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MOLECULAR SYSTEMATICS

OF NORTH AMERICAN *EUPATORIUM*

A Dissertation
Presented for the
Doctor of Philosophy Degree
The University of Tennessee, Knoxville

Kunsiri Chaw Siripun
May 2004

DEDICATION

This thesis is dedicated to my mom, Chaw Keerati Siripun

and my family in Thailand for their love, support and encouragement.

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ABSTRACT

This study had two primary goals: first, to prepare a thorough taxonomic revision of North American *Eupatorium,* and second, to conduct initial evaluations using molecular techniques of how diploid and polyploid populations of *Eupatorium rotundifolium* and *E. sessilifolium* relate to one another. Nuclear ribosomal internal transcribed spacer (ITS) region and chloroplast DNA (*trnC-psbM*) sequences were used for molecular analysis, which allowed assessment of relationships of the polyploid samples based on both a biparentally and a maternally inherited marker. The Inter Simple Sequence Repeat (ISSR) method was used for examining the relationships among diploid and polyploid populations including the genetic variation between the species. The number and variation in band-patterns between diploid and polyploid populations was examined to answer the question of whether natural hybridization was involved. The trees that were generated from the ISSR data gave evidence on the levels of genetic variation within and between populations of both species, as well as allowing evaluation of whether polyploidy has arisen from autopolyploidy or allopolyploidy.

A total of 20 species of *Eupatorium* occur in North America. The primary species concept that was employed for classification of *Eupatorium* in North America was a morphological one. Species were recognized that could be consistently distinguished by features of the capitulescence, numbers of florets per head, shape and arrangement of the involucral bracts, and features of the leaves including size, shape, margin, and venation.

This study provides an example of the successful use of ITS, chloroplast DNA sequence data, and ISSR methods to reconstruct reticulate evolution in plants and

confirms that molecular data can be highly informative, even when there is a limitation of morphological characters. The result provided the evidence to suggest that polyploid populations within both *Eupatorium rotundifolium* and *E. sessilifolium* are allopolyploids. The evidence of molecular approaches was consistent with the explanation that these two species showed reticulate evolution. Polyploid populations may have arisen many times from multiple origins, and this was particularly clear in the polyploid, apomictic species *Eupatorium godfreyanum*. Because ITS and chloroplast DNA sequence data provided low levels of variation at the intraspecific level, the analysis was supplemented with data from the ISSR methods to investigate in detail the relationships among populations within each species and to assess more thoroughly the origin of polyploidy in each.

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PART 1

INTRODUCTION

Eupatorium (L.) is placed in the family Asteraceae, one of the largest flowering plant families, with over 20,000 species (Bremer, 1994). The most distinctive character that distinguishes Asteraceae from other families is the arrangement of flowers in involucrate heads. *Eupatorium* is a member of the predominantly New World tribe Eupatorieae, which includes about 170 genera and 2400 species (King and Robinson, 1987; Bremer et al., 1994; Judd et al., 1999). Distinguishing features of the tribe are the discoid head and the elongate, blunt style branches (Heywood, 1993). Members of the Eupatorieae are normally herbs or shrubs with opposite or alternate leaves. Other features include the lack of latex ducts, the pappus usually of capillary bristles, obtuse to acute anthers and spiny pollen. The style arms are elongated while the base is hairy; style branches have long sterile appendages as well as glands between stigmatic lines. Other well-known genera in the tribe Eupatorieae: include *Ageratum*, *Mikania*, *Liatris*, and *Chromolaena* (Heywood, 1993; Bremer et al., 1994; Judd et al., 1999).

Although various relationships have been proposed for Eupatorieae with other tribes of Asteraceae, including Vernonieae and Astereae, recent molecular studies clearly place Eupatorieae as a derived clade of Heliantheae (Jansen, 1990; Panero J. L., pers. comm.). The sister clade within Heliantheae to Eupatorieae is a small group including *Perityle* and related genera that has only about 100 species. Comparison of the numbers of species in these two clades suggests that there has been a highly elevated rate of speciation in Eupatorieae. Study of species relationships in *Eupatorium* can be used to initiate the search for factors involved in this apparent burst of speciation.

Eupatorium can be recognized from other members of the same tribe that are mainly found in Central and South America such as *Austroeupatorium*, *Stomatanthes* and *Hatschbachiella* from a few characters such as the surface of achene, which is covered with many setulae (small bristles) and the presence of carpopodium (a stipe supporting an ovary) as found in *Austroeupatorium*. *Eupatorium* can be distinguished from the Central and South American by the lack of prominent carpopodium as well as the surface of the achene that is covered with numerous glands (King and Robinson, 1987).

In the classical sense, the arrangement of the leaf would be opposite or whorled. The flowers are discoid with many florets per head, which differ in colors of white, pink, blue, and purple (Radford et al., 1964), whereas in *Eupatorium s.s.* the leaves are only opposite, with relatively few florets per head and only in white color (King and Robinson, 1987; Godfrey and Wooten, 1981).

The species-level taxonomy of North American *Eupatorium s.s.* remains uncertain, partly due to the effects of natural hybridization and the presence of polyploidy and apomixis in some species (Sullivan, 1972). There are approximately 20 North American species, nine of which are only known to be diploid while 10 species are known to have multiple cytotypes and a single species, *E. godfreyanum*, is recognized as a species despite having only 1 polyploid cytotype (**Table 1-1**). Diploid populations are uniformly sexual, and produce normal pollen of regular size and shape, while the polyploids do not produce viable pollen (in fact, this is most likely due to the complete failure of chromosome pairing at meiosis), and are apomictic. Although polyploids cannot reproduce sexually because they fail to produce normal pollen, polyploids are able

to generate viable offspring that have the same genetic materials and chromosome number by an agamospermous process (Sullivan, 1976).

The patterns of geographic distribution of diploids and polyploids are distinctive. The distributions of diploids are restricted geographically (or scattered), compared to the polyploid populations, which are more widespread and overlapping with that of the diploids (Grant, 1953; Sullivan, 1976). To examine the origin of the polyploid populations, we address two hypotheses: (1) the polyploids are an autopolyploid or (2) the polyploids arise from allopolyploidy following hybridization.

If autopolyploidy is involved, the apomictic populations should exhibit a subset of genetic variation of the diploid parent, and share the same maternally (cpDNA) inherited genome. The presence of genetic variability not present in the diploid, or the presence of a different cpDNA genome, would be evidence for allopolyploidy. Previous studies that attempted to resolve this using isozyme data gave inconclusive results (Sullivan, 1976).

Currently, there is no comprehensive taxonomic treatment for *Eupatorium*. This study has two primary goals: first, to prepare a thorough taxonomic revision of North American *Eupatorium,* and second, to conduct initial evaluations using molecular techniques of how diploid and polyploid populations of a species relate to one another (Hilu and Liang, 1997; Plunkett, Soltis, and Soltis, 1997; Schilling et al., 1999).

One of the mystifying problems of *Eupatorium* is the presence of species with multiple cytotypes and reproductive systems. This occurrence combined with natural hybridization, leaves the relationship among species unclear. *Eupatorium rotundifolium* and *E. sessilifolium* were used as models for detailed population study. Both species include sexual diploid, and apomictic triploid plants (**Table 1-1**). Nuclear ribosomal

Table 1-1. A list of North American *Eupatorium* species from Kartesz (1999) and Cronquist (1980, 1985) including ploidy level, the check mark represents the species that are included in their treatment, whereas dash-line shows the species that are not included in their treatments.

No.	Species	Cronquist	Kartesz	Ploidy Level
1	*E. album*	✓	✓	diploid, triploid and tetraploid
2	*E. altissimum*	✓	✓	diploid, triploid and tetraploid
3	*E. anomalum*	✓	✓	diploid, triploid and tetraploid
4	*E. capillifolium*	✓	✓	diploid
5	*E. compositifolium*	✓	✓	diploid
6	*E. cuneifolium*	✓	-	diploid, triploid and tetraploid
7	*E. godfreyanum*	✓	✓	triploid
8	*E. hyssopifolium*	✓	✓	diploid, triploid and tetraploid
9	*E. leptophylum*	✓	✓	diploid
10	*E. leucolepis*	✓	✓	diploid, triploid and tetraploid
11	*E. mikanioides*	✓	✓	diploid
12	*E. mohrii*	✓	✓	diploid, triploid and tetraploid
13	*E. perfoliatum*	✓	✓	diploid
14	*E. pilosum*	✓	✓	diploid, polyploid
15	*E. pinnatifidum*	✓	✓	diploid
16	*E. resinosum*	✓	✓	diploid
17	*E. rotundifolium*	✓	✓	diploid, triploid
18	*E. semiserratum*	✓	✓	diploid
19	*E. serotinum*	✓	✓	diploid
20	*E. sessilifolium*	✓	✓	diploid, triploid

internal transcribed spacer (ITS) region and chloroplast DNA (*trnC-psbM*) sequences were used for molecular analysis investigating the paternal inheritance of the polyploid samples.

The Inter Simple Sequence Repeat (ISSR) method was used for examining the relationships among diploid and polyploid populations including the genetic variation between the species. The number and variation in of band-patterns between diploid and polyploid populations was examined to answer the question of whether natural hybridization was involved. Because the polyploids reproduce solely by apomixis (a method of reproduction that produces seeds without fertilization), it is possible that all individuals within a polyploid population are identical. This should be reflected by the lack of variation in ISSR banding patterns.

In addition, groups of populations (or even all of the populations within a species) may have identical ISSR patterns. The number of different ISSR patterns present within the polyploid populations of each species provides evidence on how many origins they had, and the degree of differences helped to determine whether they originated from more than a single combination of diploids. Comparison of polyploid and diploid bands was done to establish whether the polyploids included a subset of the diploid genetic variation, as would be expected if they were autopolyploid in origin, or if they included bands not represented in the conspecific diploids, as would be indicative of allopolyploidy. Note that although evidence will be presented for the presence of allopolyploidy , complete identification of the parents required sampling beyond what was projected for this study. Understanding these relationships would provide insight

into the degree to which natural hybridization has been involved in the generation of morphological diversity.

The objectives of this study were:

1. To investigate the polyploidy type of *Eupatorium rotundifolium* and *E. sessilifolium*, whether they are autopolyploid or allopolyploid.
2. To determine the origin of polyploids to assess whether they have arisen from one or multiple events.
3. To compare the relationship between diploid and polyploid populations within and between these two species.

Two majors molecular approaches were used in this study, which were DNA sequences and the ISSR marker patterns. The expected result of using the cpDNA sequences were that the polyploids would have sequences that would be similar or identical to its maternal species, whereas the ITS data might be similar to either one of its parents. The phylogenetic tree that was generated from the ITS DNA data would show the placement of the polyploid sample as sister to one of its parent if they are autopolyploid, whereas allopolyploid samples could also be placed between the two samples of its parents. The combination of using cpDNA and nrDNA may provide the history of the polyploid origin, if the polyploids are placed in different positions in the phylogenetic trees that are recovered based on the two sets of data. In practice, the DNA sequence data did not show high variation among most species of *Eupatorium*, including *E. rotundifolium* and *E. sessilifolium*. This necessitated use of a molecular tool that provided a higher level of variability. The ISSR method was chosen as an additional tool to assess relationships especially at the population level. If the polyploid is

autopolyploid, the band pattern that is generated by ISSR would show as a subset of diploid sample of the same species; in contrast, if the polyploid is allopolyploid the ISSR band patterns would be more complicated than the autopolyploid, the band pattern may not show as a part of the subset of one single species but would rather demonstrate the combination of samples (although it could be different from any of the diploids if the exact diploid parent was not sampled) and the band pattern possibly provides high diversity. The trees that would be generated from the ISSR data would be an evidence for providing the genetic variation within the same and among populations of both species, as well as to evaluate the hypotheses of whether polyploidy arises from autopolyploidy or allopolyploidy.

PART 2

REVISION OF NORTH AMERICAN *EUPATORIUM*

INTRODUCTION

Eupatorium is an example of a genus that has been treated taxonomically in dramatically different, alternative ways, and its generic delimitation has been contentious. Taxonomists have classified *Eupatorium s.l.* in various ways, depending on the emphasis placed on morphology, biogeography, phylogeny, and chromosome numbers. At the time it was initially named, Linnaeus included only 16 species (Species Plantarum, 1753; and Mantissa Plantarum, 1767). Species were added so that the genus, as broadly conceived, has been considered to include up to 1200 species (Whittemore, 1987; Mabberley, 1997; Allaby, 1998). In a series of papers culminating in King and Robinson (1987), a massive revision was proposed for the genera of the tribe Eupatorieae. This treatment strongly constricted *Eupatorium* so that only 42 species remained, including four that had been formerly segregated as a new genus, *Eupatoriadelphus* (King and Robinson, 1970b). Although there is still debate regarding the generic level classification of Eupatorieae, molecular phylogenetic studies have supported the conclusions of King and Robinson (1987) regarding *Eupatorium* (Schilling et al., 1999; Schmidt and Schilling, 2000; Ito et al., 2000a and 2000b).

The notable characteristics of *Eupatorium s.s.* include stems that are unbranched below the inflorescence, relatively few florets per head; 5 (9-14), and white corollas. The cypselae are 5-ribbed with the surface having few to many glands but no setulae and lacking a prominent carpopodium. The cells of the anther collar lack dense annular thickenings on the walls (King and Robinson, 1987, Bremer et al, 1994). King and Robinson (1987) used many features of floral microcharacters in their approach, which

have to be observed under higher magnification. The genus exists in the northern temperate zone, mainly in North America and East Asia (King and Robinson, 1970a; Bremer et al, 1994). *Eupatorium* is the only genus of the tribe that occurs naturally in Europe where it is represented by a single species (King and Robinson, 1987).

Eupatorium s.s. in eastern North America is traditionally separated into two informal groups based upon leaf and inflorescence characters: Uncasia and Traganthes.

These taxa were described as genera, but have neither been accepted at this level nor have they been transferred formally into *Eupatorium*. Uncasia species generally have opposite, linear to broad leaves, cymose inflorescences with white flowers, and are known as Bonesets or Thoroughworts. The second group Traganthes, commonly called Dog–Fennels, has finely dissected leaves with paniculate inflorescences and a coastal plain distribution (Godfrey and Wooten, 1981; King and Robinson, 1987). The species that have verticillate leaves with purplish flowers, formerly known as the "Eutrochium Group" of *Eupatorium* (*Eupatorium* section *Verticillata* Lamont (1995)) and commonly called Joe-Pye-Weeds, are now segregated as *Eupatoriadelphus* (King and Robinson, 1970b). *Eupatoriadelphus* is removed from *Eupatorium* based on morphological characteristics (the arrangement of leaves and the presence of anthocyanic flower pigments; King and Robinson, 1970b). Recent comparative studies of chloroplast DNA (Schilling et al., 1999) and the internal transcribed spacer (ITS) region of ribosomal DNA show that *Eupatoriadelphus* is distinct from *Eupatorium* (Schmidt and Schilling, 2000). In contrast to *Eupatoriadelphus*, molecular phylogenetic data do not clearly support the recognition of Uncasia and Traganthes as distinct groups (Ito et al., 2000a; Schmidt and Schilling, 2000).

LINNAEAN TYPES

During the mid eighteenth century Linnaeus named the *Eupatorium* specimens that were collected from North America. From a total of 14 species that were included in Species Plantarum (1753), only five North American species still remain in the treatment. Most of the type specimens are well preserved in the Linnaean Herbarium but are relatively inaccessible. The only species that is available as a plate is *E. hyssopifolium*. The others could be accessed in the microfiche format: *E. album* (**Fig. 2-1**), *E. altissimum* (**Fig. 2-2**), *E. perfoliatum* (**Fig. 2-3**), *E. rotundifolium* (**Fig. 2-4**) and *E. sessilifolium* (**Fig. 2-5**). The type species, *E. cannabinum* (**Fig. 2-6**), is the only member of the tribe that occurs in Europe, the others are found in the southeastern United States (King and Robinson, 1987). Several species have been removed from the genus since Linnaeus named them, such as *Conoclinium coelestinum* (L.) DC (= *Eupatorium coelestinum*), or *Eupatoriadelphus purpureus* (= *Eupatorium purpureum*) (King and Robinson, 1970b; Schmidt and Schilling, 2000).

MATERIALS AND METHODS

Herbarium specimens of Eupatorium were borrowed from several herbaria: University of North Carolina Herbarium, R. K. Godfrey Herbarium of Florida State University, New York State Herbarium, Pullen Herbarium of the University of Mississippi, Texas A&M University, University of Georgia Herbarium, University of South Florida, Massey Herbarium Virginia Polytechnic Institute and State University, and University of Louisiana at Monroe. Morphological observations were made for the whole plant and for individual mature florets taken from the specimens. Florets were



Figure 2-1. Linnaean type specimen of *Eupatorium album* L.



Eupatorium altissimum L.

Figure 2-2. Linnaean type specimen of *Eupatorium altissimum* L.



Figure 2-3. Linnaean type specimen of *Eupatorium perfoliatum* L.

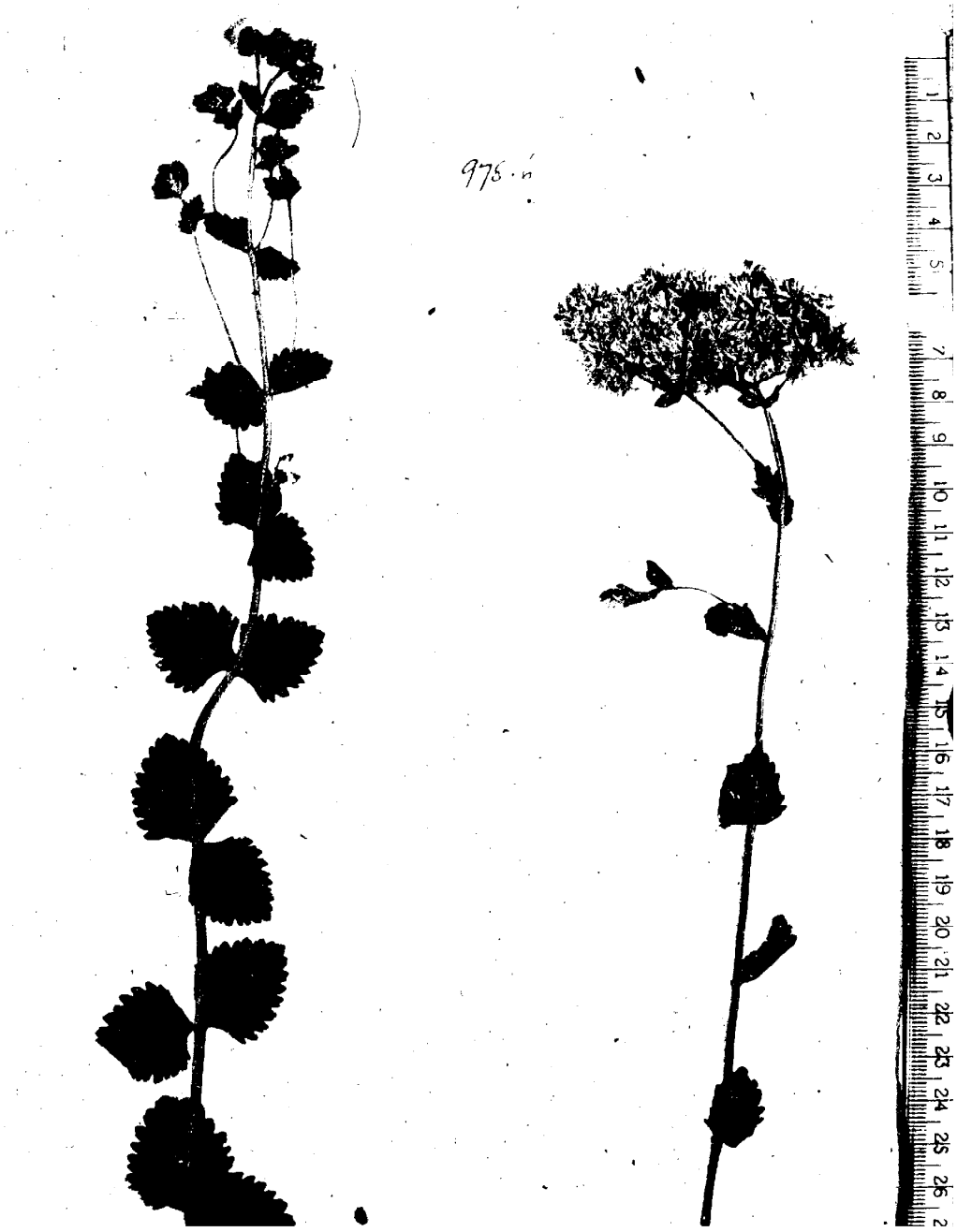
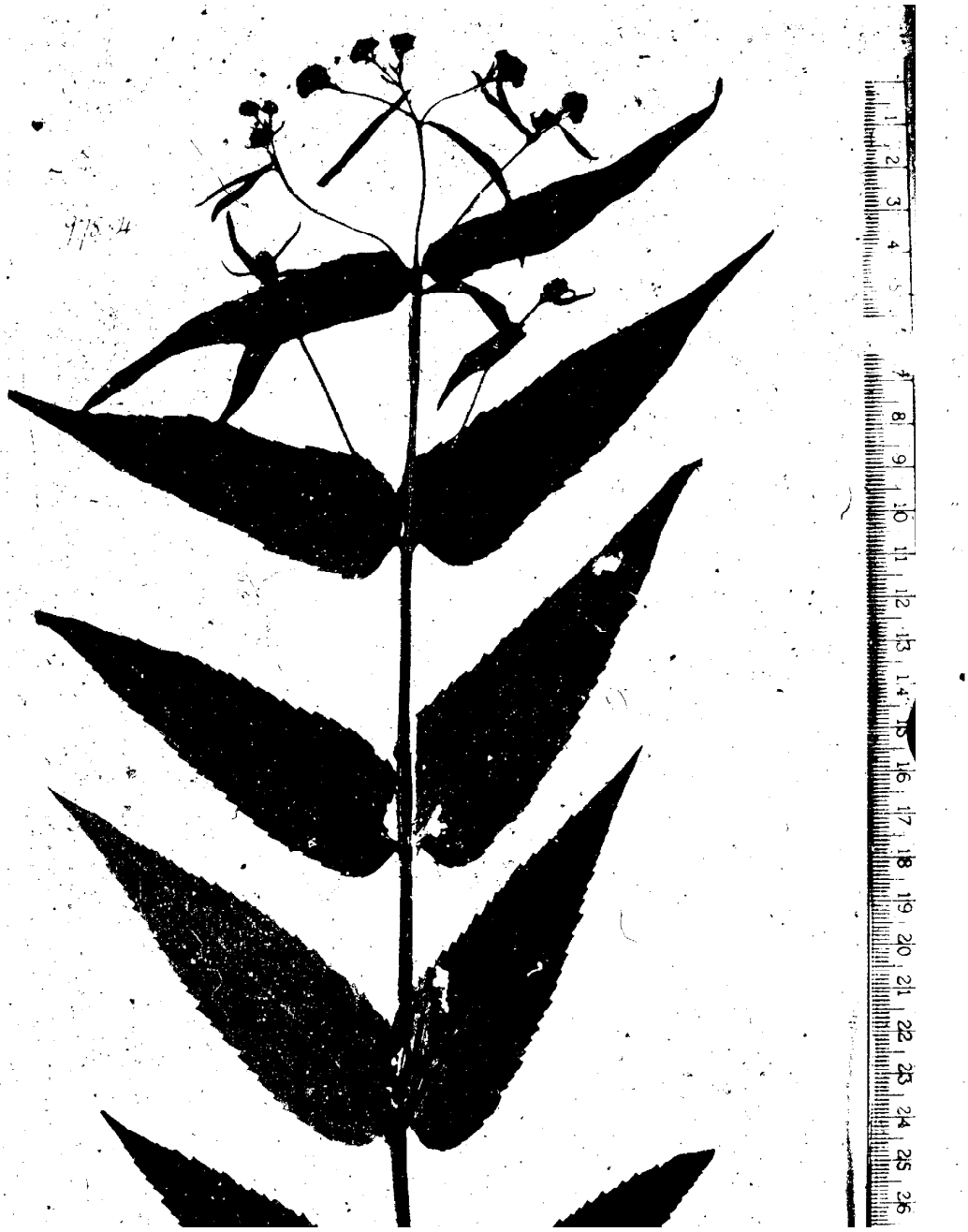


Figure 2-4. Linnaean type specimen of *Eupatorium rotundifolium* L.



