogenetic position of *H. chondrilloides* (in the *chondrilloides* clade) as sister to the remaining South American species of *Hypochaeris* suggests that its karyotype (based on chromosome morphology) might have been directly derived from the *H. angustifolia* karyotype. Mechanisms may include those suggested above for the *apargioides* group, followed by the loss of a fragment of the short arm of chromosome number 3. Only two individuals from a single population have been examined and more data are needed to establish if the observed karyotype is typical for the species, and to be able to trace effectively its evolutionary history.

Species possessing karyotypes of the *tenuifolia* or *acaulis* groups do not form monophyletic clades, but are nested in clades including species from the *apargioides* group (*pampasica* clade, *microcephala* clade, *tenuifolia* clade). This agrees well with the proposed cytological mechanisms involved, namely initial deactivation (*tenuifolia* group) followed by eventual loss of the whole terminal segment on chromosome 2 (*acaulis* group). Significantly, in

H. chillensis and *H. tenuifolia*, a few individuals analysed still possessed a visible secondary constriction on chromosome 2 (H. Weiss-Schneeweiss, unpubl. res.), perhaps indicating that the silencing of this locus can be reversible or is still ongoing in this species (see also Hasterok and Małuszyńska, 2000*b*). Silencing or loss of rDNA loci is known mainly in polyploids and hybrids (Vaughan *et al.*, 1993; Chen and Pikaard, 1997; Hasterok and Małuszyńska, 2000*a*; Dadejová *et al.*, 2007), but has also been observed in diploids possessing more than one locus (Małuszyńska *et al.*, 1998).

Multiple origins of the *acaulis* group karyotypes are suggested from genome size data (Ch. König, Department of Systematic and Evolutionary Botany, University of Vienna, unpubl. res.). Thus *Hypochaeris pampasica* and *H. megapotamica*, members of the large clade including *H. apargioides*, *H. chillensis* and *H. alba*, have considerably larger genomes (4·5 pg and 4·6 pg DNA [2C]) than *H. acaulis* and *H. palustris* (3·5 pg and 3·7 pg DNA), which group with *H. tenuifolia* and *H. taraxacoides* (Figs 2 and 3).

A third, probably early, derivative of the *apargioides* group is the *caespitosa* group (*H. caespitosa*). This evolved via loss of the rDNA locus on chromosome number 3 (Fig. 3). The karyotype is only known from the tetraploid condition, but so far only one population has been analysed, and the diploids might exist. It remains unclear if the loss is connected to polyploidization of the *apargioides* group (see 'Evolution in polyploids'), or follows the same trend seen in the *tenuifolia* and *acaulis* groups.

In conclusion, the exact nature of chromosomal rearrangements relative to the putative ancestral karyotype seen in *H. angustifolia* remains unclear. However, interpretation of the karyotypic information in a phylogenetic context suggests that the early evolution of *Hypochaeris* in South America was characterized by considerable karyotypic differentiation resulting from independent derivations from the ancestral karyotype and marked diversification with respect to the position and evolution of the 35S rDNA locus on chromosome 3. By contrast, the congruent position in all South American species of the 35S rDNA locus on the long arm of chromosome 2 (compared to its short arm localization in *H. angustifolia*; Fig. 3), suggests that this change occurred only once, and pre-dated those of chromosome 3. Among the different karyotypes, the *apargioides* group and its clear derivatives (*tenuifolia*, *acaulis*, *sessiliflora* and probably also *caespitosa* group) are the most species-rich. Further data are required to unravel whether and how the karyotypic constitution has contributed to the diversification of these species. Additionally, there is also a need for meiotic studies of intraspecific hybrids to elucidate relationships between species and to unravel potential chromosomal rearrangements.

SUPPLEMENTARY INFORMATION

Supplementary Information is available online at http://aob.oxfordjournals.org/ and lists the plant material of *Hypochaeris* analysed for AFLP in this study, including collector(s), and number and location of voucher specimens.

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