Eupatorium sessilifolium L.

Figure 2-5. Linnaean type specimen of *Eupatorium sessilifolium* L.



Figure 2-6. Linnaean type specimen of *Eupatorium cannabinum* L.

kept in boiling water for a few minutes prior to observation to make them soft and easy to dissect. At least 10 specimens were measured, and pictures were taken from the complete specimens (with flowers). The whole plant and enlarged images of florets were scanned and saved as a set of .tif files, which are included in the manuscript. The descriptions in this manuscript follow the guidelines for contributors of treatments of Asteraceae for the Flora of North America.

RESULTS AND DISCUSSION

A total of 20 species of *Eupatorium* occur in North America. In general, species are clearly distinguished by flower and leaf morphology, although some species such as *E. mohrii* may require an extra observation from the rhizome to verify the identity. Images of each species and variety (if present) are provided in **appendix A** (**Figs. 2-7 – 2-31**).

KEY TO NORTH AMERICAN SPECIES OF *EUPATORIUM*

1. Blades of principal leaves pinnatifid, ternate, or pinnately dissected
    2. Capitulescence subcorymbose to paniculate; flowers 7-9 per head; leaves with broad base and not linear……..…… *E. pinnatifidum* (15)
    2. Capitulescence paniculate; flowers mostly 5 per head; leaves with narrow base and linear
        3. Capitulescence branches recurved and secund ……....……*E. leptophyllum* (9)
        3. Capitulescence clustered, the branches not recurved or secund
            4. Leaves less than 0.5 mm wide, leaf linear or ternate, cypselae 0.5 – 1.0 mm… ...…….……....……*E. capillifolium* (4)

4. Leaves 0.5 mm or more wide, leaf linear, ternate or pinnatifid, cypselae 1-1.5 mm……*E. compositifolium* (5)

1. Blades of principal leaves entire to serrate, but not pinnatifid, ternate, or pinnately dissected

5. Leaves with an evident petiole usually 1 cm or more long

6. Flowers usually 5 per head; leaf blades oriented vertically; stems green; endemic to FL……..……..... *E. mikanioides* (11)

6. Flowers usually 9-15 per head; leaf blades oriented horizontally; stem sometimes reddish to purplish; widespread...…….*E. serotinum* (19)

5. Leaves sessile to subsessile, the petiole usually less than 1 cm long

7. Leaves strongly perfoliate; flowers 9-11 per head..…….……*E. perfoliatum* (13)

7. Leaves not perfoliate; flowers 5-14 per head

8. Flowers more than 5 per head, usually 9-14 ……....…*E. resinosum* (16)

8. Flowers usually 5 per head

9. Plants with a conspicuous tuberous rhizome; leaves sessile to subsessile, often branching near the ground-level into two or more aerial stems

10. Leaves triplinerved, the main lateral veins diverging above the base; involucre less or equal to 3 mm long, phyllary subimbricate…… ……..……*E. mohrii* (12)

10. Leaves pinnately veined, only the midrib conspicuously larger, involucre less or equal to 5 mm long, phyllary strongly imbricate… ……..……*E. anomalum* (3)

9. Plants with a short or stout rhizome, but not tuberous; leaves sessile, mostly solitary stem from the rhizome

    11. Leaves trinerved or semi-triplinerved, the basal-most lateral veins conspicuous

        12. Phyllary apex acuminate to attenuate with sharp mucronate tip

            13. Leaf base slightly oblique to attenuate, leaves mostly 3-4 times as long as wide ……..……..……..…..*E. album* (1)

            13. Leaf base rounded to broadly cuneate, leaves mostly 1-2 times as long as wide …….……...…..*E. pilosum* (14)

        12. Phyllary apex rounded to acute, tip not mucronate

            14. Leaves strongly trinerved, the main lateral veins diverging at the base of the blade, and pubescent throughout; production of 2 or more pairs of leaves from lateral buds without elongation of the axis giving the appearance of many leaves per node.… …….*E. altissimum* (2)

            14. Leaves semi-triplinerved to triplinerved, the main lateral veins diverging above the base of the blade; lateral buds dormant or producing only 1 pair of leaves, giving the appearance of few leaves per node

                15. Leaves mostly 1-2 times as long as wide……..…... ……...*E. rotundifolium* (17)

15. Leaves mostly more than 2 times as long as wide

16. Cypselae 1.5--2 mm; involucre less or up to 3 mm long; phyllaries and leaves densely punctate with glandular trichomes ……*E. semiserratum* (18)

16. Cypselae 2.5--3 mm; involucre less or up to 5 mm long; phyllaries and leaves sparsely punctate with glandular trichomes...……..*E. cuneifolium* (6)

11. Leaves pinnately veined, only the midrib conspicuously larger

17. Phyllaries 6-8 mm long, the apex acuminate to attenuate, strongly mucronate; leaves ascending ..……..…….*E. leucolepis* (10)

17. Phyllaries 4-5.5 mm long, the apex rounded to acute, not mucronate; leaves spreading

18. Leaves linear, mostly 6-40 times as long as wide…… ……....…….……..……..*E. hyssopifolium* (8)

18. Leaves oblong or elliptic, mostly 2-5 times as long as wide

19. Stems pubescent throughout, leaf bases obtuse to cuneate.....……..*E. godfreyanum* (7)

19. Stems glabrous below capitulescence, leaf bases rounded….....…….*E. sessilifolium* (20)

EUPATORIUM DESCRIPTION

EUPATORIUM L., Sp. Pl. 2: 836. 1753.

[Dedicated to Mithridates Eupator, King of Pontus 132-63 BC.]

Uncasia Greene Leaf. Bot. Observ. Crit. 1: 13 1903.

Cunigunda Bub. Fl. Pyren. ii. 273 (1900).

Traganthes Wallr. Sched. Crit. i. 456, in nota (1822).

Herbs perennial. **Stems** erect, 3--20 dm, clustered or branched from crown buds or tuberous rhizome, unbranched below the capitulescence, terete, pubescent or glabrous. **Leaves** opposite, sometimes alternate within capitulescence, sessile or petiolate, blades entire, toothed, variously linear to ovate, deltoid, or dissected, surfaces scabrous or glabrous, abaxially and/or adaxially with compressed, subsessile glandular trichomes. **Capitulescences** discoid, 5--9 (--14) on each branch of capitulescence, corymbose or diffusely to densely paniculate, pedicellate. **Phyllaries** conic to elliptic, 1--9 □ 0.5--1.5 mm. **Phyllaries** 7--15, subimbricate to imbricate in 2--3 (--4) series, adjacent strongly connate, usually green, inner longer than outer, margins scarious, hyaline and with glandular hairs, apex sometimes shortly mucronate. **Receptacle** naked. **Disc florets** 4--15: bisexual, fertile; corollas white sometimes purplish, corolla tubes slender with expanded throat, limb campanulate, lobes 5; style branches elongate, stigmatic lines near the base, papillate. **Cypselae** prismatic, 2--5 mm long, brownish to black, glandular, 5 ribbed, pappi of 20--50 whitish, minutely barbed bristles 2--5 mm. $\underline{X}$= 10

Species 42 (20 in the flora): eastern North America, Europe, eastern Asia. (King, R. M. and H. Robinson. 1987. The genera of the Eupatorieae (Asteraceae). Monograph.

Syst. Bot. Mo. Bot. Garden. 22: 1-581; Cronquist, A. 1980. *Eupatorium*. *In*: Vascular flora of the southeastern United States 1: 180-193. Univ. N. C. Press, Chapel Hill.)

Eupatorium can be found in disturbed habitats, abandoned areas and along roadsides. The appearance of white disk florets makes them easy to identify, especially when the plants are in flower in mid-summer through fall. *Eupatorium* is often encountered growing in large clumps, but can be found as a single stem. The abundance and the wide range of this genus make it possibly considered as weeds (such as *E. serotinum*, *E. perfoliatum*) but some species are rare and have a restricted distribution (such as *E. resinosum*). Several species have habitat preferences – e.g. *E. altissimum* is associated with limestone; *E. sessilifolium* and *E. album* are more likely to be in wooded areas; *E. rotundifolium* could be easily found in wet and moist habitat. The characters of the leaf and involucral bracts are most often used to distinguish between otherwise similar species.

Generally, diploid species are outcrossing and have viable pollen therefore in habitats where the diploids overlap, hybrids can be found. In such cases, it is difficult to place the hybrid into a species because the hybrid might share the characters of two species. Nonetheless, apomictic polyploids are never found to overlap with the diploids even where the plants are found together, because the polyploids reproduce asexually (Sullivan, 1972). In contrast, sexual diploids have the potential to hybridize, so the hybrids that share characters between the two species might be formed (Stebbins, 1950).

For example, one of the collected specimens was found to be the hybrid between *E. serotinum* and *E. sessilifolium*, when both species were found in Graham Co., North Carolina. The hybrid had subpetiolate leaf with 7 florets per head, whereas 5 and 9

florets per head are found in *E. sessilifolium* and *E. serotinum*, respectively. Another collection, which was found at Little River Canyon State Park, Dekalb Co., Alabama, was a hybrid between *E. album* and *E. perfoliatum* as the base of the leaves were more perfoliate than *E. album* but less than *E. perfoliatum*. Moreover, the leaf texture was rougher, which is similar to *E. perfoliatu*m than *E. album*.

1. *Eupatorium album* L. Mantissa Plantarum 1: 111. 1767.

Perennials single-stemmed from short caudex. **Stems** erect, 4--10+ dm, pubescent throughout and with subsessile glandular trichomes. **Leaves** mostly opposite, seldom alternate within capitulescence, sessile, elliptic to oblanceolate, 3--12 □ 1--3.5 cm, setulose; the setulae denser on the midrib and veinlets, venation pinnate, net-like, apex rounded to acute, base slightly oblique to attenuate, margins subentire, serrate to serrulate. **Capitulescence** corymbose. **Phyllaries** 9--15, subimbricate to imbricate in 2--4 series, linear, 1.5--9 □ 0.6--1 mm, apex acuminate to attenuate, strongly mucronate, pubescent throughout. **Disc florets** (4--) 5: corollas 4--4.5 mm. **Cypselae** 2.5--3.5 mm, pappi of 40--50 bristles, 3.5--4.5 mm long. 2n = 20, 30, 40 [Grant, W. 1953. A cytotaxonomic study in the genus *Eupatorium*. American Journal of Botany 40: 729-742; Sullivan, V. 1976. Diploidy, polyploidy, and agamospermy among species of *Eupatorium* (Compositae). Can. J. Bot. 54: 2907-2917.]

Flowering summer to fall (Jul-Sep). Dry, open or wooded, disturbed sites and sandy pinelands; Ala., Conn., Del., Fla., Ga., La., Md., Miss., N. J., N. Y., N. C., Ohio, S. C., Tenn., Va., W. Va.

***Eupatorium album* varieties**

a. Leaves coarsely serrate, apex obtuse to rounded, pubescent throughout and with subsessile glandular dots .....var. *album*

a. Leaves subentire to serrate, apex acute, pubescent to subglabrous

b. Principal pair of lateral veins of the leaf diverging from the base of the midrib, margins slightly entire to subentire; not deeply tooth, 4--7 × 1--2 cm.....var. *subvenosum* A. Gray

b. Principal pair of lateral veins of the leaf diverging above the base of the midrib, margins serrate; evenly serrate, 4--7 × 1--2 cm……..

…….var. *vaseyi* (Porter) Cronquist

***2. Eupatorium altissimum* L.** Species Plantarum 2: 837. 1753.

Uncasia altissima (L.) Greene Leafl. Bot. Observ. Crit. 1: 13. 1903.

Eupatorium saltuense Fernald Rhodora 44:461, tab. 738, fig. 1,2. 1942.

Perennials single-stemmed from short caudex. **Stems** erect, 5--15+ dm, pubescent throughout and with subsessile glandular trichomes, nodes sometimes associated with galls. **Leaves** mostly opposite (but nodes often appearing leafy because of the development of leaves on lateral buds without axis elongation), sessile to subsessile, lance-elliptic to oblanceolate, 5--12 × 0.5--2 cm, pubescent and with glandular dots, strongly trinerved, apex acuminate, base slightly attenuate, margin entire proximally and serrate distally. **Capitulescence** corymbose. **Phyllaries** 8--10, subimbricate to imbricate in 2--3 series, oblong and tapering at the base, 1--4 × 0.5--1.5 mm, apex rounded to acute, pubescent throughout. **Disc florets** 5, corollas 3--3.5 mm. **Cypselae** 1.5--2 mm, pappi of

30--40 bristles 3.5--4 mm. 2n = 20, 30, 40 [Sullivan, V. 1976. Diploidy, polyploidy, and agamospermy among species of *Eupatorium* (Compositae). Can. J. Bot. 54: 2907-2917.]

Flowering summer to fall (Jul – Sep). Clearings, open woods, and thickets; Ala., Ark., Conn., Fla., Ga., Ill., Ind., Kans., Ky., La., Md., Mass., Mich., Minn., Miss., Mo., Nebr., N. J., N. Y., N. C., Ohio, Okla., Pa., S. C., Tenn., Tex., Va., West Va., Wis.

3. *Eupatorium anomalum* Nash. Bull. Torrey Bot. Club 23: 106. 1896.

Uncasia anomala (Nash.) Greene Leafl. Bot. Observ. Crit.1: 13. 1903.

Perennials single-stemmed from conspicuous, tuberous rhizome. **Stems** erect and clustered at the top of the plant, 8--15+ dm, pubescent throughout, pubescence much denser on the capitulescence branches, with subsessile glandular trichomes. **Leaves** mostly opposite, seldom alternate within capitulescence (small shoots often develop from lateral buds with elongated branches), sessile to subsessile, oblong to elliptic, 1.5--5 × 0.5--2 cm, pubescent, venation pinnate, apex rounded to acute, base cuneate, margins entire to serrate; unevenly toothed. **Capitulescences** corymbose. **Phyllaries** 8--12, strongly imbricate in 2--3 series, oblong to lanceolate, 2.5--5 × 0.5--0.7 mm, apex rounded to acute, pubescent throughout. **Disc florets** 5: corollas 3--3.5 mm. **Cypselae** 1.8--2 mm, pappi of 20--35 bristles 3.5--4.5 mm. 2n = 20, 30, 40 [Gleason, H. A. and A. Cronquist. 1991. Manual of Vascular Plants of Northeastern United States and Adjacent Canada, N. Y. Botanical Garden.]

Flowering summer to fall (Jul - Aug). Wet, low ground; Ala., Fla., Ga., N. C., S. C.

4. *Eupatorium capillifolium* (Lam.) Small. Mem. Torrey Bot. Club 5: 311. 1894.

Artemesia capillifolia Lamarck in J. Lamark et al., Encycl. 1: 267. 1758

Perennials clustered from short caudex. **Stems** erect, 5--20 dm, pubescent throughout and with subsessile glandular trichomes, highly branched. **Leaves** opposite proximally alternate distally (nodes often appearing leafy because of the development of leaves on lateral buds without axis elongation), sessile, linear, ternate, 0.5--10 cm × 0.2--0.5 mm, pubescent, apex rounded to acute, base slightly cuneate, margins entire. **Capitulescences** densely paniculate. **Phyllaries** 8--10, imbricate in 2--3 series, oblong, 0.5-- 2.5 × 0.2--0.5 mm, apex acuminate and shortly mucronate, pubescent almost throughout. **Disc florets** 5: corollas 2--2.5 mm. **Cypselae** 0.5--1 mm, pappi of 20--30 bristles 2--2.5 mm. 2n = 20 [Grant, W. 1953. A cytotaxonomic study in the genus *Eupatorium*. American Journal of Botany 40: 729-742.]

Flowering mid summer to fall (Aug-Oct). Old fields, open sites, and along road side; Ala., Ark., Del., Fla., Ga., Ky., La., Md., Mass., Miss., Mo., N. J., N. C., Okla., Pa., S. C., Tenn., Tex., Va., W. Va.

5. *Eupatorium compositifolium* Walter Flora Caroliniana 199. 1788.

Traganthes compositifolia (Walter) Greene Leafl. Bot. Observ. Crit. 1: 13. 1903.

Perennials single stemmed from short caudex, upper stems clustered. **Stems** erect, 5--20 dm, pubescent throughout and with glandular trichomes (lateral buds often develop fertile and infertile branches). **Leaves** opposite proximally, alternate distally, sessile, linear, ternate or pinnatifid, 2--8 cm × 1--2.5 mm, pubescent, venation pinnate, apex acuminate; base cuneate, margins entire. **Capitulescences** elongate and paniculate.

Phyllaries 7--10, imbricate in 2--3 series, elliptic to oblong, 1--3 × 0.5--0.8 mm, apex acuminate and shortly mucronate; sometimes purple, hyaline, pubescent along midvein. **Disc florets** 5: corollas sometimes with purple throat, 2.5--3 mm. **Cypselae** 1--1.5 mm, pappi of 20--30 bristles 3--3.5 mm. 2n = 20 [Grant, W. 1953. A cytotaxonomic study in the genus *Eupatorium*. American Journal of Botany 40: 729-742.]

Flowering midsummer to fall (Aug-Oct). Open or slightly shaded sites, sand dunes, disturbed area and along the roadside; Ala., Ark., Fla., Ga., Ky., La., Miss., N. C., Okla., S. C., Tenn., Tex., Va.

6. *Eupatorium cuneifolium* Willd. Species Plantarum iii 1753.

Uncasia cuneifolia (Willd.) Greene Leafl. Bot. Observ. Crit. 1: 13. 1903.

Eupatorium glaucescens Ell. Sketch, ii 297.

Eupatorium tortifolium Chapm. In Coult. Bot. Gaz. iii. (1878) 5.

Perennials single-stemmed from short caudex, branched near ground level. **Stems** erect, 3--10+ dm, pubescent throughout and with subsessile glandular trichomes. **Leaves** mostly opposite, sometimes alternate within capitulescence, sessile to subsessile, oblong to lance-oblong, 2--4.5 × 0.5--1 cm, scabrous, triplinerved, apex acute, base cuneate, margins entire to serrate, when serrate, teeth mostly proximal. **Capitulescences** corymbose. **Phyllaries** 8-10, imbricate in 1--2 series, lanceolate, tapering toward the apex, 2--5 × 0.5--1 mm, apex rounded to acute, pubescent throughout. **Disc florets** 5: corollas 3--3.5 mm. **Cypselae** 2.5--3 mm, pappi of 30--40 bristles 3--5 mm. 2n = 20, 30, 40 [Grant, W. 1953. A cytotaxonomic study in the genus *Eupatorium*. American

Journal of Botany 40: 729-742; Sullivan, V. 1976. Diploidy, polyploidy, and agamospermy among species of *Eupatorium* (Compositae). Can. J. Bot. 54: 2907-2917.]

Flowering summer to fall (Jul-Sep). Dry, sandy soil, pine and oak woods and old fields; Ala., Del., Fla., Ga., Miss., N. C., S. C., Tex.

7. *Eupatorium godfreyanum* Cronquist Brittonia 37(3): 238-240. 1985.

Perennials single-stemmed from short caudex. **Stems** erect, 6--10+ dm, pubescent throughout and with subsessile glandular trichomes. **Leaves** mostly opposite, seldom alternate within capitulescence, sessile, elliptic, 5--10 × 1.5--4 cm, scabrous, venation strongly pinnate, apex acute, base obtuse to cuneate, margins serrate. **Capitulescences** corymbose. **Phyllaries** 7--10, strongly imbricate in 2--3 series, conic and tapering at the tip, 2--6 × 1--1.5 mm, apex acute, pubescent throughout. **Disc florets** (4--) 5: corollas 3--3.5 mm. **Cypselae** 2--3 mm, pappi of 20--50 bristles 3.5--4.5 mm. 2n = 30, 40 [Grant, W. 1953. A cytotaxonomic study in the genus *Eupatorium*. American Journal of Botany 40: 729-742.]

Flowering summer to fall (Jul–Sep). Dry, open, disturbed sites, and along the edge of woods; Ky., Md., N. C., N. J., Ohio, Tenn, Va., West Va. [*Eupatorium vaseyi* Porter, misapplied.]

8. *Eupatorium hyssopifolium* L. Species Plantarum 2: 836. 1753.

Uncasia hyssopifolia (L.) Greene Leafl. Bot. Observ. Crit. 1: 13. 1903.

Eupatorium lecheaefolium Greene Pittonia 3: 177. 1897.

Eupatorium linearifolium (Walter) Fernald Rhodora 44:460. 1942.

Eupatorium torreyanum Short & Peter var. *laciniatum* Gray Fl. N. Am. ii 84.

Perennials single-stemmed from short caudex. **Stems** erect, 5--10+ dm, pubescent throughout and with subsessile glandular trichomes. **Leaves** mostly opposite or whorled proximally, sometimes alternate within capitulescence (small shoots often develop from lateral buds with elongated branches), sessile, linear to lance-oblong, 2--6 × 0.5--1 cm, scabrous, venation pinnate, apex acute, base cuneate, margins entire to serrate. **Capitulescences** corymbose. **Phyllaries** 8--10, subimbricate in 2--3 series, elliptic to oblong, 1.5--5 × 1--1.5 mm, apex obtuse to acute, pubescent throughout, more densely at apex. **Disc florets** 5: corollas 3--3.5 mm. **Cypselae** 2--3 mm, pappi of 20--30 bristles 3.5--4 mm. 2n = 20, 30, 40 [Grant, W. 1953. A cytotaxonomic study in the genus *Eupatorium*. American Journal of Botany 40: 729-742.]

Flowering mid-summer to fall (Aug-Oct). Dry, open, disturbed sites, and along roadsides; Ala., Ark., Conn., Del., Fla., Ga., Ill., Ky., La., Md., Mass., Miss., Mo., N. J., N. Y., N. C., Ohio, Pa., R. I., S. C., Tenn., Tex., Va., West Va., Wis.

9. *Eupatorium leptophyllum* DC. Prodromus Systematis Naturalis Regni Vegetabilis 5: 176. 1836.

Eupatorium capillifolium var. *leptophyllum* (DC.) H. E. Ahles. J. Elisha Mitchell Sci. Soc. 80: 173. 1964.

Perennials single stem from short caudex. **Stems** erect, 5--20 dm, glabrous throughout but with subsessile glandular trichomes. **Leaves** opposite proximally, alternate distally, (nodes often appearing leafy because of the development of leaves on lateral buds without axis elongation), linear and sometimes ternate, 2--10 cm × 0.5--1

mm, pubescent, venation pinnate, apex rounded to acute, base slightly perfoliate, margins entire. **Capitulescences** paniculate, the branches recurved and secund. **Phyllaries** 6--10, imbricate in 1--2 series, lanceolate, 1.5--3 × 0.5--0.7 mm, apex acuminate and shortly mucronate, pubescence mostly restricted to midrib. **Disc florets** 5: corollas 2--2.5 mm. **Cypselae** 1--1.5 mm, pappi of 15--30 bristles 2--2.5 mm. 2n = 20 [Grant, W. 1953. A cytotaxonomic study in the genus *Eupatorium*. American Journal of Botany 40: 729-742.]

Flowering mid-summer to fall (Aug-Oct). Pond margins; wet, low places, sometimes in shallow water; Ala., Fla., Ga., Miss., N. C., S. C.

10. *Eupatorium leucolepis* (DC.) Torr. & A. Gray A Flora of North America: 2(1): 84. 1841.

Uncasia leucolepis (DC.) Greene Leafl. Bot. Observ. Crit. 1: 13. 1903.

Perennials single-stemmed from short caudex. **Stems** erect, 4--10+ dm, pubescent throughout and with subsessile glandular trichomes, pubescence denser within capitulescence. **Leaves** mostly opposite, seldom alternate within capitulescence, ascending, sessile, lance-oblong to linear-oblong, 2--6 × 0.4--1 cm, setulose and with glandular dots, venation pinnate, apex acute, base rounded to cuneate, margins entire to serrate. **Capitulescences** corymbose. **Phyllaries** 8--10, imbricate in 2--3 series, narrow elliptic, 2.5--8 × 0.8--1.2 mm, acuminate and sharply mucronate, pubescent throughout. **Disc florets** 5: corollas 3--3.5 mm. **Cypselae** 2--3 mm, pappi of 30--40 bristles 4.5--5 mm. 2n = 20, 30, 40 [Sullivan, V. 1976. Diploidy, polyploidy, and agamospermy among species of *Eupatorium* (Compositae). Can. J. Bot. 54: 2907-2917.]

Flowering summer to fall (Jul-Sep). Pine barrens, wet meadows, margins of ponds in sandy soil; Ala., Del., Fla., Ga., La., Mass., Md., Miss., N. C., N. J., N. Y., R. I., S. C., Tenn., Tex., Va.

***Eupatorium leucolepis* varieties**

a. Leaf blades folded along the midrib, more ascending, leaf width less than or equal to 1 cm.....var. *leucolepis*

a. Leaf blades spread along the midrib, less ascending, leaf width less than or equal to 1.5m.....var. *novae-angliae* Fernald

11. *Eupatorium mikanioides* Chapm. Flora of the Southern United States 195. 1860.

Uncasia mikanioides (Chapm.) Greene Leafl. Bot. Observ. Crit. 1: 13. 1903.

Perennials single-stemmed from short rhizome. **Stems** erect, 5--10+ dm, pubescent throughout and with subsessile glandular trichomes. **Leaves** opposite, petioles 1--3 cm, blades deltoid to rhombic, 2.5--8 × 2--6 cm, puberulent, semi-triplinerved to triplinerved, venation palmate, apex acute; base broadly cuneate, margins serrate with uneven teeth, sometimes crenate. **Capitulescences** corymbose. **Phyllaries** 8--12, imbricate in 2--3 series, elliptic to oblong, 1.5--5 × 0.8--1 mm, apex acute to acuminate, pubescent throughout. **Disc florets** 5: corollas 3.5--4 mm. **Cypselae** 1.2--1.5 mm, pappi of 20--35 bristles 3.5--4.5 mm. $2\underline{n} = 20$ [Grant, W. 1953. A cytotaxonomic study in the genus *Eupatorium*. American Journal of Botany 40: 729-742]

Flowering summer to fall (Jul-Sep). Moist or wet, low, often saline places; Fla.

12. *Eupatorium mohrii* Greene Contributions from the U. S. National Herbarium 6: 762. 1901.

Uncasia mohrii (Greene) Greene Leafl. Bot. Observ. Crit. 1: 13. 1903.

Eupatorium recurvans Small Fl. S.E. U.S. 1167, 1338. 1903.

Perennials branching near ground level from conspicuous, tuberous rhizome. **Stems** erect, 3--10+ dm, pubescent throughout and with subsessile glandular trichomes. **Leaves** mostly opposite, sometimes alternate within capitulescence, sessile to subsessile, oblanceolate, 2--8 × 0.5--2 cm, pubescent, triplinerved, apex acute, base cuneate, margins entire distally; serrate proximally, with tooth size increasing gradually to the apex. **Capitulescences** corymbose. **Phyllaries** 7--10, subimbricate in 1--2 series, oblanceolate, 1--3 × 0.2--0.5 mm, apex rounded, pubescent throughout. **Disc florets** 5: corollas 2--2.5 mm. **Cypselae** 1--2 mm, pappi of 20--30 bristles 2.5--3 mm. 2n = 20, 30, 40 [Grant, W. 1953. A cytotaxonomic study in the genus *Eupatorium*. American Journal of Botany 40: 729-742.]

Flowering summer to fall (Jul-Sep). Moist, low ground; margins of ponds in sandy soil; Ala., Fla., Ga., La., Miss., N. C., S. C., Tex., Va.

13. *Eupatorium perfoliatum* L. Species Plantarum 838 (-839). 1753.

Uncasia perfoliata (L.) Greene Leafl. Bot. Observ. Crit. 1: 13. 1903.

Cunigunda perfoliata (L.) Lunell Amer. Midl. Naturalist 5: 35. 1917.

Perennials single-stemmed from short caudex. **Stems** erect, 4--10+ dm, pubescent throughout and with subsessile glandular trichomes. **Leaves** opposite, sessile, oblong; blade slightly tapering toward the apex, 5--15+ × 1.5--4 cm, scabrous, venation pinnate,

apex acute, base connate-perfoliate, margins serrate. **Capitulescences** corymbose. **Phyllaries** 7--10, imbricate in 1--2 series, oblong, 2--4.5 □ 0.6--1 mm, apex acute to acuminate, pubescent throughout. **Disc florets** 9--11: corollas 2.5--3 mm. **Cypselae** 1.5 --2 mm, pappi of 20--30 bristles 3--3.5 mm. 2n = 20 [Grant, W. 1953. A cytotaxonomic study in the genus *Eupatorium*. American Journal of Botany 40: 729-742.]

Flowering mid-summer to fall (Aug-Oct). Moist, low ground, especially along roadsides; Man., N.B., N.S, Ont., P. E. I., Que.; Ala., Ark., Conn., Del., Fla., Ga., Ill., Ind., Iowa, Kans., Ky., La., Maine, Md., Mass., Mich., Minn., Miss., Mo., Nebr., N. H., N. J., N. Y., N. C., N. Dak., Ohio, Okla., Pa., R. I., S. C., S. Dak., Tenn., Tex., Vt., Va., West Va., Wis.

14. *Eupatorium pilosum* Walter Flora Caroliniana 199. 1788.

E. rotundifolium var. *saundersii* (Porter ex Britton) Cronquist Rhodora 50: 29. 1948.

Perennials stem single from short rhizome. **Stems** erect, 3--10+ dm, pubescent throughout and with subsessile glandular trichomes. **Leaves** mostly opposite, sometimes alternate within capitulescence, sessile to subsessile, lanceolate, lance-ovate to elliptic, 3 --9 □ 2--4.5 cm, pubescent, semi-triplinerved to triplinerved, apex acute to cuneate; base rounded to rounded-cuneate, margins unevenly serrate. **Capitulescences** corymbose. **Phyllaries** 7--10, imbricate in 2--3 series, oblong but tapering to the tip, 2--7 □ 1--1.5 mm, apex acuminate and shortly mucronate, pubescent throughout. **Disc florets** 5: corollas 3.5--4 mm. **Cypselae** 3--4 mm, pappi of 30--50 bristles 4--5 mm. 2n = 20, 30, 40 [Sullivan, V. 1976. Diploidy, polyploidy, and agamospermy among species of *Eupatorium* (Compositae). Can. J. Bot. 54: 2907-2917.]

Flowering summer to fall (Jul-Sep). Moist, low ground; margins of ponds in sandy soil; Ala., Conn., Del., Fla., Ga., Ky., La., Md., Mass., Miss., N. J., N. Y., N. C., Pa., R. I., S. C., Tenn., Va., West Va.

15. *Eupatorium pinnatifidum* Elliott Sketch, ii. 295.

Traganthes pinnatifida Greene Leafl. Bot. Observ. Crit. 1: 13. 1903.

Eupatorium pectinatum Small Fl. S.E. U.S. 1165, 1338. 1903.

Eupatorium smithii Greene & C. Mohr Contr. U.S. Natl. Herb. 6:761, pl.10 1901.

Perennials stem single from short caudex, **Stems** erect, 4--10+ dm, pubescent throughout and with subsessile glandular trichomes, highly branched distally. **Leaves** opposite proximally, alternate distally, (nodes often appearing leafy because of the development of leaves on lateral buds without axis elongation), sessile, linear, ternate, pinnatifid to bi-pinnatifid, 2--10 cm × 0.5--1.5 mm, pubescent, venation pinnate, apex acute; base narrowly cuneate to attenuate, margins entire to serrate. **Capitulescences** subpaniculate to subcorymbose. **Phyllaries** 10--12, imbricate in 2--3 series, elliptic to oblong, 1--3.5 × 0.5--1 mm, apex acute to acuminate and shortly mucronate, pubescent almost throughout. **Disc florets** 7--9: corollas 2--2.5 mm. **Cypselae** 1--1.5 mm, pappi of 20--30 bristles 2.5--3 mm. $2\underline{n} = 20$ [Grant, W. 1953. A cytotaxonomic study in the genus *Eupatorium*. American Journal of Botany 40: 729-742.]

Flowering mid-summer to fall (Aug-Oct). Dry to wet areas, along roadsides, around ponds; Ala., Fla., La., Miss., S. C., Tex., TN, Va. [This species may represent a recurrent series of hybrids between *E. capillifolium* (Lam.) Small and *E. perfoliatum* L. or *E. compositifolium* Walter and *E. perfoliatum* L.]

16. *Eupatorium resinosum* Torr. ex DC. Prodromus Systematis Naturalis Regni Vegetabilis 5: 176. 1836.

Uncasia resinosa (Torr.) Greene Leafl. Bot. Observ. Crit. 1: 13. 1903.

Perennials stems single from short rhizome. **Stems** erect, 4--10+ dm, pubescent throughout and with subsessile glandular trichomes. **Leaves** mostly opposite, sometimes alternate within capitulescence, sessile, narrow elliptic, 4--10+ × 0.5--2 cm, pubescent, venation pinnate, apex acute to acuminate, base cuneate, margins serrate. **Capitulescences** corymbose. **Phyllaries** 10--12, imbricate in 2--3 series, elliptic to oblong, 1.5--4 × 0.8--1 mm, apex broadly acute to acute, pubescent throughout. **Disc florets** 9--14: corollas 3--3.5 mm. **Cypselae** 2--3 mm, pappi of 20--30 bristles 3--3.5 mm. $2\underline{n} = 20$ [Grant, W. 1953. A cytotaxonomic study in the genus *Eupatorium*. American Journal of Botany 40: 729-742.] This taxon listed by the Center For Plant Conservation.

Flowering mid summer to fall (Aug-Sep). Moist, low ground; pocosins, bogs, cutover woodland; N. J., N. C., S. C.

17. *Eupatorium rotundifolium* L. Species Plantarum 837. 1753.

Uncasia rotundifolia (L.) Greene Leafl. Bot. Observ. Crit. 1: 13. 1903.

Perennials stems single from short rhizome. **Stems** erect, 4--10+ dm, pubescent throughout and with subsessile glandular trichomes. **Leaves** mostly opposite, sometimes alternate within capitulescence, sessile to subsessile, deltoid to sub-rotund; sometimes ovate, 1.5--5 × 1.5--4 cm, pubescent, semi-triplinerved to triplinerved, apex acute, base broadly rounded to truncate, margins crenate to serrate. **Capitulescences** corymbose.

Phyllaries 8--10, strongly imbricate in 2--3 series, oblanceolate but tapering at the tip, 2--6 × 0.5--1.5 mm, apex acute, pubescent throughout. **Disc florets** 5: corolla 3--4 mm. **Cypselae** 2--3 mm, pappi of 30--40 bristles 3.5--4 mm. 2n = 20, 30, 40 [Grant, W. 1953. A cytotax onomic study in the genus *Eupatorium*. American Journal of Botany 40: 729-742; Sullivan, V. 1976. Diploidy, polyploidy, and agamospermy among species of *Eupatorium* (Compositae). Can. J. Bot. 54: 2907-2917.]

Flowering summer to fall (Jul-Aug). Moist, low ground; along roadsides, margins of ponds, in sandy soil; Ala., Ark., Conn., Del., Fla., Ga., Ind., Ky., La., Maine, Md., Mass., Miss., Mo., N. H., N. J., N. Y., N. C., Ohio, Okla., Pa., R. I., S. C., Tenn., Va., West Va.

***Eupatorium rotundifolium* varieties**

a. Leaves with broadly rounded, clasping base with unevenly serrate.....……...……

…….……var. *cordigerum* Fernald

a. Leaves with broadly rounded, but not clasping base

b. Principal pair of lateral veins of the leaf diverging from the base of the midrib, base broadly cuneate to subtruncate, margin crenate……

.....……..……var. *rotundifolium*

b. Principal pair of lateral veins of the leaf diverging above the base of the midrib, base broadly cuneate to cuneate, margin serrate

c. The broadest part of the leaf is about half of the leaf, 3--7 × 3--6 cm……..

……..…..var. *ovatum* (Bigelow) J. D. Montgom. & Fairbrothers

c. The broadest part of the leaf rather close to the base or about 1/3 of the leaf (from the base), 2--5 × 1.5--3 cm.....……..

……..…….var. *scabridum* (Elliott) A. Gray

The synonyms of *E. rotundifolium* varieties are:

Eupatorium rotundifolium var. *ovatum* = *Eupatorium pubescens* Muhl. Ex Willd.

Eupatorium rotundifolium var. *rotundifolium* = *Eupatorium verbenifolium* Reichard)

18. *Eupatorium semiserratum* DC. Prodromus Systematis Naturalis Regni Vegetabilis 5: 177. 1836.

Eupatorium lancifolium Small Fl. S. E. U. S. 1167, 1338. 22 July 1903.

Eupatorium cuneifolium var. *semiserratum* (DC.) Fernald & Griscom Rhodora 37: 179. 1935.

Perennials stems single from short rhizome. **Stems** erect, 4--10+ dm, highly branched distally, pubescent throughout and with subsessile glandular trichomes. **Leaves** mostly opposite, sometimes alternate within capitulescence, sessile to subsessile, elliptic to elliptic-lanceolate, 3--7 × 0.5--2.5 cm, pubescent and with dense glandular dots, semi-triplinerved, apex acute, base narrowly cuneate, margins entire to unevenly serrate. **Capitulescences** corymbose. **Phyllaries** 7--10, imbricate in 2--3 series, elliptic, 1--3 × 0.5--1 mm, apex rounded to acute, pubescent throughout. **Disc florets** 5: corollas 2.5--3 mm. **Cypselae** 1.5--2 mm, pappi of 30--40 bristles 2.5--3 mm. 2n = 20 [Grant, W. 1953. A cytotaxonomic study in the genus *Eupatorium*. American Journal of Botany 40: 729-742.]

Flowering mid-summer to fall (Aug-Sep). Dry, sandy loam; along roadsides, shade to sun, and along pine woods; Ala., Ark., Del., Fla., Ga., Ky., La., Miss., Mo., N. C., Okla., S. C., Tenn., Tex., Va.

19. *Eupatorium serotinum* Michx. Flora Boreali-Americana 2: 100. 1803.

Uncasia serotina (Michx.) Greene Leafl. Bot. Observ. Crit. 1: 13. 1903.

Perennials single-stemmed from short caudex. **Stems** erect, 5--15+ dm, pubescent throughout and with subsessile glandular trichomes. **Leaves** opposite, petioles 1--2.5 cm, lanceolate, 2--9+ × 0.5--4 cm, puberulent, triplinerved to trinerved, apex acute; base rounded to slightly oblique, margins entire to serrate. **Capitulescences** corymbose. **Phyllaries** 8--12, subimbricate in 1--2 series, elliptic to oblong, 1--3 × 0.5--1 mm, apex slightly rounded to acute, pubescent throughout. **Disc florets** 9--15: corollas 2.5--3 mm. **Cypselae** 1--1.5 mm, pappi of 20--30 bristles 2--2.5 mm. 2n = 20 [Grant, W. 1953. A cytotaxonomic study in the genus *Eupatorium*. American Journal of Botany 40: 729-742]

Flowering mid-summer to fall (Aug-Oct). Moist or dry, open sites, along roadsides; Ala., Ark., Conn., Del., Fla., Ga., Ill., Ind., Iowa, Kans., Ky., La., Md., Mass., Mich., Minn., Miss., Mo., Nebr., N. J., N. Y., N. C., Ohio, Okla., Pa., R. I., S. C., Tenn., Tex., Va., West Va., Wis.

20. *Eupatorium sessilifolium* L. Species Plantarum 2: 837. 1753.

Uncasia sessilifolia (L.) Greene Leafl. Bot. Observ. Crit. 1: 13. 1903.

Eupatorium sessilifolium var. *brittonianum* Porter Bull. Torrey Bot. Club 19: 129. 1892

Perennials single-stemmed from short caudex. **Stems** erect, 5--10+ dm, glabrous proximally, pubescent within capitulescence and with subsessile glandular trichomes. **Leaves** opposite, seldom alternate within capitulescence, sessile, lanceolate to oblong, 7--

15+ × 1--3 cm, scabrous, venation pinnate, apex narrowly acute to acuminate; base rounded, margins serrate. **Capitulescences** corymbose. **Phyllaries** 10--15, strongly imbricate in 2--3 series, elliptic to oblong, 2--5 × 0.5--1.5 mm, apex rounded to acute, pubescent throughout. **Disc florets** 5: corollas 3--3.5 mm. **Cypselae** 2--3 mm, pappi of 30--40 bristles 3--4 mm. 2n = 20, 30 [Grant, W. 1953. A cytotaxonomic study in the genus *Eupatorium*. American Journal of Botany 40: 729-742; Sullivan, V. 1976. Diploidy, polyploidy, and agamospermy among species of *Eupatorium* (Compositae). Can. J. Bot. 54: 2907-2917]

Flowering summer to fall (Jul - Sep). Dry, open, edges of mesic woods and at high elevations; Ala., Ark., Conn., Del., Ga., Ill., Ind., Iowa, Ky., Md., Mass., Mich., Minn., Miss., Mo., N. J., N. Y., N. C., Ohio, Pa., R. I., S. C., Tenn., Vt., Va., West Va., Wis.

Eupatorium vaseyi Porter has been placed as a variety of *E. sessilifolium* by some authors.

THE SPECIES CONCEPT

The morphological data are often used for adjudicating species boundaries (Cracraft, 2000). The primary species concept that was employed for classification of *Eupatorium* in North America was a morphological one. Species were recognized that could be consistently distinguished by features of the capitulescence, numbers of florets per head, shape and arrangement of the involucral bracts, and features of the leaves including size, shape, margin, and venation. Many of the species recognized would also be biological species, and be reproductively isolated from each other. Strict application of the biological species concept for *Eupatorium* is not uniformly possible because of the

presence of apomixis within several species (Montgomery and Fairbrothers, 1970). In many cases, apomictic and sexual populations with species of *Eupatorium* were not distinguishable, other than by examination of pollen. In some cases, however, there were groups of populations within species that exhibited slight but consistent differences and were associated with apomixis. This can be expected because apomicts, when successful, will produce what are essentially large clones, and they can be very consistent even for relatively minor differences. The results presented in the molecular analyses suggest that variation among apomictic populations of a given species can mimic that of what would be expected through variation and recombination within a sexually reproducing species, even though their origin was through repeated production of apomicts from different combinations of parental populations. In cases where the variants were widespread, but were clearly alignable with a sexually reproducing species because of their major features, they have been recognized as varieties. In the case of *E. godfreyanum*, however, the origin appears clearly to have been an interspecific hybrid that has become stabilized through apomixis, and it is sufficiently distinct from either putative parent that it is recognized as a distinct species.

The International Code of Botanical Nomenclature; St. Louis Code (2000) (section 5,article 25) noted that "for nomenclatural purposes, a species or any taxon below the rank of species is regarded as the sum of its subordinate taxa, if any." In the genus *Eupatorium* certain species are solely sexual and diploid (e.g. *E. serotinum* and *E. perfoliatum*), but some species are composed of both diploid and apomictic polyploid (e.g. *E. rotundifolium* and *E. sessilifolium*) (Grant, 1953). Hybridization in this genus is rather common and hybrid derivatives of diverse morphology may occur (Sullivan, 1972;

Godfrey and Wooten, 1981). The degree of morphology varies because of the apomictic form and so some accessions are placed as variety. If the ranges of major morphology (as mentioned earlier) are the same but shows slightly different minor characters, such as the leaf base and the leaf venation, the latter would be listed as variety. Cronquist (1985) noted that taxonomy of polyploid-apomictic group has a large arbitrary component and taxonomist would try to compare the morphology of the apomictic polyploid with the sexual diploid as nearly as they could, hence understanding the polyploid origin of the apomictic is useful.

PART 3

MOLECULAR ANALYSIS

INTRODUCTION

Even when narrowly delimited, *Eupatorium* is challenging to classify because its species exhibit such a high degree of morphological diversity. Species circumscription is complicated further by the presence of polyploidy, apomixis, and hybridization (Sullivan, 1976; Godfrey and Wooten, 1981). Some of the North American species of *Eupatorium* have more than one cytotype: including diploids (2n = 20) and polyploids (2n = 30 or 2n = 40) (**Table 1-1**). A detailed analysis of the relationship among cytotypes of two species was undertaken in the hope that it would help to understand morphological variation in *Eupatorium* and may provide insight into whether hybridization is involved in the production of polyploids.

Sullivan (1976) investigated the relationship between conspecific diploid and polyploid cytotypes of *Eupatorium* and noted that the cytotypes often have distinct and different geographic distributions. In general, the diploids have smaller distribution ranges than the polyploids of the same species (**Figs. 3-1, 3-2**). In addition, there is an association between the polyploid cytotype and the presence of apomixis; as far as has been determined, all diploids are sexual and all polyploids (whether 3x or 4x) are apomictic.

Knowledge of how polyploid cytotypes relate genomically to diploid cytotypes will shed light on whether or not hybridization has been involved in their production. There are two major possibilities: (1) polyploids are merely autopolyploids, which have more than two similar genomes (e.g. AAAAaa); (2) polyploids are allopolyploids, which have at least one genome from a different diploid species (AAaaBB).

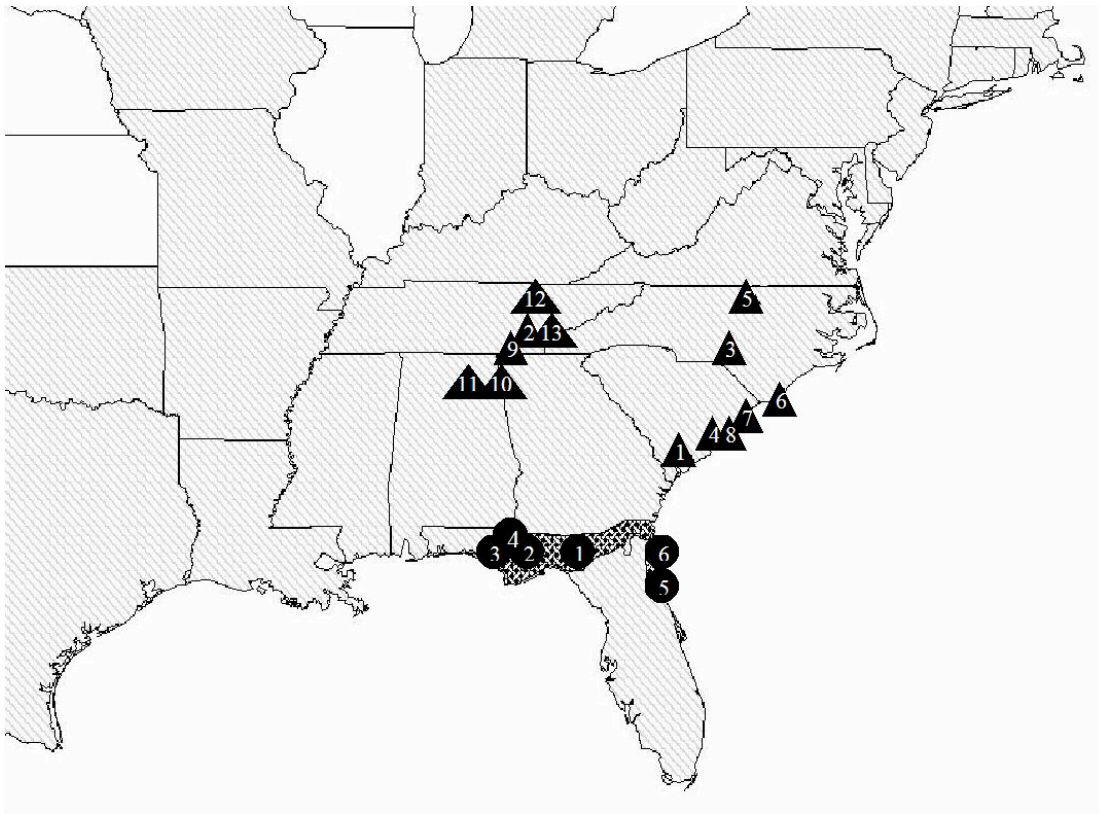


Figure 3-1. The distribution of diploid and polyploid populations of *Eupatorium rotundifolium* and their collection sites

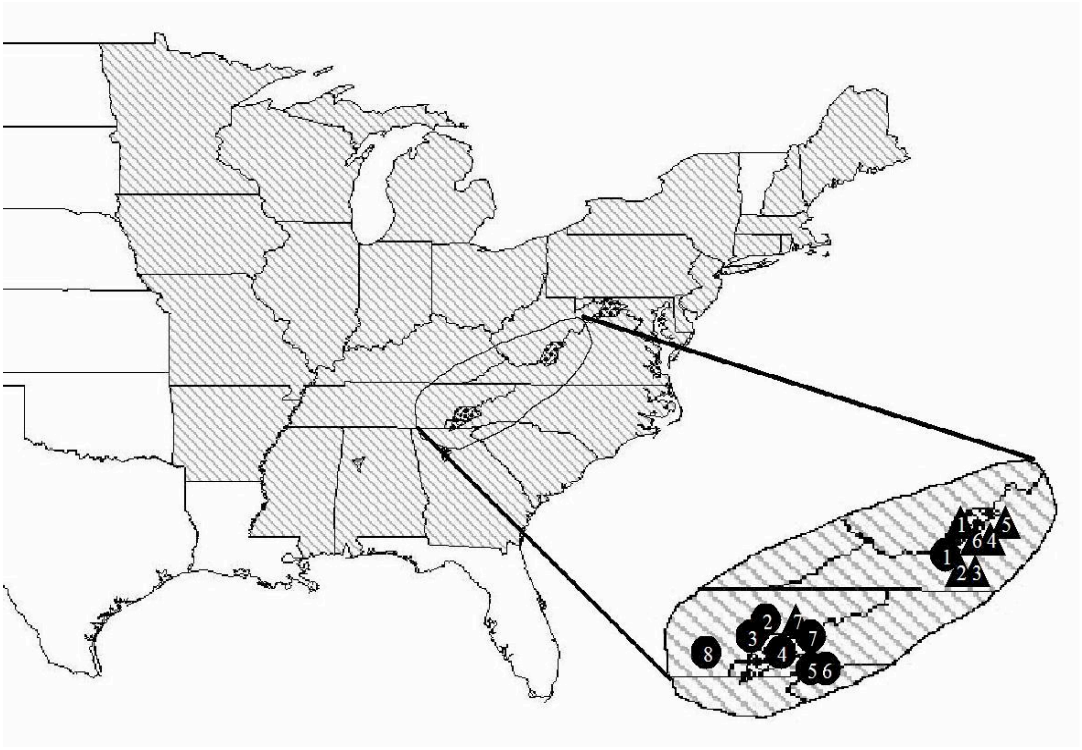


Figure 3-2. The distribution of diploid and polyploid populations of *Eupatorium sessilifolium* and their collection sites

The most common situation is that most polyploids in nature are thought to be allopolyploids, and many plant species originated from ancient hybridization events (Stebbins, 1950; Grant, 1981; Hartl and Clark, 1997). However, naturally occurring autoploidy has been documented for at least some species such as *Tolmiea menziesii* (Saxifragaceae), *Heuchera grossulariifolia* (Saxifragaceae), and *Allium nevii* (Alliaceae) (cited by Soltis et al., 2003).

The combination of hybridization, polyploidy, and apomixis can lead to some of the most complex taxonomic situations. Similar other well-known examples are found in *Rubus* (Alice et al., 2001) and *Taraxacum* (Kirschner et al., 2003).

There is some reason to suspect that hybridization may be involved in production of apomicts within *Eupatorium*. *Eupatorium* interspecific F1 hybrids have been reported (Sullivan, 1978), and are frequently seen on herbarium specimens (personal observation). The morphology of the hybrids may be similar to one of its parental species, or intermediate between its parents (Sullivan, 1978).

Initial studies to examine the origins of polyploids of North American *Eupatorium* have been done by Sullivan (1976, 1978). Geographic ranges of various diploid species were investigated in her studies. To investigate the parents of the hybrids, she made the hybrids through artificial crosses this, the diploid parental species and the hybrids had to be collected from the places where there was a high probability to find an intermediate hybrid for example in the disturbed contiguous habitats. Seeds were collected from putative hybrids and the parental species, subsequently; the test for pollen compatibility among the collected samples and the progeny were made in the greenhouse. Cytological and morphological characteristics of the progenies were observed and

compared with the parental species. However, due to the complexity within this genus, the progenies from the hybrid plants produced higher morphological diversity than their parent species, which made it more difficult to compare the relationship among them (Sullivan, 1972; Sullivan, 1976). Previous attempts to elucidate the genomic relationships of diploids and polyploids using phytochemistry and allozymes have also been inconclusive because there is little variation among species.

Molecular methods have been useful in resolving many aspects of how species are related to one another, including the genomic relationships between diploids and polyploids. One of the surprising outcomes of detailed studies of polyploids is that many allopolyploids have multiple, recurrent origins (Schwarzbach and Rieseberg, 2002). Multiple origins of polyploids may be demonstrated if the chloroplast DNA markers of the diploid ancestors can be discriminated and can be recognized in different populations of the derived polyploids (Vogel et al., 1999). The first demonstration of multiple origins of polyploid species using molecular analyses was in the pteridophyte genus *Asplenium* (Werth et al., 1985). Later Doyle et al. (2003) verified that several allopolyploids of perennial soybeans (*Glycine* subgenus *Glycine*) originated by hybridization among a subset of genome groups, which were formed from a large interconnected polyploid complex. It is important to examine the relationships among diploids to be able to potentially detect incongruence between markers or variability in placement of different populations, which would provide evidence of hybridization.

Another potential application of this study will be an assessment of whether some species of *Eupatorium* might be considered rare and thus deserving of some level of protection. Diploid *Eupatorium* species are outcrossing and produce viable pollen

(Sullivan, 1972). It is these sexually reproducing populations that continue to have evolutionary potential to produce new lineages (Richards, 1997). In contrast, polyploid individuals of *Eupatorium* produce no pollen or abnormal pollen (Sullivan, 1972) and seeds are produced only via agamospermy (the production of fertile seeds in the absence of sexual fusion between gametes), which basically produces offspring from the same clone and could be a sign of an evolutionary dead-end (Soltis et al., 1991). As mentioned above, it may be only the diploid populations of a species that give it long-term viability as a biological entity. This raises the possibility that a species with more than one cytotype could be widespread, yet still endangered if the diploid populations are few and limited in occurrence as may be the case in some species of *Eupatorium*. Examination of levels of variability within and between diploid populations will allow assessment of whether their numbers have been drastically reduced so that a genetic bottleneck has been produced.

HYBRIDIZATION

Hybridization is widespread in higher plants and may be one of the major factors that produces variation in plant evolution (Grant, 1981). Stebbins (1950) defined the term hybridization in the broad sense as the crossing of any two genetically unlike individuals and called the offspring a hybrid. Hybridization could be an important creative force in plant diversity, where one of the consequences of hybridization is the fusion of two species through interspecific gene flow (introgression), to create a complex pattern of variation, and in some cases to weaken the morphological distinctiveness of species (Judd et al., 2002). There are two outcomes of hybrid types: (1) the sorting of chromosomal rearrangements in the later generation hybrids, which could lead to new

population systems that are homozygous for a unique combination of chromosomal sterility factors. In this case, the new hybrid population would be fertile, stable and at the same ploidy level as its parents, and would be able to continue to vary through outcrossing, (2) if the progenies that are partially reproductively isolated arise due to the chromosomal sterility barriers from both parental species, the hybrid would be sterile (Rieseberg and Carney, 1998).

Hybridization has traditionally been considered to be more common in plants than animals (Grant, 1981). Part of the reason is that when plant hybrids are produced, they may survive for a long time and even reproduce vegetatively. Even if they are sterile, this gives a longer time interval for a change such as polyploidy that could restore fertility as compared to animals (Stebbins, 1950). Furthermore, plants can tolerate polyploidy better than many animals so that hybrid plants are more likely to have evolutionary futures through the restoration of fertility via polyploidy (McDade, 1995).

Hybrids can be defined in various ways e.g. Stebbins (1950) proposed that hybrids could possibly be formed between members of different, partially isolated organisms. Rieseberg and Carney (1998) described hybrid as an organism that formed by cross-fertilization between individuals of different species or could be the offspring between individuals from populations, where the offspring could be distinguished on the basis of one or more heritable characters. However, recent papers suggest that a hybrid could be an organism or lineage of a reticulate history that arise when divergence is expected, which means that hybrids could be formed between individuals from isolated or differentiated populations (McDade, 1995).

POLYPLOIDY

Polyploidy is the phenomenon in which an organism has more than two sets of chromosomes, and is one of the most widespread and distinctive features of the higher plants (Stebbins, 1950; Grant, 1981; Soltis et al., 2003). Traditionally, it has been considered that there are two types of polyploidy, one involves the simple multiplication of the same chromosome set and is termed autopolyploidy; the second involves the doubling of structurally dissimilar chromosome sets and is called allopolyploidy (cited by Otto and Whitton, 2000). Allopolyploids that occur in nature are associated with hybridization, either at an interspecific level or between different subspecies or races of the same species, whereas autopolyploids may arise without hybridization as found in *Heuchera* (Saxifragaceae) (Soltis et al., 1989).

The frequency of polyploidy has been of interest for some time, especially for the flowering plants (Soltis et al., 2000; Soltis et al., 2003). Stebbins (1950) stated that polyploidy was found more frequently in perennial herbs than in annuals and was relatively rare in woody plants. Darlington (1937) estimated that about one half of flowering plants were polyploid, whereas Stebbins (1950) proposed that only 30 – 35% of angiosperms were polyploid. However, Grant (1981) hypothesized that flowering plants that have chromosome number (n) equal or higher than 14 might have a polyploid origin; based on this he speculated that 47% of angiosperms were polyploid (cited by Soltis et al., 2003). Recently, Cook et al. (2003) proposed that 50% of angiosperm species have a polyploid origin. The evidence of the role of polyploidy in evolution was estimated by Otto and Whitton (2000), who proposed that ploidy changes may represent from 2 – 4% of speciation events in angiosperms and about 7% in ferns, respectively. It

could be considered that all of the species in Eupatorieae are polyploid (Yahara et al., 1989), but may stem from a single ancient polyploidization. If all of *Eupatorium* is ancestrally polyploid (= paleoplolyploid), secondary polyploidy (= neopolyploidy) has occurred within about half of the species (**Table 1-1**).

MOLECULAR APPROACHES

Molecular approaches have enhanced greatly our ability to estimate phylogeny. Examining both nuclear and organellar markers in combination offers an advantage for phylogenetic inference because they potentially have separate phylogenies, and discrepancies between these phylogenies can provide an indication that hybridization is involved. Chloroplast DNA (cpDNA) data is useful to investigate the maternal contribution in plant hybridization and polyploidy (Soltis et al., 1991). In contrast, nuclear ribosomal DNA (nrDNA) is biparentally inherited, so that analyses of nrDNA may provide evidence of one or both of the parental contributions to polyploid species (Soltis and Soltis, 1990). The complementarity of using cpDNA and nrDNA data has benefited to several analyses, such as in *Heuchera* group (Saxifragaceae) (Soltis and Kuzoff, 1995), tribe Eupatorieae (Asteraceae) (Ito et al., 2000a), and Trilliaceae (Farmer, 2000). Doyle et al. (1990) used combined data to present a better explanation of the complex evolutionary origins of polyploid species in *Glycine* subgenus *Glycine* (Leguminosae).

NUCLEAR RIBOSOMAL DNA

The internal transcribed spacer (ITS) region of 18S-26S nuclear ribosomal DNA (nrDNA) has been widely used to infer phylogeny at lower taxonomic levels in many angiosperm families. As cited by Baldwin et al. (1992, 1995) the ITS region is highly

repeated in the plant nuclear genome, thus this high copy number promotes detection, amplification, cloning, and sequencing of nrDNA. In addition this gene family may undergo rapid concerted evolution through an unequal crossing-over and gene conversion, a property that promotes intragenomic uniformity of repeat-units. Most of all the small size of the ITS region (<700 bp in angiosperms) and the presence of highly conserved sequences flanking each of the two spacers make this region easy to amplify using the universal primers designed by White et al. (1990). ITS has been demonstrated to be a useful molecular marker at the intergeneric level to determine phylogenies of the tribe Eupatorieae (Schmidt and Schilling, 2000; Ito et al., 2000a, 2000b). Since there may be little variation in ITS at the intraspecific level (see below), in this study a single individual from each population was used for the analysis as a representative of each population, which were mainly from *E. rotundifolium* var. *rotundifolium* and *E. sessilifolium* and some other species where they were available.

CHLOROPLAST DNA

Chloroplast DNA (cpDNA) data is an effective tool for phylogenetic analysis among genera and at higher taxonomic levels (Downie and Palmer, 1992; Olmstead and Palmer, 1994). Analyses of chloroplast genome evolution showed a high degree of conservation in length, structure, gene content and linear order of genes among major lineages of land plants (Palmer et al., 1985), thus any structural changes that occurred in the chloroplast genome might reveal evolutionary relationships (Downie and Palmer, 1992). Gene arrangement and size (the range of 148~151 kb) of the chloroplast genome are relatively the same throughout the Asteraceae (cited by Downie and Palmer, 1992). Most initial studies of cpDNA utilized the approach of restriction fragment analysis. The

polymerase chain reaction (PCR) provides the ability to amplify specific sequences. Because each cpDNA is typically single copy within a given cpDNA molecule, and there are numerous, presumably identical cpDNA molecules in each cell, which are basically identical, it is technically easy to amplify and sequence specific cpDNA regions for phylogenetic studies (Olmstead and Palmer, 1994).

The uses of DNA sequences (both ITS and cpDNA) in this study revealed differences among even closely related species, but few to no differences at the intraspecific level, so another appropriate approach had to be taken as an additional tool for investigating relationships at the population level.

INTER SIMPLE SEQUENCE REPEAT (ISSR)

Inter Simple Sequence Repeat (ISSR) is a molecular marker technique that has been developed to study genetic variation (Wolfe and Liston, 1998) and was first published by Gupta et al. (1994). It can be used to infer phylogenetic relationships within and among species (e.g. parasitic plant genus *Hyobanche* (Orobanchaceae) (Wolfe and Randle, 2001)). Tsumura et al. (1996) used the ISSR technique to study genetic variation in the closely related Douglas-fir (*Pseudotsuga menziesii*) and sugi (*Cryptomeria japonica*). Because it reveals a high level of genetic differentiation, ISSR is widely used particularly in horticultural applications, mostly for breeding purpose such as for the Asiatic hybrid lily (Yamagishi et al., 2002) and the cultivated grain *Amaranthus* (Xu and Sun, 2001). This method has also been used to verify the varieties of sorghum and banana (Godwin et al., 1997).

The ISSR approach has been shown to be a particularly effective tool when analyzed together with other molecular markers. For example, it was used in

combination with nrDNA (ITS) to study biogeography of the alpine plant genus *Raoulia* (Smissen et al., 2003); with random amplified polymorphic DNA (RAPD) to detect genetic diversity in conifers (Tsumura et al., 1996), and with allozyme techniques to study genetic variations in cryptoviviparous mangrove *Aegiceras corniculatum* (Myrsinaceae) (Ge and Sun, 1999). ISSR is not only useful for the study of vascular plants, but also could be applied to analyze nonvascular plants. Vis (1998) used ISSR molecular markers to distinguish gametophytes of red algae in genus *Batrachospermum* (Rhodophyta). Furthermore, the ISSR method has been proved to have a great potential testing natural hybridization among natural populations of *Penstemon* (Scrophulariaceae) (Wolfe et al., 1998), and the two varieties in wild bergamot *Monarda fistulosa* (Lamiaceae) in West Virginia (Kimball et al., 2001).

The conventional approach to employing ISSR markers involves using single primers complementary to microsatellites or simple sequence repeats (SSRs), which are composed of tandem repetitive DNA sequences with a repeat length of short motifs (1–5 base pairs) scattered throughout the entire plant genome (cited by Tsumura et al., 1996). The ISSR markers are generated from single-primer PCRs where the primer is developed from a microsatellite motif, e.g. $(GT)_{x,}(GA)_{x}$. Primers can be anchored on the 5′ or 3′ end with one to three nucleotides such as CAA-$(GA)_{x}$. Each primer amplifies the region between SSRs, which is why they are referred to as inter-simple sequence repeats. ISSR approaches are used to detect polymorphisms and can be used as a fingerprint to identify closely related species (Tsumura et al., 1996; Wolfe and Randle, 2001). Because of the high potential of the ISSR method to reveal genetic variation, it was chosen as a

potentially useful tool for analyzing *Eupatorium* genetic differences within and among populations of species and as an indicator of ploidy origin.

For this study *Eupatorium rotundifolium* and *E. sessilifolium* specimens were collected from diploid and polyploid populations. DNA extracts from these specimens were used in molecular studies. The molecular approach consisted of two parts: the amplification of DNA for generating nuclear and chloroplast sequences, and the ISSR studies. The molecular data were used for (1) determining the relationship among species and (2) evaluating the origin of polyploidy; that is whether polyploids are autopolyploids or allopolyploids, and if they are allopolyploids, then what might be their genomic content, and (3) addressing the question of the origin of an apomictic species: *Eupatorium godfreyanum.*

MATERIALS AND METHODS

Fieldwork was performed in the southeastern United States, where both diploid and polyploid populations of *E. rotundifolium* var. *rotundifolium* and *E. sessilifolium* are distributed (**Figs. 3-1** and **3-2**). Locations to be visited were sought based on the specimen holdings at the herbaria of the University of Tennessee, Florida State University, and Virginia Tech. Three major trips were taken during late summer and early fall of 2001 – 2003. Six of the 19 populations collected for *E. rotundifolium* and eight of the 15 collections for *E. sessilifolium* were diploid. A single specimen of each population was used as a voucher (or a photocopy of the specimens was made before removal of the leaves for DNA analysis).

Eupatorium rotundifolium diploid and polyploid samples were collected from 6 southeastern states in the U.S. (**Table 3-1**): Alabama (AL), Florida (FL), Georgia (GA), North Carolina (NC), South Carolina (SC) and Tennessee (TN). Six of the 19 populations collected in FL were diploids; RD1 was collected from Jefferson Co., RD2, RD3 and RD4 were from Calhoun Co. and the last two populations were collected along the coastal line, St. Johns Co.

Thirteen polyploid populations of *E. rotundifolium* were collected from 5 states. Two polyploid populations were from AL: RP10 (Dekalb Co.), RP11 (Marshall Co.), and only one population from GA, RP9 (Dade Co.). Three populations were from NC (RP3 (Richmond Co.); RP5 (Orange Co.); RP6 (Brunswick Co.) and four populations RP1 (Colleton Co.), RP4 (Berkeley Co.), RP7 (Georgetown Co.) and RP8 (Berkeley Co.) were collected from SC. The last three polyploid populations were from TN: RP2 (Bledsoe Co.), RP12 (Cumberland Co.) and RP13 (Bledsoe Co.) (**Fig. 3-1**).

Eight of the 15 populations of *E. sessilifolium* were diploids (**Table 3-2**) and were collected from NC, TN, and Virginia (VA). Populations of *E. sessilifolium* were more scattered and smaller than the populations of *E. rotundifolium*. SD1 was the only diploid population that was collected from Brush Mountain, Montgomery Co., VA; the others were from the NC and TN border area (**Fig. 3-2**). Four of diploid populations were collected from TN: SD2, SD3 and SD4 were from Blount Co., and only SD8 was from Bledsoe Co. Of the last three diploid populations collected from NC, two were from Graham Co. and one from Swain Co. Each population was at least 5 miles apart and they were not part of a continuous population.

Table 3-1 Population codes and sample sizes of *Eupatorium rotundifolium* used for molecular approaches.

Population Code	Location (county, state)	Sample Size (#plant)
Diploid Population		
RD1	Jefferson, FL	3
RD2	Calhoun, FL	10
RD3	Calhoun, FL	10
RD4	Calhoun, FL	9
RD5	St. Johns, FL	8
RD6	St. Johns, FL	10
	Total	50
Polyploid Population		
RP1	Colleton, SC	7
RP2	Bledsoe, TN	6
RP3	Richmond, NC	10
RP4	Berkeley, SC	10
RP5	Orange, NC	5
RP6	Brunswick, NC	8
RP7	Georgetown, SC	6
RP8	Berkeley, SC	4
RP9	Dade, GA	3
RP10	Dekalb, AL	3
RP11	Marshall, AL	5
RP12	Cumberland, TN	7
RP13	Bledsoe, TN	4
	Total	78

Table 3-2 Population codes and sample sizes of *Eupatorium sessilifolium* used for molecular approaches.

Population Code	Location (county, state)	Sample Size (#plant)
Diploid Population		
SD1	Montgomery Co. VA	3
SD2	Blount Co. TN	9
SD3	Blount Co. TN	8
SD4	Blount Co. TN	4
SD5	Graham Co. NC	2
SD6	Graham Co. NC	6
SD7	Swain Co. NC	5
SD8	Bledsoe Co. TN	6
	Total	43
Polyploid Population		
SP1	Craig Co. VA	1
SP2	Floyd Co. VA	4
SP3	Franklin Co. VA	5
SP4	Botetourt Co. VA	6
SP5	Bedford Co. VA	3
SP6	Roanoke Co. VA	6
SP7	Jefferson Co. TN	3
	Total	28

Most of the *E. sessilifolium* polyploid populations were collected from VA along the Blue Ridge Parkway: SP2 (Floyd Co.), SP3 (Franklin Co.), SP4 (Botetourt Co.) and SP5 (Bedford Co.). The other two populations collected in VA were from very isolated populations in the mountains, SP1 (Craig Co.) was close to the borderline of West Virginia and SP6 was located in Poor Mountain, Roanoke Co. SP7 was the only population that was collected in Jefferson Co., TN.

During the collection trip in 2002, 5 samples of *Eupatorium godfreyanum* were collected from Floyd Co., VA and another single sample, which was noted as a TN state record, was found in Cumberland Co. during field collection in 2003. The overall morphology of *E. godfreyanum* is quite similar to *E. sessilifolium*, including the habitat, but there are some exceptions: first, the base of the leaf is acute (not as round as *E. sessilifolium* or rotund as *E. rotundifolium*); second the plant is pubescent throughout; and third, the flowers do not produce pollen.

Flowers from each specimen were collected and tested for pollen appearance with cotton blue solution, to estimate ploidy levels (**Figure 3-3**). The sample sizes and locations of both species are shown in **Tables 3-1 and 3-2**. DNA samples were extracted principally from young leaves with the CTAB protocol of Doyle & Doyle (1987)(**Appendix B-3**). To decrease the interference from phenolic compounds, DNA extractions were cleaned up using the Wizard Kit protocol (Promega, Madison, Wisconsin, USA) (**Appendix B-4**). The purified DNA extracts were quantified and the concentration was adjusted to between 25 – 100 ng/ μl and kept in –20°C freezer until used.

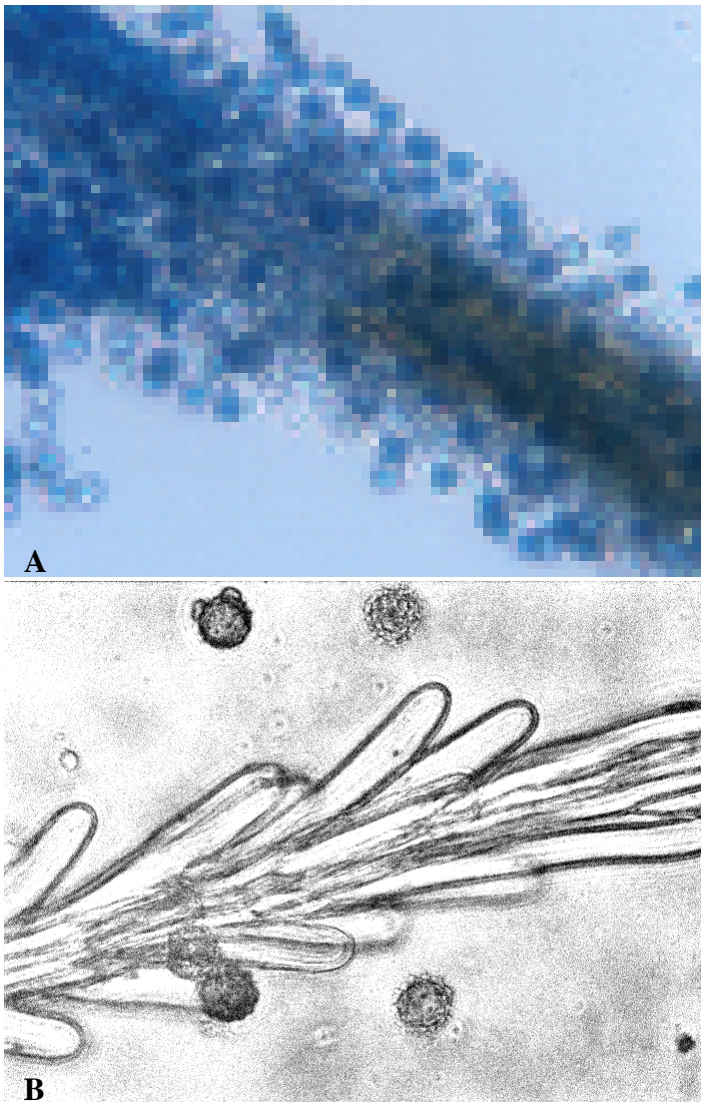


Figure 3-3. *Eupatorium rotundifolium* (A) and *E. sessilifolium* (B) pollen grains stained with cotton blue solution (400X).

NUCLEAR RIBOSOMAL DNA; ITS

Amplification was performed by PCR in a thermal cycler (PTC-100™ Programmable Thermal Controller, MJ Research, INC.). One of the DNA samples from each population was analyzed and used as a representative for its population. The 20 µl PCR reaction mix was composed of 13.8 µl ddH_2O, 2.0 µl 10X Buffer (Promega), 0.9 µl 50 mM $MgCl_2$, 0.4 µl dNTP (A, C, G, T), 0.4 µl of both ITS primers 5 (5'-GGAAGTAAAAGTCGTAACAAGG-3') and 4 (5'-TCCTCCGCTTATTGATATGC-3') (White et al., 1990), 0. 4 µl BSA, and 0.1 µl *Taq* polymerase (Promega) while the mixture was still kept on ice. One µl DNA extract (template) was added just before placing in the thermo cycler with the following program (**Table 3-3**).

Four of the 19 samples of *E. rotundifolium* (RD2, RD3, RP5 and RP11) had to be cloned (**Appendix B-8**) because of contamination of fungal DNA in the plant samples. Additional ITS sequences of *Eupatorium* that were used in this study were downloaded from Gen-Bank, a list of the species names and Gen-Bank accessions number is summarized in **Appendix B-10**.

Table 3-3 The thermocycler program for the ITS PCR protocol.

Step	Temperature (°C)	Time (min)	Stage
1	94	2	melt
2	94	1	denaturation
3	50	1	primer annealing
4	72	1	extension of DNA
5	Go to step 2	39 times	multiply
6	72	3	final extension
7	4	-	storage

CHLOROPLST DNA: *trnC – psbM*

To choose an appropriate cpDNA region, a preliminary study was performed, using *Eupatorium hyssopifolium, E. rotundifolium* and *E. capillifolium* (the outgroup), as representatives of this genus. Twenty-one non-coding cpDNA regions (*trnH-psbA; psbA-3′trnK; 3′trnK-matK; matK-5′trnK; rps16 intron; trnS-trnG; trnG* intron*; rpoB-trnC; trnC-ycf6; ycf6-psbM; psbM-trnD; trnD-trnT; trnS-trnfM; trnS-rpS4; rpS4-trnT; trnT-trnL; trnL* intron*; trnL-trnF; 5′rpS12-rpL20; psbB-psbH; and rpL16* intron) were examined for sequence variation among the 3 samples. The survey suggested that the *trnC-ycf6* region was likely to be the most useful region for molecular analysis in *Eupatorium* (Total Potential Informative Character (PIC) = 14; % variability = 2.34) (Shaw et al., in press).

Amplification was performed by PCR in a thermal cycler (PTC-100™ Programmable Thermal Controller, MJ Research, INC.). One of the DNA samples from each population was used as a representative for its population and was the same sample that were used in ITS analysis. The 20 μl PCR reaction mix was composed of 13.8 μl ddH_2O, 2.0 μl 10X Buffer (Promega), 0.9 μl 50 mM $MgCl_2$, 0.4 μl dNTP (A, C, G, T), 0.4 μl of $trnC^{GCA}$F: CCA GTT CRA ATC YGG GTG (modified from Demesure et al., 1995) and psbMR: ATG GAA GTA AAT ATT CTY GCA TTT ATT GCT (Shaw et al., in press), 0.4 μl BSA, and 0.1 μl *Taq* polymerase (Promega, Madison WI) while the mixture was on ice. One μl DNA extract (template) was added just before the sample was placed in the thermal cycler with program from **Table 3-4**.

Table 3-4 The thermocycler program for cpDNA: *trnC -psbM* PCR protocol.

Step	Temperature (°C)	Time (min)	Stage
1	80	5	melt
2	94	1	denaturation
3	50	1	primer annealing
4	72	3.5	extension of DNA
5	Go to step 2	34 times	multiply
6	72	5	final extension
7	4	-	storage

GEL ELECTROPHORESIS

PCR products were tested by 1% agarose gel electrophoresis, which contained 0.5 g/ml ethidium bromide (**Appendix B-7**). A molecular weight marker (100 bp ladder Fisher BioReagents Molecular Weight Marker) was run in the first lane followed by PCR products in the adjacent lanes and the last one was left for the negative control. The gel was run for 45 min or until the blue marker migrated approximately 6 cm at ~100 V.

PURIFICATION AND SEQUENCING

The PCR product was purified using the ExoSAP-IT protocol (USB Corporation, Cleveland, OH). ITS sequencing reaction was performed by two primers (ITS5 and ITS4). The sequencing of cpDNA region used two forward primers (*trnC* and *ycf6*) on a single amplification product (*trnC-psbM* PCR). *trnC* was used as an initial primer, which sequenced ~600 bp long; part of its sequence overlapped (~50 bp) the *ycf6* region (internal primer), which sequenced ~490 bp long. Both ITS and cpDNA sequencing reactions were performed with the ABI Prism Dye Terminator Cycle Sequencing Ready

Reaction Kit, v. 3.1(Perkin-Elmer/Applied Biosystems, Foster City, CA) and followed by Centri-Sep (**Appendix B-5**). Reaction products were sent to the University of Tennessee Molecular Biology Resources Facility, where they were read by the automated sequencing machine (ABI Prism model 3100 sequencer).

ALIGNMENT

The sequences were received from the sequencing lab as *.abi files. The program Sequencher was used for comparing the assigned nucleotide bases with the chromatograms, and manual adjustments were made where needed. The corrected sequences of nucleotide bases were saved as a text file. Chloroplast DNA and ITS sequences were aligned initially with the Clustal X (Thompson et al., 2001) program. The alignment parameters were set as Pairwise Parameter: Slow-accurate, Gap Opening: 10.0 and with Gap Extension: 0.1. The complete alignment was run and the output files were saved as Clustal and NEXUS formats. However, the alignments were scanned by eye and adjusted manually as needed, with particular attention to gap assignment.

ITS & CHLOROPLAST DATA ANALYSIS

Phylogenetic analysis used a distance method (neighbor-joining) and the maximum parsimony method, implemented with the computer program PAUP 4.0b10 (Swofford, 2002). Heuristic searches were performed with random sequence addition with replicates to increase the probability of finding all the shortest trees. Gaps were treated as missing data. A resampling method, the bootstrap analysis (Felsenstein, 1985), was used to assess the relative support for individual clades, using fast stepwise-addition replicates for each of 10,000 bootstrap replicates with the maximum number of trees set

at 100. A consensus trees was generated which retained groups with a frequency of more than 50%.

The outgroup taxa used in this study were selected from *Eupatoriadelphus* because it is sister to *Eupatorium* (unpublished result, per. com. with Schilling, E. E.). The available data of *Eupatoriadelphus maculatus* and *E. fistulosus* from other studies (Schmidt and Schilling, 2000; Ito et al., 2000b) were used as outgroups for ITS and *E. fistulosus* and *E. purpureus* were used as outgroups for cpDNA.

The trees that were generated from nuclear and cpDNA data sets were compared, and the presence of incongruence between these trees was considered as potential evidence for the occurrence of hybridization.

ISSR ANALYSIS

Analysis for ISSR included both diploid and polyploid populations. Each population was represented by ten individuals at maximum, although most had fewer because of the small sizes of populations; in some cases, amplification of some samples within a population did not occur for unknown reasons which reduced the number of available samples. PCR reactions (**Table 3-5**) were performed using standard protocols (**Appendix B-6**), which were developed by Dr. Andrea Wolfe (http://www.biosci.ohio-state.edu/~awolfe/ISSR.html).

Table 3-5 The thermocycler program for ISSR protocol

Step	Temperature (°C)	Time		Stage
		min	second	
1	94	1	30	melt
2	94	-	45	denaturation
3	45	-	45	primer annealing
4	72	1	30	extension of DNA
5	Go to step 2	35 times		multiply
6	94	-	45	melt
7	45	-	45	annealing
8	72	5	-	final extension
9	4	-		storage

An initial screening was performed to determine the most useful primers and to optimize the protocols for *Eupatorium*. A total of 21 primers (**Appendix B-6**) was chosen variously from the "800" series (University of British Colombia primer set #9) and those listed on Dr. Andrea Wolfe's ISSR web page (http://www.biosci.ohio-state.edu/~awolfe/ISSR.html). Several protocols were tested in order to yield the maximum quality of reproducible bands. Four primers were chosen because they were easily to amplify, gave a relatively large number of bands that could be readily scored, and appeared to exhibit variability between the diploid and polyploids samples chosen for initial screening. These four ISSR primers were used in single-primer reactions: 808 $(AG)_8$-C, 810 $(GA)_8$-T, 811$(GA)_8$-C and MANNY $(CAC)_4$-RC.

The PCR products were run on 2% agarose gels, which contained 0.5 □g/ml ethidium bromide. The gel was run for 2 – 2.5 hrs or until the blue marker migrated approximately 8 cm at ~96 – 98 V. The molecular weight marker (100 bp ladder Fisher

BioReagents Molecular Weight Marker) was loaded in the first and last lane, the PCR products were mixed with the loading dye before loading to the gel, the very last lane was left for the negative control, which included all ingredients except a DNA extract (**Fig. 3-4**). The gel, with a plastic fluorescent ruler next to the marker for scale, was photographed with a digital camera KODAK DC 120 (KODAK Digital Science 1D), the image was saved as *.tif format, and a high quality print was obtained. Two replicate PCR reactions were conducted for each primer/taxon pair. Only bands that appeared in both replicates were scored.

The bands were manually scored and sized by comparing the migration distances with those of the molecular weight markers. The data on band presence and distance were imported into a spreadsheet using Excel (Microsoft® Excel X for Mac® Program, Bloomsbury Publishing Plc.). The program function was used to plot the graph between Rf (Y-bar) and molecular weight (X-bar) and to calculate the distances of bands of known molecular weight with exponential mode. The graph represents the equation y = aLn (x) + b, where y = Rf and x = molecular weight. Distances of unknown bands were measured and the molecular weights of the bands were estimated using the equation above. They were scored as 1 if the band was present and 0 if the band was absent. Data was imported into a new spreadsheet with rows = loci (band size) and columns = taxa. The fragments were arranged by size. The largest one was on the first row and the smallest size was at the bottom in order of primers 808, 810, 811 and MANNY, respectively. Number of bands per primer, number of bands per species, number of bands that were shared by both populations within species, and the percent of polymorphic bands were recorded.

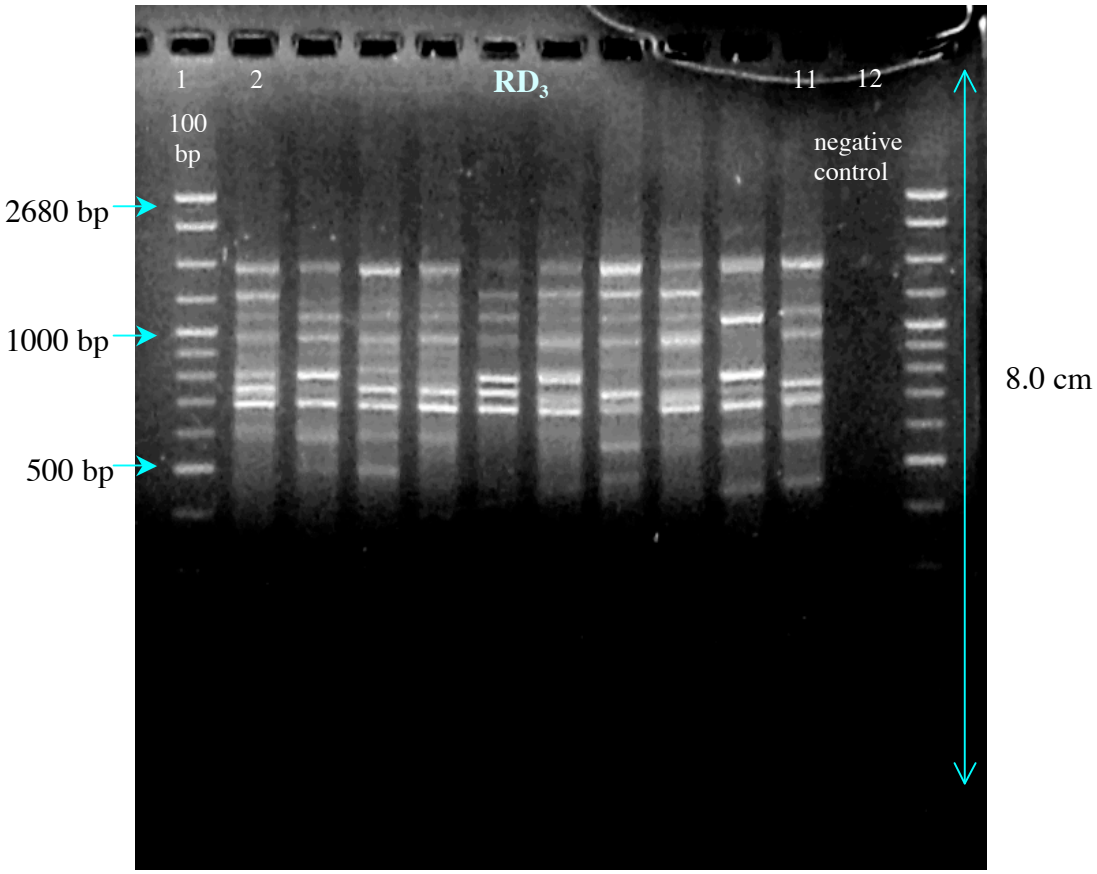


Figure3-4. Banding profiles of *Eupatorium rotundifolium*, diploid population#3 by primer 810. The first lane shows 100 bp ladder, lane#2-11 are different individuals from the same population, lane#12 is a negative control and the last lane is 100 bp ladder.

ISSR DATA ANALYSIS

Analysis of ISSR data was based on the distance matrix method (standard distances of mean character difference coefficient was used to compute the pairwise distances neighbor-joining), implemented with the computer program PAUP v. 4.0 b10 (Swofford, 2002). Bootstrap analysis (Felsenstein, 1985) was performed with PAUP v.4.0b10, using fast stepwise-addition replicates for each of 10,000 bootstrap replicates with the maximum number of trees set at 100. The ISSR trees generated from *Eupatorium rotundifolium* diploid and polyploid populations data set were compared to the trees that were derived from *E. sessilifolium* diploid and polyploid populations profile to investigate the evidence of polyploid origins. Additionally, both data sets were combined to study the relationship of both species.

RESULTS AND DISCUSSION

The overall result of this study indicates that there was genetic variation within and between both diploid and polyploid populations of both species; that ITS and cpDNA gave different levels of variation in *E. rotundifolium* and *E. sessilifolium*; that ISSR data showed less variation in polyploids than in diploids, but showed variation between populations; and that allopolyploidy is probably involved in the formation of polyploids. It was found possible that in both *E. rotundifolium* and *E. sessilifolium* polyploid populations had multiple origins and the relationship between diploids and polyploids involved reticulation.

ITS SEQUENCE

The length of the ITS sequence in both *Eupatorium rotundifolium* and *E. sessilifolium* was the same, 654 bp (base pairs). ITS1 was 272 bp, which was longer than ITS2 (220 bp). In both species the 5.8S region was 162 bp in length; because it was also invariant, it was excluded from the phylogenetic analyses. The neighbor-joining tree (**Fig. 3-5**) generated from ITS sequence data included a total of 36 samples, 19 of *E. rotundifolium*, 15 of *E. sessilifolium*, and two additional samples representing two species of *Eupatoriadelphus* that were used as the outgroup. The neighbor-joining tree illustrated that there was more variation among *E. rotundifolium* samples than *E. sessilifolium*, where there was only a single change in SD8 that was uninformative (autapomorphic).

Figure 3-5 demonstrated that the diploid samples of *Eupatorium rotundifolium* varied in ITS sequence, and the data analysis suggested that the variation was contained in 5 major subtypes. However, the polyploid samples were not all uniform, but instead some were closer to at least one diploid sample than to another polyploid. Because there was variability for ITS within polyploid samples of *E. rotundifolium*, it is possible that the ITS in the polyploids may have come from various diploids. A single sample, RP11, was placed as the most basally diverging sequence, followed by another single sample, RP12, which was a sister to all other *E. rotundifolium* samples. The composition of one of the clades suggested that a polyploid sample RP1 might share a similar nuclear genome with the diploid RD2. RP1 and RP4, which are in the same clade, were collected from SC and both locations were close to each other (**Fig. 3-1**). RD1 and RD3 were collected from different counties but were most closely related to each other based on ITS

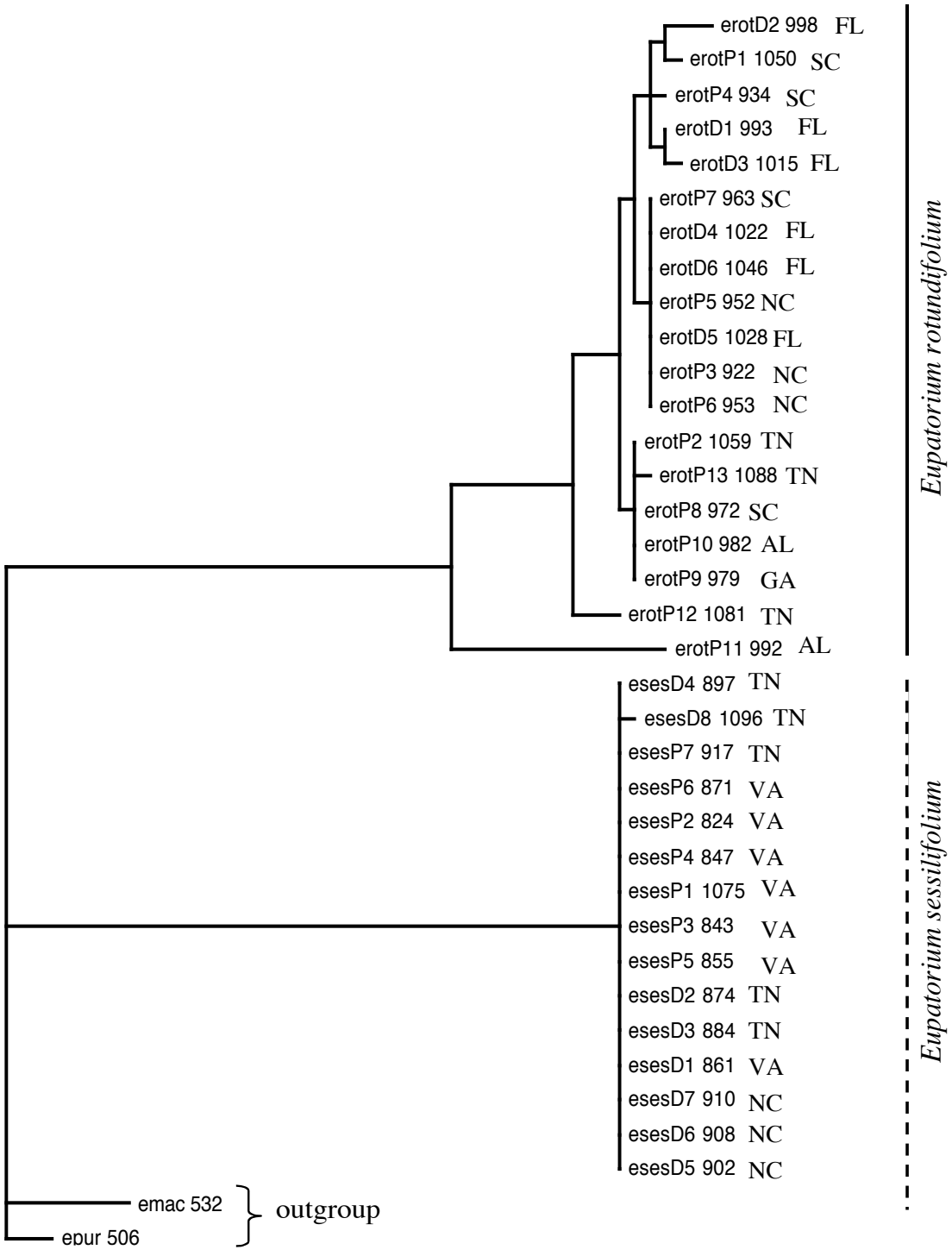


Figure 3-5. Neighbor-joining tree based on ITS DNA sequences of *Eupatorium rotundifolium* and *E. sessilifolium*, used *Eupatoriadelphus maculatus* and *E. purpureus* as the outgroup taxa.

sequences. Another clade included the diploids RD4, RD5, and RD6 together with the polyploid samples: RP3, RP5, RP6, and RP7. Notably, these populations were variously from NC and SC, with two populations that were collected along the coast and the other two in the piedmont. The other clade was composed of only polyploid samples: RP2, RP8, RP9, RP10, and RP13. Of these samples, only RP8 was farther away, the other polyploid populations were located near each other in AL, GA, and TN. It was clear that *E. rotundifolium* showed more variation in its ITS region than *E. sessilifolium*.

The ITS sequence data were also analyzed by parsimony analysis, and the result gave 36 shortest trees (47 informative characters; CI = 0.95, RI = 0.98). A strict consensus tree (**Fig. 3-6)** showed that *E. rotundifolium* was monophyletic with one polyploid sample (RP11), which was different from all other samples. *E. sessilifolium* diploid and polyploid populations were also monophyletic with a high bootstrap value showing 100% support. The levels of genetic diversity in nrDNA of *E. rotundifolium* and *E. sessilifolium* were unequal, even though they are very closely related species. The origin of polyploidy of *E. sessilifolium* and *E. rotundifolium* has not been resolved by the ITS sequences due to the low levels of ITS region sequence divergence among these samples.

There are several possible explanations for the lack of variation in *E. sessilifolium*. One possibility involves concerted evolution; and a second is that the species is of very recent origin. Because both the diploids and the polyploids lacked variation, it is not a matter of concerted evolution after the formation of the polyploids.

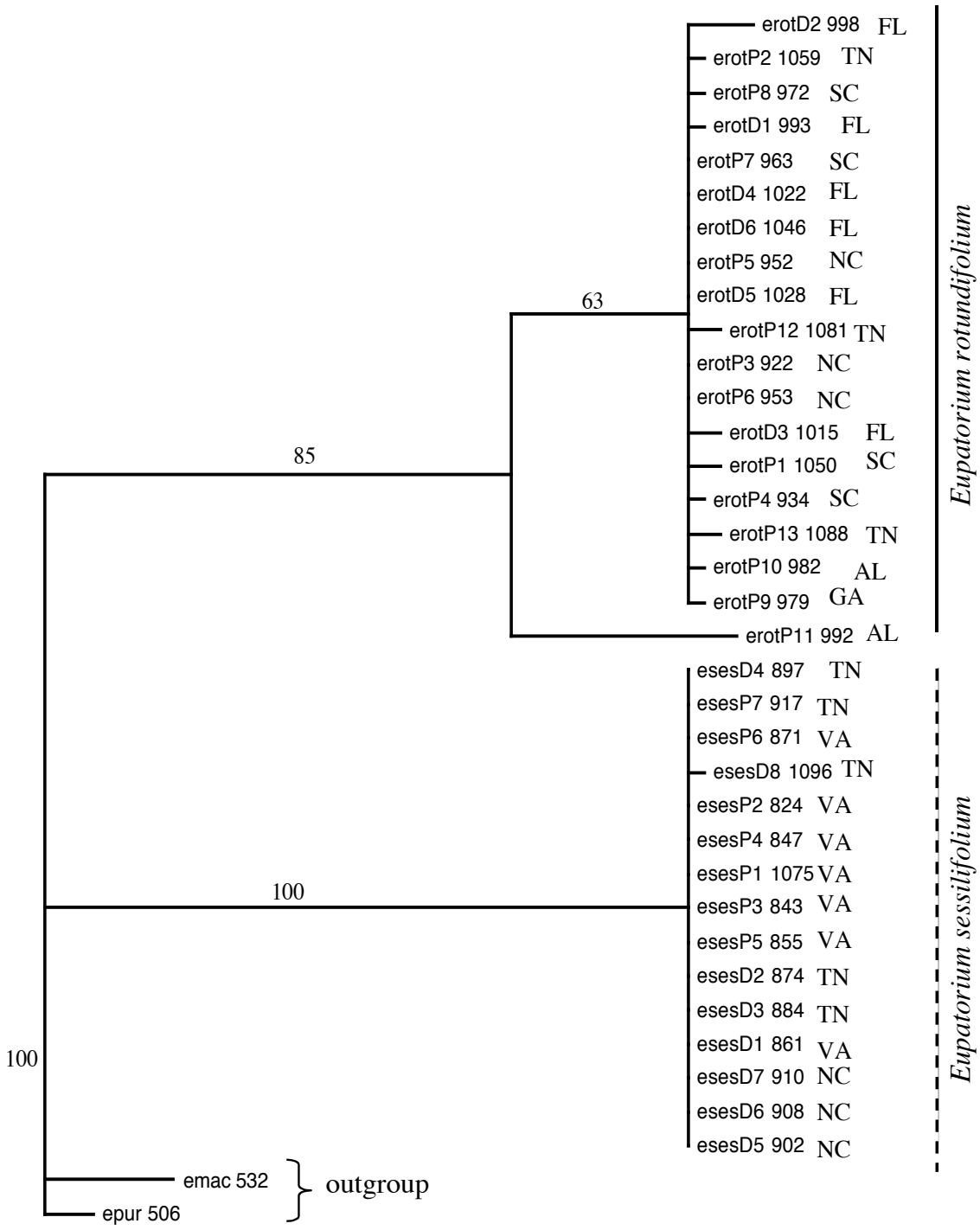


Figure 3-6. Strict consensus tree generated from maximum parsimony analysis of ITS sequences of *Eupatorium rotundifolium* and *E. sessilifolium*, using *Eupatoriadelphus maculatus* and *E. purpureus* as the outgroup taxa with the bootstrap value on the branch.

Concerted evolution, a process of gene conversion or unequal crossing-over, will be able to homogenize different parental genomes in a hybrid or in a polyploid so that one parental genome type may be seen in them (Sang et al., 1995). The distinctive placement of RP11 in the ITS tree prompted a critical reexamination of its morphology, but it was not distinguishable from other *E. rotundifolium* samples. The presence of a somewhat distinctive ITS sequence in RP11 raised the possibility that its origin might have involved another diploid species of *Eupatorium*.

To investigate this hypothesis, a broader set of ITS sequences of *Eupatorium* was compiled by downloading those available from GenBank, which originally came from the works of Ito et al. (2000b) and Schmidt and Schilling (2000), as well as adding some additional new sequences. The resulting data set included ITS sequences of samples from across the entire range of the genus including North America, Asia, and Europe. It was not possible to include some of the North American species (e.g. *E. anomalum*, *E. resinosum*) because of the lack of plant material, but these were mostly relatively rare species with restricted geographic ranges. The tree was again rooted by the outgroup: *Eupatoriadelphus fistulosus*, *E. maculatus*, *E. purpureus,* and *E. dubius*.

A total of 77 samples were included in the analysis, and the neighbor-joining tree of the combined ITS sequence data (**Fig. 3-7**) showed that *Traganthes* group, which is composed of *E. capillifolium*, *E. compositifolium,* and *E. leptophyllum,* was a basal clade. Another clade included the European (*Eupatorium cannabinum*) and all of the Asian species, from which were available *Eupatorium variabile*, *E. tashiroi*, *E. makinoi*, *E. lindleyanum*, *E. japonicum*, *E. glehnii*, and *E. formosanum*, suggesting that these are monophyletic and possibly derived from within the North American *Eupatorium*.

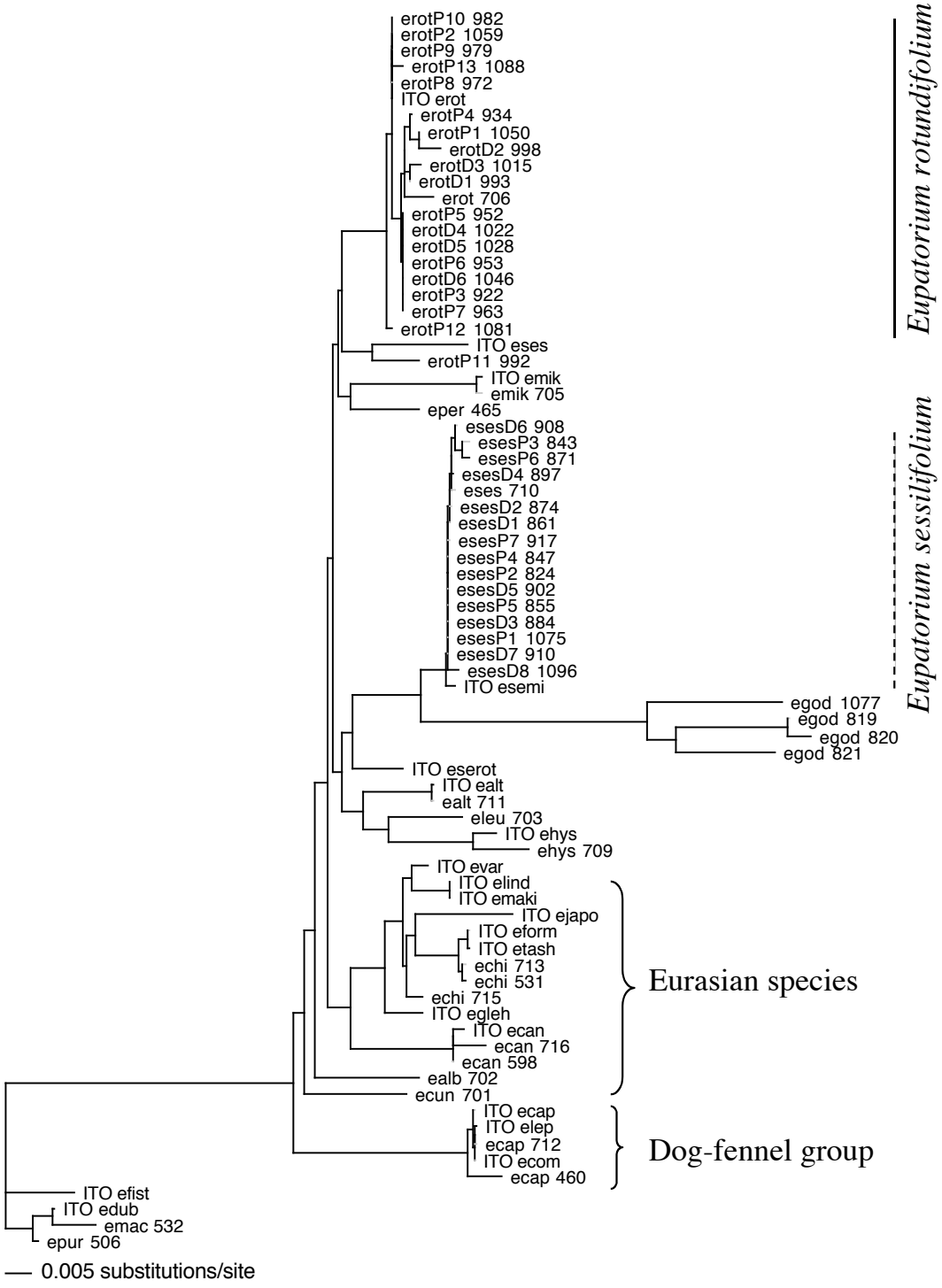


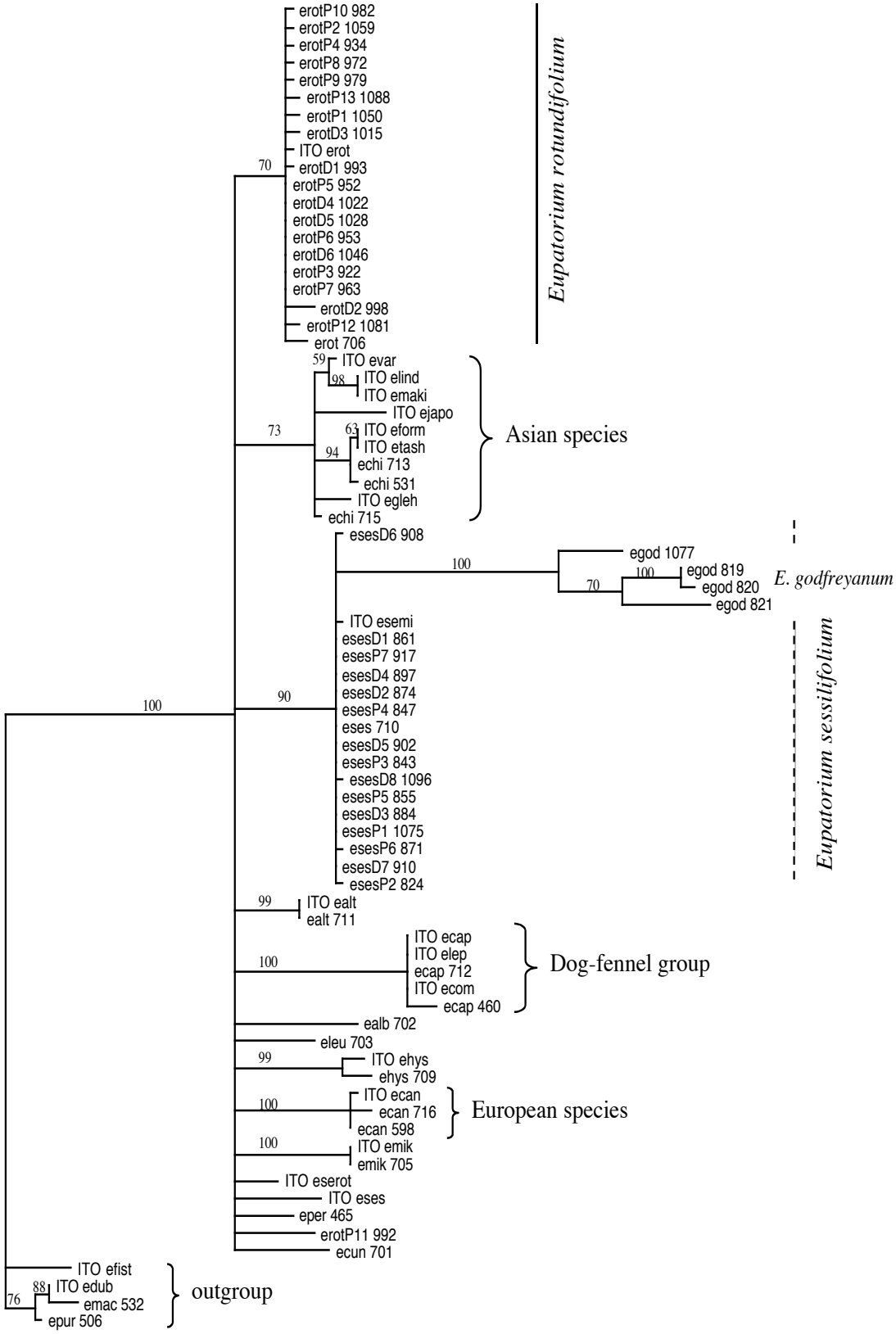
Figure 3-7. Neighbor-joining tree based on ITS DNA sequences of the genus *Eupatorium*, using *Eupatoriadelphus maculatus* and *E. purpureus* as the outgroup taxa.

Surprisingly, RP11 was in a clade with a diploid sample of *E. sessilifolium* that was collected by Ito et al. (2000b) from Graham Co., NC, the same county from which the other *E. sessilifolium* diploid populations used in this study were found. With the exception of *E. rotundifolium* (RP11), and *E. sessilifolium* that was collected by Ito et al. (2000b), *E. rotundifolium* and *E. sessilifolium* were both monophyletic. However, the inability to confirm the identity of the sample reported by Ito et al. as *E. sessilifolium* suggests caution in interpreting this result.

One of the mysterious problems of the apomictic species *Eupatorium godfreyanum* was the identification of its possible parental species. Cronquist (1985) proposed a new species called *E. godfreyanum* based on material that had been previously but erroneously referred to by the name *Eupatorium vaseyi*. Although *E. godfreyanum* appears to be entirely apomictic, this species was rather frequent in many localities of different states such as VA and WV, so it needed to have an appropriate name. However, the origin through which this species has arisen was ambiguous. One possibility, suggested by Sullivan (cited by Cronquist, 1985), was that *E. godfreyanum* is possibly a hybrid between *E. sessilifolium* and *E. pilosum*. Alternatively, Cronquist (1985) suggested that *E. rotundifolium* var. *rotundifolium* might be one parent of *E. godfreyanum*. **Fig. 3-7** showed the placement of *E. godfreyanum* accessions in clade that was sister to the *E. sessilifolium* clade. The ITS sequences of *E. godfreyanum* were slightly longer and had more sequence differences than the other species that were used in this study, probably because of poor sequence quality.

The large data set for *Eupatorium* was also analyzed by parsimony analysis, which produced a strict consensus tree (**Fig. 3-8**) of 325 trees. The aligned length was

Figure 3-8. Strict consensus tree generated from ITS sequences of the genus *Eupatorium* with the bootstrap value on the branch. The names that have ITO in front were from Ito et al. (2000b) samples. *Eupatoriadelphus maculatus, E. perpureus, E. fistulosus* and *E. dubius* were used as the outgroup taxa.


erotP10 982
erotP2 1059
erotP4 934
erotP8 972
erotP9 979
erotP13 1088
erotP1 1050
erotD3 1015
ITO erot
erotD1 993
erotP5 952
erotD4 1022
erotD5 1028
erotP6 953
erotD6 1046
erotP3 922
erotP7 963
erotD2 998
erotP12 1081
erot 706
Eupatorium rotundifolium
ITO evar
ITO elind
ITO emaki
ITO ejapo
ITO eform
ITO etash
echi 713
echi 531
ITO egleh
echi 715
Asian species
esesD6 908
egod 1077
egod 819
egod 820
egod 821
E. godfreyanum
ITO esemi
esesD1 861
esesP7 917
esesD4 897
esesD2 874
esesP4 847
eses 710
esesD5 902
esesP3 843
esesD8 1096
esesP5 855
esesD3 884
esesP1 1075
esesP6 871
esesD7 910
esesP2 824
Eupatorium sessilifolium
ITO ealt
ealt 711
ITO ecap
ITO elep
ecap 712
ITO ecom
ecap 460
Dog-fennel group
ealb 702
eleu 703
ITO ehys
ehys 709
ITO ecan
ecan 716
ecan 598
European species
ITO emik
emik 705
ITO eserot
ITO eses
eper 465
erotP11 992
ecun 701
ITO efist
ITO edub
emac 532
epur 506
outgroup

524 at of which, 346 were constant characters, 133 characters were potentially informative and 45 were variable but uninformative. The CI = 0.69 and the RI was 0.89. The differences between the strict consensus tree and the neighbor-joining tree are in the placement of the RP11 and the *E. sessilifolium* sample, which was collected by Ito et al. (2000b). These two samples formed a polytomy to *E. rotundifolium*, *E. sessilifolium* and other species such as *E. perfoliatum*, *E. serotinum*, and *E. leucolepis*.

CHLOROPLAST SEQUENCE

The DNA samples used in chloroplast analysis were the same set used to obtain the ITS sequence data. As with the ITS sequence data, cpDNA data were obtained for *E. rotundifolium* and *E. sessilifolium* by analyzing one individual for each of the populations. Sequences were obtained for the *trnC-psbM* region of a total of 34 samples of *Eupatorium rotundifolium* and *E. sessilifolium*, and for two additional samples of *Eupatoriadelphus fistulosus* and *E. purpureus*, which were used as the outgroup.

The aligned sequence matrix included a total of 1061 base pairs, of which there were 29 parsimony informative changes and two positions that were variable but uninformative. Inspection of the sequences revealed two regions for which alignment was problematic (**Fig. 3-9**). One was a region of 23 bp with an inversion: 14 samples had one form and the remaining 22 samples had the other, which was the same as the orientation of *Eupatoriadelphus*. This region was coded as a single change, and the individual base pairs were omitted from the analysis to avoid inappropriate weighting of what is likely a single change. The second was a poly A/T region at positions 184 – 199, for which there was variation in both numbers of adjacent A and T nucleotides, making it

Inversion Region (23 bp)

```
SD2         GTTCTGCTG ggtaaaatttggttgagttatat AGCAGAACAG
SP2         GTTCTGCTG ggtaaaatttggttgagttatat AGCAGAACAG
erot_926    GTTCTGCTG atataactcaaccaaatttacc AGCAGAACAG
RD2         GTTCTGCTG atataactcaaccaaatttacc AGCAGAACAG
eper_465    GTTCTGCTG atataactcaaccaaatttacc AGCAGAACAG
ecap_461    GTTCTGCTG atataactcaaccaaatttacc AGCAGAACAG
```

Poly A/T Region (~15 bp)

```
SD2         GATAGAGAAT aaaaaaatttttt- GAAACCCCTC
SP2         GATAGAGAAT aaaaaaatttttt- GAAACCCCTC
erot_926    GATAGAGAAT aaaaaaatttttt- GAAACCCCTC
RD2         GATAGAGAAT aaaaaaatttttt- GAAACCCCTC
eper_465    GATAGAGAAT aaaaaatttttttt GAAACCCCTC
ehys_870    GATAGA---- aaaaatttttttt- GAAACCCCTC
ecomp_1129  GATAGA---- aaaatttttttt- GAAACCCCTC
```

Figure 3-9. The inversion and the poly A/T regions of some samples of genus *Eupatorium* that were found throughout in cpDNA: *trnC-ycf6* region.

difficult to distinguish base pair substitutions from other possible types of changes. This region was omitted from the analysis. The length of the *trnC-psbM* region was very similar in both *E. rotundifolium* and *E. sessilifolium*, although one indel (3 bp) was found in many samples of *E. rotundifolium*, whereas 2 indels were found in several samples of *E. sessilifolium* (1 bp and 6 bp, respectively).

Parsimony analysis of the chloroplast DNA sequence data for *Eupatorium rotundifolium* and *E. sessilifolium* produced 3 shortest trees (29 informative characters; L = 33, CI = 0.97, RI = 0.98). The strict consensus tree is shown in **Fig. 3-10**, and was identical to the neighbor-joining tree (not shown). Inspection of **Fig. 3-10** indicates that there was more variation in *E. sessilifolium* than in *E. rotundifolium*. Furthermore, *E. sessilifolium* was paraphyletic and were placed in three different clades, whereas all *E. rotundifolium* samples were placed in one clade and mixed with a few samples of *E. sessilifolium*.

Fig. 3-10 showed that in *Eupatorium rotundifolium*, RP2, RP9, and RP12 shared the same clade with RP6, which was collected from Brunswick, NC, while the other samples of *E. rotundifolium* formed a polytomy with *E. sessilifolium*. RP2 and RP12 were collected from TN and RP9 was from Dade Co., GA; however, but the sequence data suggested that these populations are genetically close to each other.

Fig. 3-10 also demonstrated that four of the *E. sessilifolium* polyploid populations (SP2, SP3, SP4, and SP5), which were collected along the Blue Ridge Parkway, possibly had the same maternal origin (SD3 and SD8) with bootstrap value support of 86%. Four diploid populations (SD2, SD5, SD6, and SD7) formed a clade with the rest of the

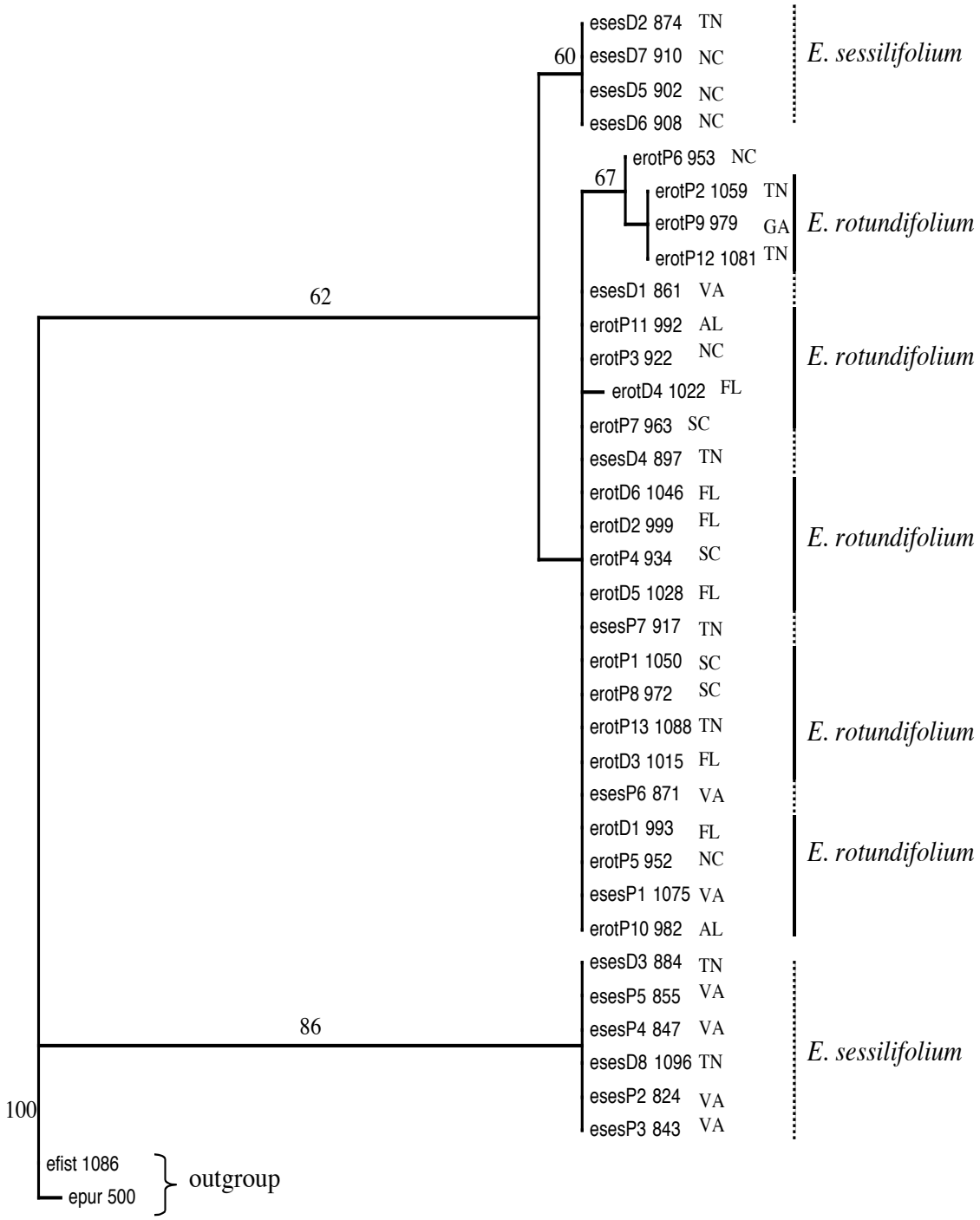


Figure 3-10. Strict consensus tree of *Eupatorium rotundifolium* and *E. sessilifolium* based on cpDNA: *trnC-psbM* sequences using parsimony analysis with bootstrap value on the branch.

E. sessilifolium populations, SD1 and SD4 with the other three polyploid populations; SP1, SP6, and SP7 formed a polytomy with *E. rotundifolium*.

A broader survey of *trnC-psbM* region was conducted for *Eupatorium* to investigate the ploidy origin of polyploids of *E. sessilifolium* since they appear to be paraphyletic. A total of 58 samples, including two species of *Eupatoriadelphus* used as the outgroup, were analyzed. The aligned cpDNA data set included 1061 bp, of which 39 bp were excluded because they were part of the inversion or the poly A/T region. The total character of 1061 bp was the same length as in the previous analysis; adding additional samples (e.g. *E. album*, *E. altissimum*, *E. perfoliatum*) in the analysis did not change the alignment length. However, there were an additional 2 indels that were required with the addition of *E. compositifolium*, including a small deletion of 10 bp and a much larger deletion of 107 bp.

Of the 1061 characters, 39 were potentially informative and additional 21 varied only within a single sample. Parsimony analysis produced 84 minimum length trees of 435, with a CI = 0.75 and RI = 0.88. The strict consensus tree is shown in **Fig. 3-11**.

In the tree the sample of *Eupatorium capillifolium* was placed as the basal most clade within *Eupatorium*. The next diverging clade included *E. album* (North American), *E. cannabinum* (European species), and *E. chinense* (Asian species), which suggested that Asian and European species might have derived from within the North American *Eupatorium*, a finding that is similar to the ITS result (Neighbor-joining tree, **Fig. 3-7**).

Samples of two species were placed in different clades within the cpDNA-based tree. One sample of *E. pilosum*, collected from FL, was placed in the large clade that

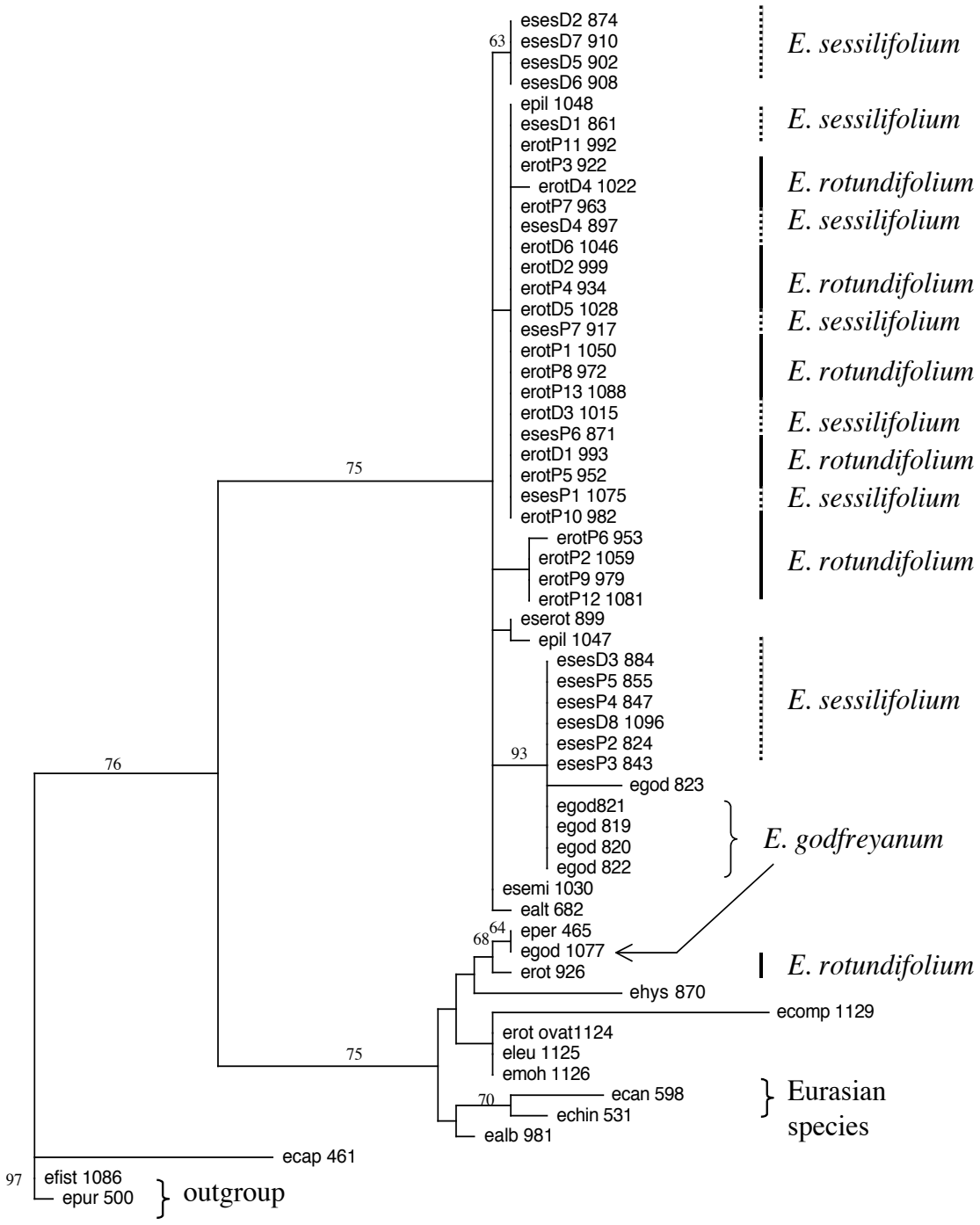


Figure 3-11. The phylogram generated from cpDNA: *trnC-psbM* sequences of genus *Eupatorium*, using *Eupatoriadelphus fistulosus* and *E. purpureus* as the outgroup taxa.

contained most of the samples of *E. rotundifolium*, whereas the other sample of *E. pilosum* that was collected from TN was placed in a separate clade with *E. serotinum*. The samples of *E. godfreyanum* that were collected from the Blue Ridge Parkway were placed in a clade with *E. sessilifolium*.

The four samples of *E. sessilifolium* polyploid populations (SP2-SP5) that were placed in the same clade were also collected along the Blue Ridge Parkway. However, the other sample of *E. godfreyanum*, which was collected in Cumberland Co. TN, was placed in a different clade together with samples of *E. perfoliatum* (TN) and *E. rotundifolium* (NC). The disparate placement of the samples *E. pilosum* and *E. godfreyanum* suggested that they have multiple origins, which presumably must involve reticulate evolution or hybridization/introgression in this genus.

ISSR RESULTS

There are variations in numbers of bands and sizes that are produced by the ISSR method in both *Eupatorium rotundifolium* and *E. sessilifolium*. Each of the primers chosen for use in this study produced similar numbers of bands. The range in band number was 49 – 61 in *Eupatorium rotundifolium* and 32 – 46 in *E. sessilifolium*. Every primer that was used in this analysis generated more bands in *E. rotundifolium* that *E. sessilifolium*. The number of bands or loci that were shared between diploid and polyploid populations of is shown in **Fig. 3-12** for *E. rotundifolium*, in **Fig. 3-13** for *E. sessilifolium*, and is summarized in **Table 3-6**.

The number of bands produced in polyploid populations was higher than for diploid populations in *E. rotundifolium*; in contrast the number of bands that were present

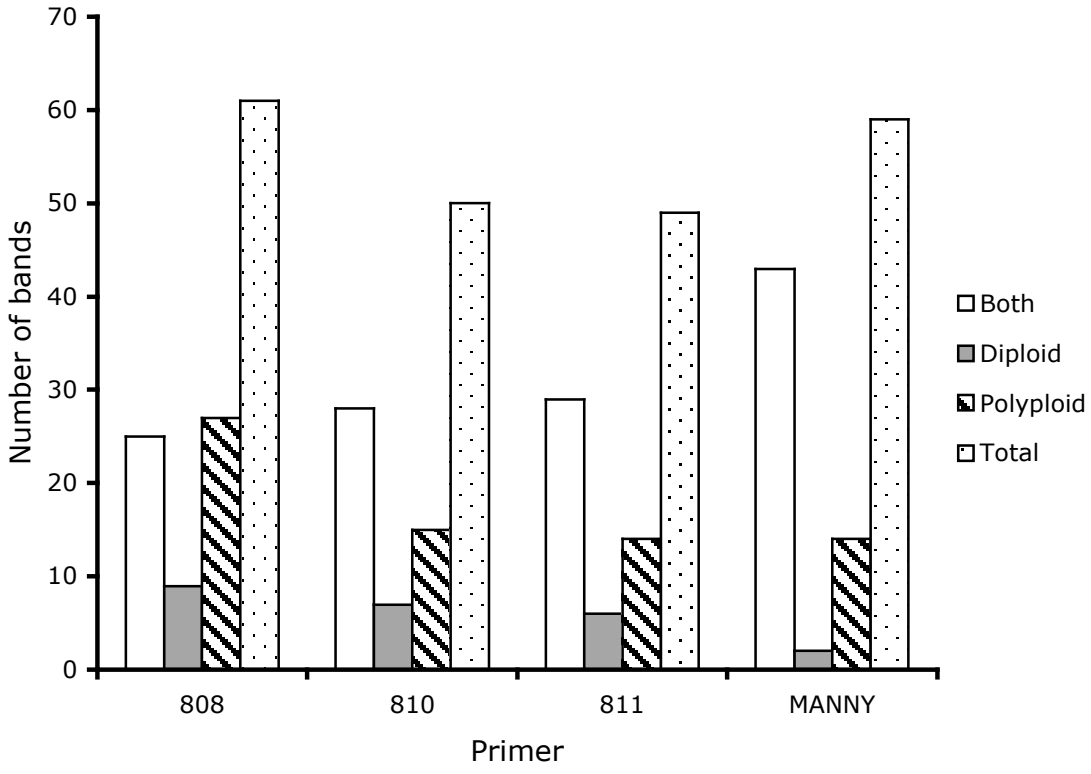


Figure 3-12. Number of bands produced in *Eupatorium rotundifolium* using 4 primers by ISSR method.

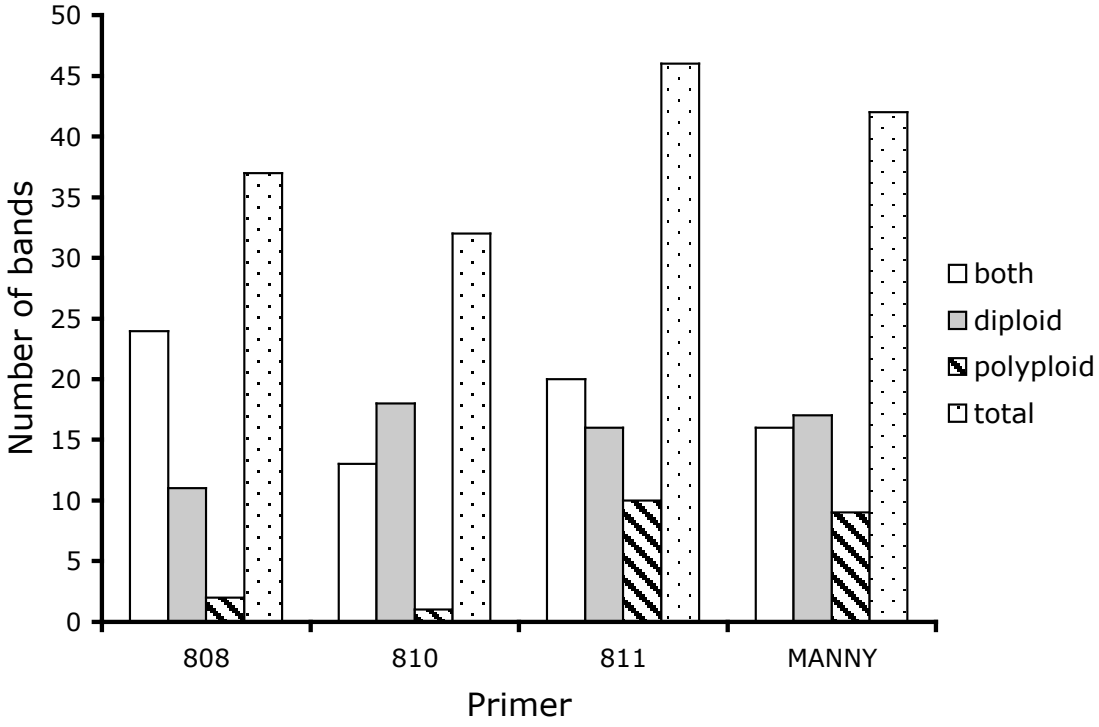


Figure 3-13. Number of bands produced in *Eupatorium sessilifolium* using 4 primers by ISSR method.

Table 3-6 Summary of ISSR band profiles used in this study.

Primer	Primer sequence	Total no. of bands		Size range of bands (bp)
		E. rotundifolium	*E. sessilifolium*	min- maximum
808	$(AG)_8$-C	61	37	335 - 1970
810	$(GA)_8$-T	50	32	322 – 2500
811	$(GA)_8$-C	49	46	300 – 2200
MANNY	$(CAC)_4$-R*C	59	42	219 - 2200

*R = A,G

in diploid populations was higher than in polyploids in *E. sessilifolium*. The number of loci indicates that there is more variation in *E. rotundifolium* than in *E. sessilifolium*, which is similar to the ITS result that there was more variation in *E. rotundifolium* than *E. sessilifolium.*

ISSR BANDS

A notable aspect of the results from the ISSR data was that there was variation within and between diploid and polyploid populations in both *E. rotundifolium* and *E. sessilifolium*. Within the same population, diploid individuals (**Fig. 3-14A)** showed higher diversity than the polyploids (**Fig. 3-14B**) and this was found in both species. The number of bands and presence or absence of bands varied between diploids more than the polyploids. In both species the band-pattern in the polyploids showed less variation among individuals within each population, but the variation was greater when compared among polyploid populations of the same species.

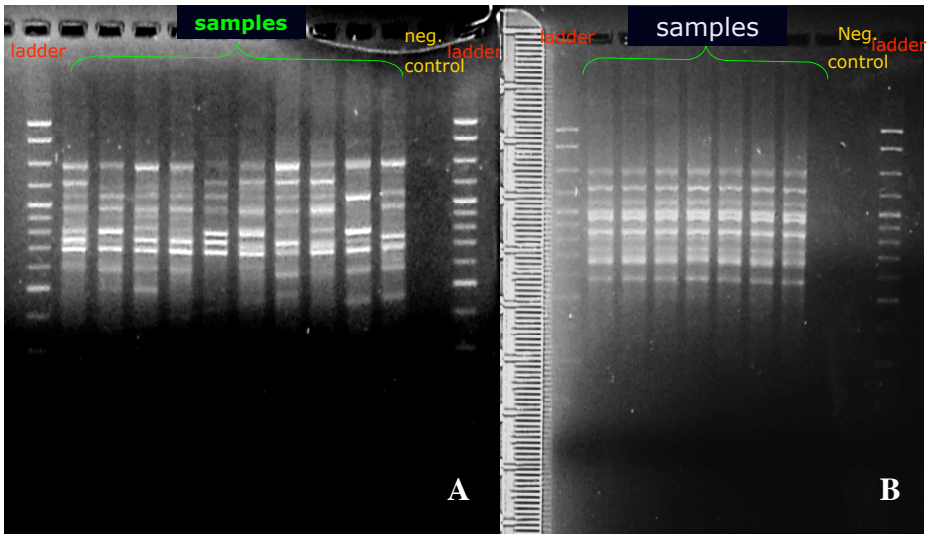


Figure 3-14. The pattern of ISSR bands that present in diploid population, RD6 (A) and in polyploid population, RP6 (B) with primer 808, showed higher variation in diploid than the polyploid.

The polymorphism is the coexistence of the bands among each individual within the same population. For example, if each sample produces 5 bands, but one sample has 4 bands, in that case the polymorphism will be 20% (or equal to 1/5). The percentages of polymorphisms of *Eupatorium rotundifolium* and *E. sessilifolium* are shown in **Figs. 3-15 and 3-17**. **Figure 3-15** showed that the polymorphisms of all diploid populations of *E. rotundifolium* were high, except for RD1 due to the small sample size. Some of the polyploid populations (RP3, RP4, RP7, RP8 and RP13) showed a slightly higher polymorphism and more diversity among individuals than other polyploid populations, which showed a low or 0% polymorphism.

Figure 3-16 summarized the average polymorphisms of each primer, and a t-test showed that diploid populations showed significantly higher variation than polyploid populations. The percent of polymorphisms of *E. sessilifolium* (**Fig. 3-17**) diploid populations were high, but SD1 and SD5 had very low diversity because only a few samples were used (3 and 2 samples in SD1 and SD5, respectively). The polyploid populations showed a high variation among individuals but yet less than the diploids. The statistical analysis by t-test confirmed that diploid populations showed significantly higher variation than polyploid populations (**Fig. 3-18**).

Monomorphic bands, the bands that occur in all individuals, was zero in *E. rotundifolium*, but there were 2 bands that were found in every population of both diploids and polyploids in *E. sessilifolium* with two primers: 810 shared a single band size approximately 1075 bp and primer MANNY shared a band of 1200 bp, respectively.

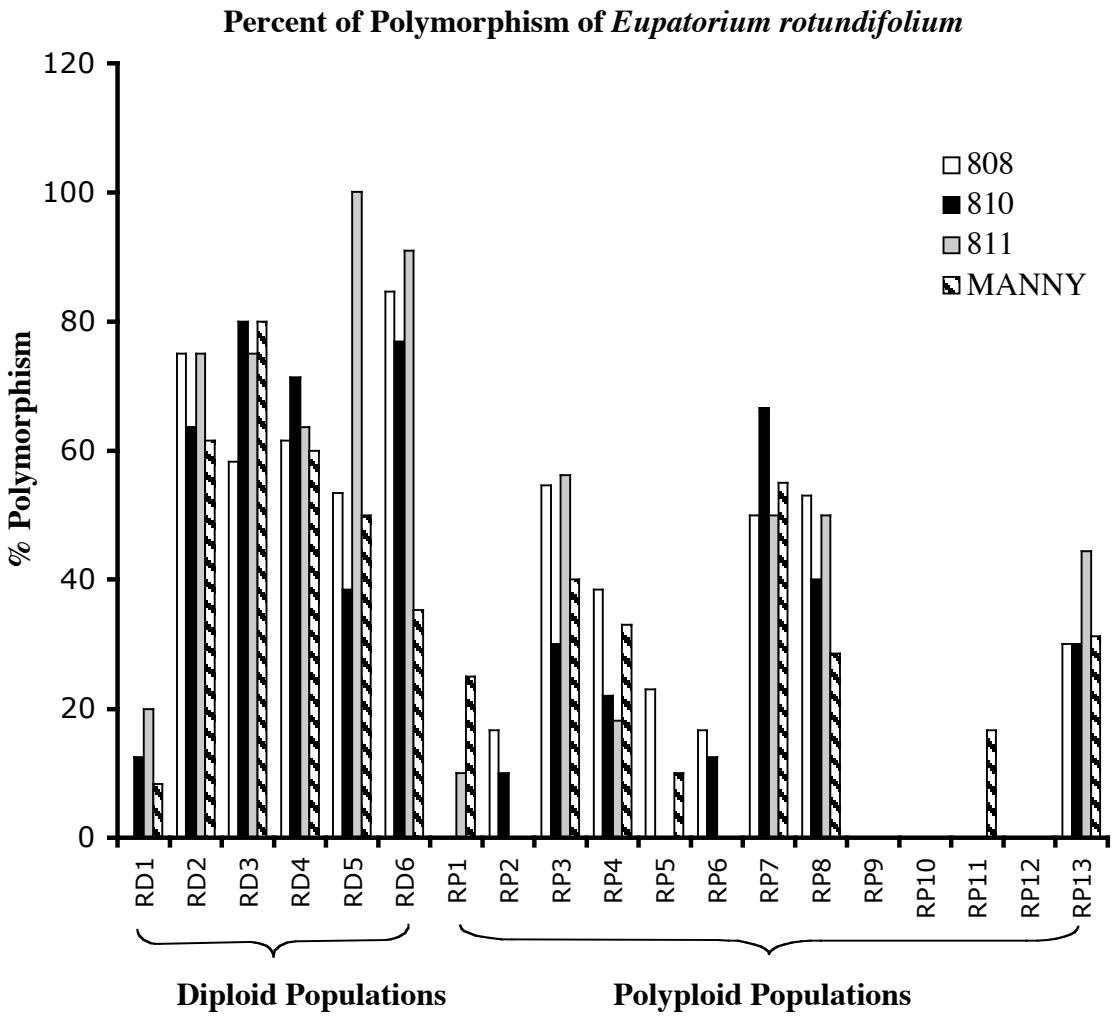


Figure 3-15. Percent of polymorphisms between diploid and polyploid populations of *Eupatorium rotundifolium* using the ISSR molecular method.

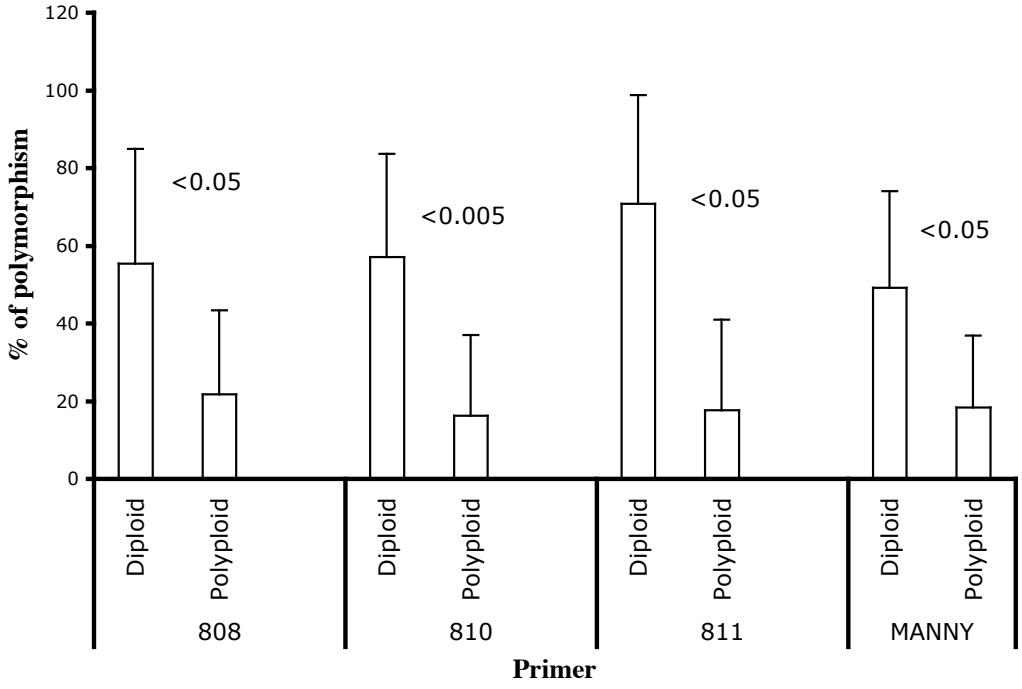


Figure 3-16. Percent of polymorphisms between diploid and polyploid populations of *Eupatorium rotundifolium* using the ISSR molecular method.

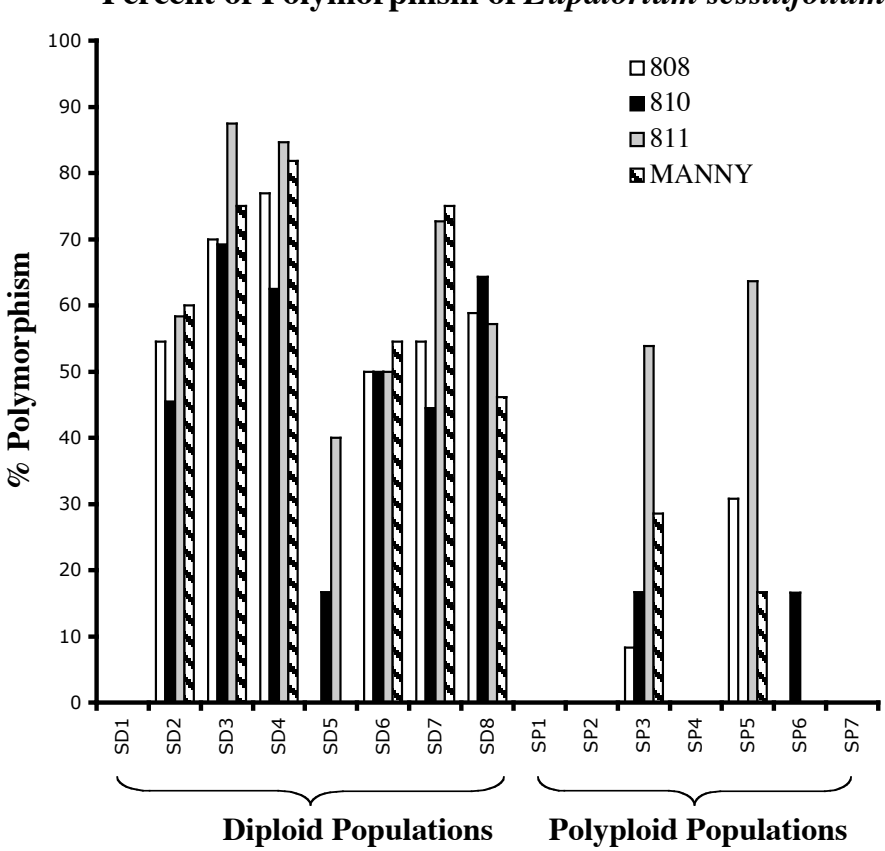


Figure 3-17. Percent of polymorphisms between diploid and polyploid populations of *Eupatorium sessilifolium* using the ISSR molecular method.

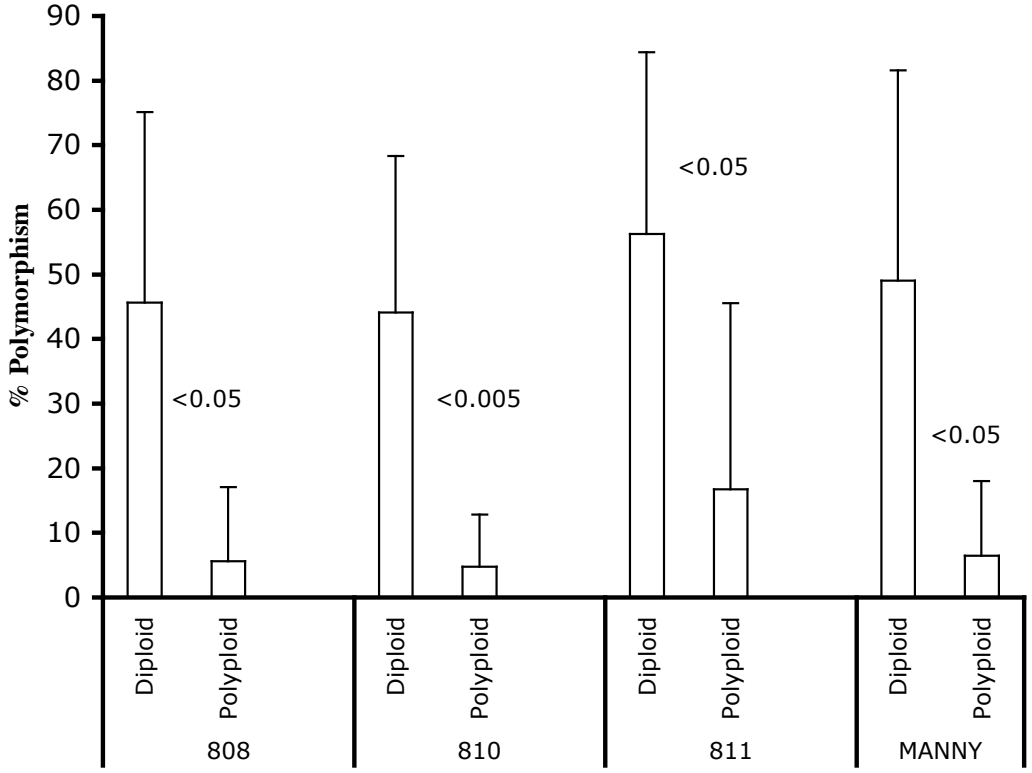


Figure 3-18. The average percent of polymorphisms between diploid and polyploid populations of *Eupatorium sessilifolium* used ISSR molecular method.

The invariance of banding patterns among individuals within many polyploid populations when compared among individuals of both species suggested that all members of these populations were from a single clone. Note, however, that no pair of polyploid populations had identical band patterns. There are two possible explanations for this. One is that there has been variation subsequent to their formation. It seems more likely, though, that they have arisen from different origins. It is also notable that the bands of the polyploids were not a subset of the bands of the diploids. This would not be expected if the polyploids were of autopolyploid origin. Because none of the polyploid patterns was identical to any diploid populations, it seems likely that the polyploids are allopolyploid in origin.

The level of genetic diversity and how the diversity is distributed in diploid and polyploid populations are of interest. Three aspects of genetic diversity were obtained from ISSR data. One is simply comparing the amount of variation to what might be expected from the banding patterns: (1) the diploid populations of both *E. rotundifolium* and *E. sessilifolium* are variable. This suggests that they are large enough to continue to harbor significant variation, so perhaps we can say that they are not critically endangered from the genetic diversity viewpoint; (2) the polyploid populations are generally of low variability. This would be expected for a population relying on asexual reproduction – the entire population is really just a clone; (3) the presence of differences between polyploid populations suggest that they are not just a big widespread clone, but rather that they have variability, which could have various multiple origins; migration between populations; variation that emerges after polyploid formation.

TREES FROM ISSR DATA

Eupatorium rotundifolium

A neighbor-joining tree (**Fig. 3-19**) was constructed using distance methods to summarize the relationships based on ISSR data among the 50 diploid individuals of *Eupatorium rotundifolium*. The neighbor-joining tree showed that in almost all cases all of the individuals within a population formed a cluster that is distinct from other populations. The only exception was an individual from population RD4 (RD4_1027), which was placed in the same cluster with the RD5 population. The individuals from RD2 and RD4 were more closely related to each other than to individuals from RD1, RD3, RD5, and RD6. RD1 showed a low amount of genetic diversity due to the small sample size (range of polymorphism was between 8 – 20%), which precluded rigorous analysis of population differences between RD1 and other diploids (Smissen et al., 2003). The individuals in RD2, RD3, RD4, RD5, and RD6 appeared to be genetically diverse with an average polymorphism of 49% to 70% in the four primers used (**Fig. 3-15**).

An additional set of ISSR data from 13 polyploid populations of *Eupatorium rotundifolium* was added to the analysis. The neighbor-joining tree, constructed from distances among all individuals (128 samples) in 19 populations of *E. rotundifolium* is shown in **Fig. 3-20**. For the diploid samples, the position of the RD6 population was the only difference that was found when the tree from the larger sample set was compared with the one based only on diploid populations (**Fig. 3-19**).

The pattern of polyploid populations in this tree is of interest for several reasons: (1) individuals within populations tended to cluster together (similar to diploid

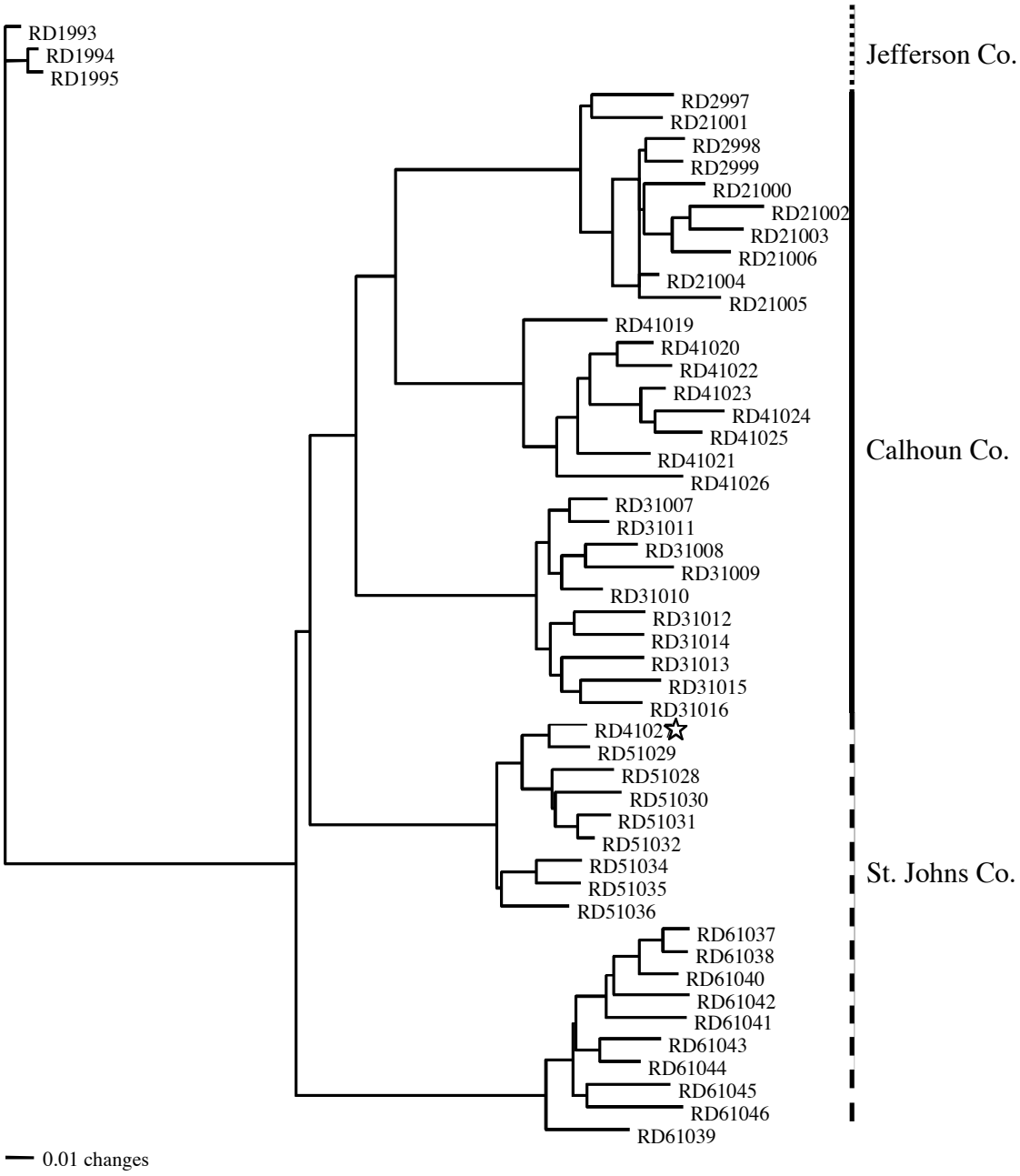


Figure 3-19. Neighbor-joining tree of *Eupatorium rotundifolium* diploid populations generated from ISSR profiles. The star shows the placement of one sample from RD4 that was placed within RD5 population.

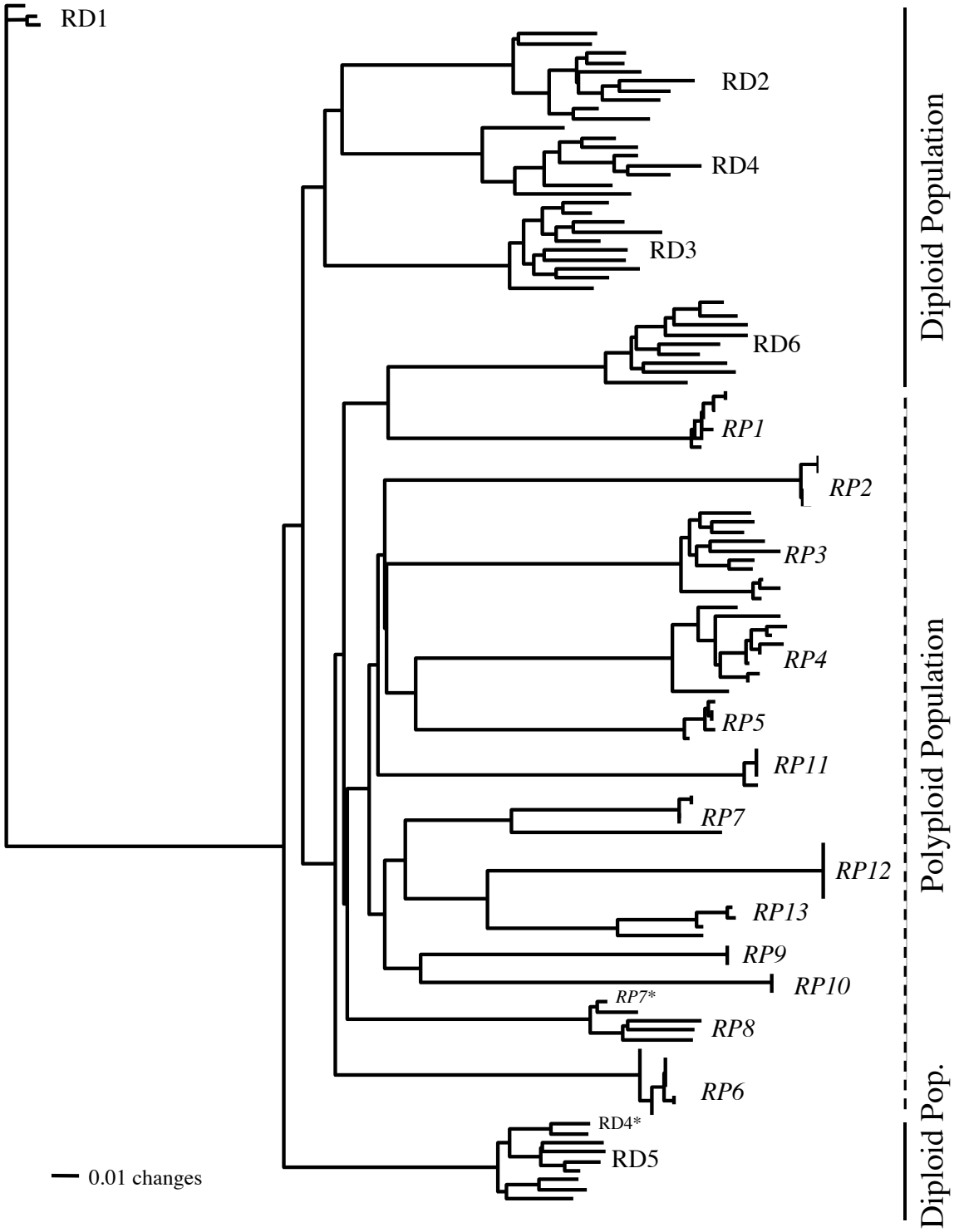


Figure 3-20. Neighbor-joining tree of the ISSR profiles generated from 19 populations of *Eupatorium rotundifolium.*

populations); (2) not all polyploids were placed as a single group; (3) at least one polyploid population is placed closer to a diploid population than to other polyploids; and (4) the other polyploids seemed to form a cluster that contains more than one population.

The population RP6 was placed basal to a large group that contained all of the other polyploid populations, as well as a single diploid population, RD6. The placement of RP1 in a cluster with RD6 suggests that RP1 is closer to a diploid population than to other polyploids. The neighbor-joining tree also indicates that there were 2 major subsets of polyploid populations: one subset included RP2, RP3, RP4, RP5 and RP11; the second included RP7, RP9, RP10, RP12, and RP13.

Neither subset appeared to be based on geographic distribution, however four of the five populations (RP9, RP10, RP12, and RP13) in the second subset are from geographically proximate areas. Interestingly, one sample of the RP7 population was placed within the cluster of RP8 samples, (which was similar to the placement of RD4 within RD5 in the diploids), the explanation of this case might come from the gene flow between RP7 and RP8, and most likely through seed dispersal. In these two subsets, neither of them showed any close relationship with any diploid populations. It is not certain whether they might have come from other diploid populations that were not included in this study or possibly from another species. Unlike diploid populations, the genetic diversity among individuals within polyploid populations was relatively low: the average polymorphism was in the range between 16 to 21% (**Fig. 3-16**). This result was not unexpected because of the asexual reproduction that is found in the polyploids.

Eupatorium sessilifolium

A neighbor-joining tree (**Fig. 3-21**) was constructed using distance methods among 43 individuals representing 8 diploid populations of *Eupatorium sessilifolium*. All 8 diploid populations that were used in this study were collected from NC, TN, and VA. In general the individuals within a population were always placed in distinct clusters, which indicated that they were more similar to one another than to individuals in another population, a result similar to that seen in *E. rotundifolium* diploids.

There were three distinctive groups in the neighbor-joining tree (**Fig. 3-21**). One group included SD2, SD3, SD4, and SD6; a second small group was composed of SD7 and SD8; and a third group included SD1 and SD5. SD1, the only diploid population collected in VA, showed the highest difference of all diploids, and it was the most isolated population. The variation of individuals in each population was relatively high (the average of polymorphism = 44 – 56%; **Fig. 3-18**), which is similar to the diploid populations of *E. rotundifolium*.

An additional set of ISSR data from seven polyploid populations of *Eupatorium sessilifolium* was added to the analysis. The neighbor-joining tree, constructed from distances among all individuals (78 samples) in 15 populations of *E. sessilifolium*, is shown in **Fig. 3-22**. The pattern of polyploid populations in *E. sessilifolium* shares several similarities to the pattern of *E. rotundifolium*. The overall results are: (1) within the polyploids all of the individuals within the population formed single, distinct clusters (similar to the diploid populations); (2) not all polyploids were placed in a single cluster; (3) at least one polyploid population was placed closer to a diploid population than to

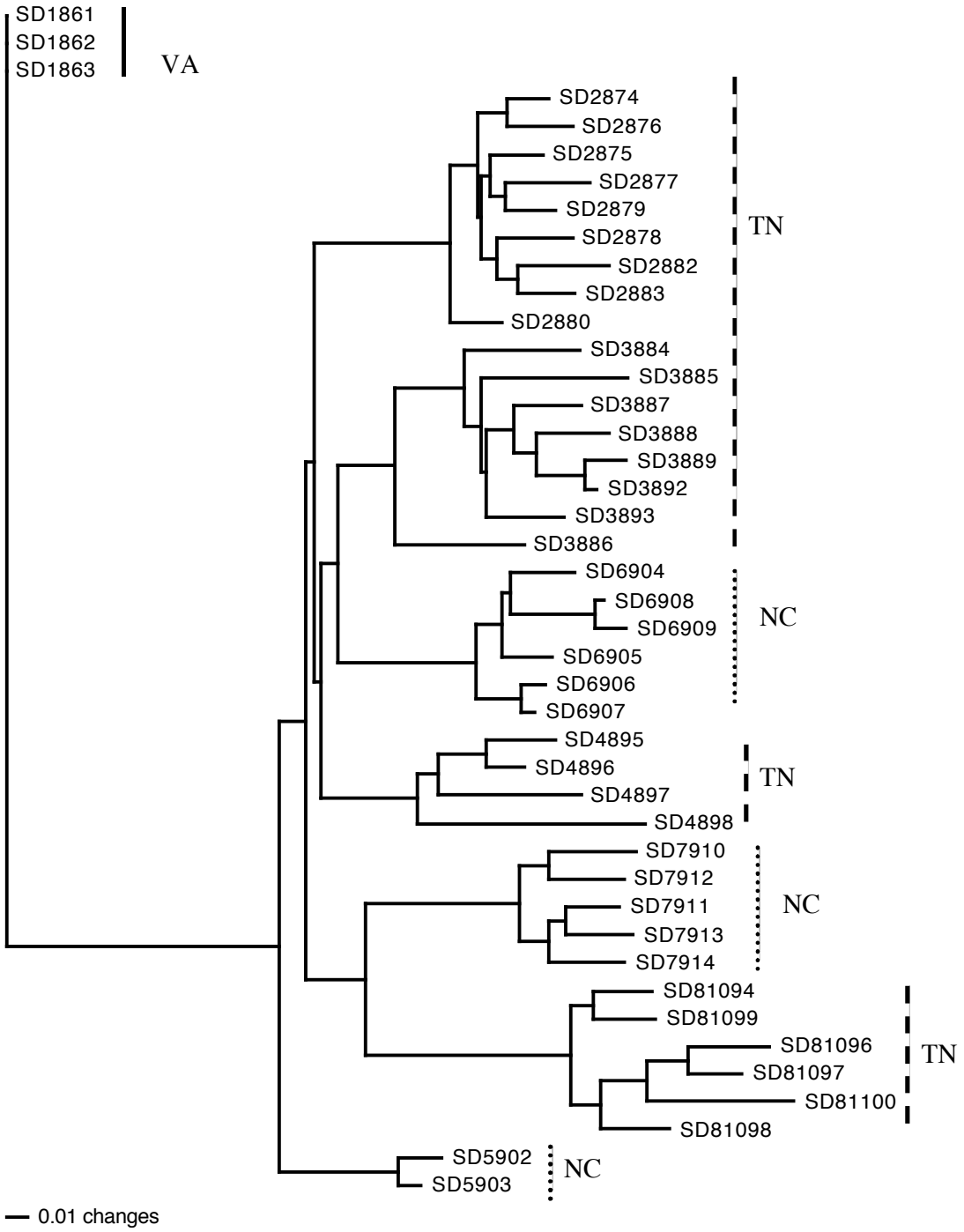


Figure 3-21. Neighbor-joining tree of *Eupatorium sessilifolium* diploid populations constructed from a distance method using ISSR data.

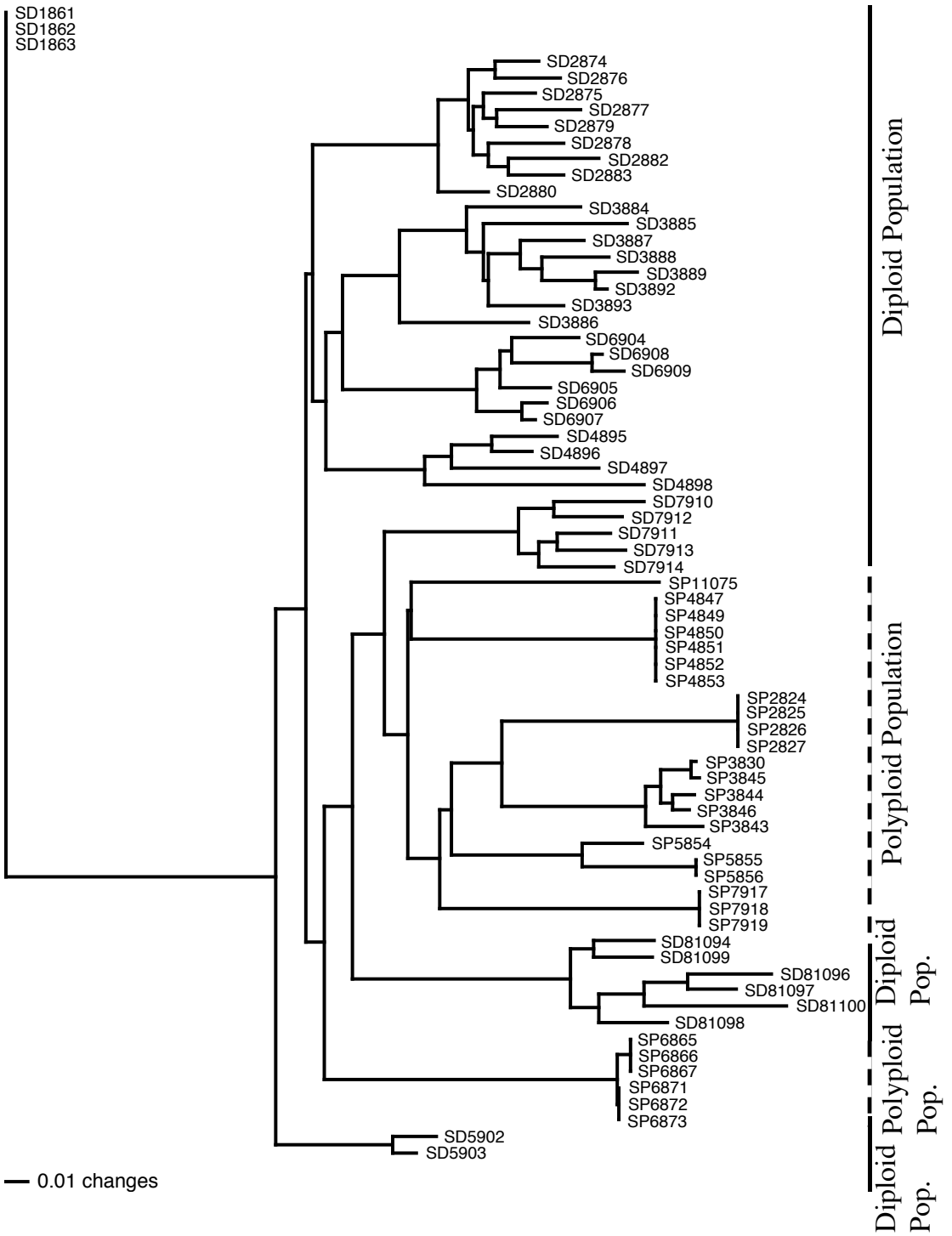


Figure 3-22. Neighbor-joining tree generated from *Eupatorium sessilifolium* diploid and polyploid populations combining data of ISSR band profiles.

other polyploids (the placement of SP6); and (4) the other polyploids seemed to be placed within a cluster that was not identical to any diploids that were used in this study.

The neighbor-joining tree of the combined data including *E. sessilifolium* diploids and polyploids looks like **Fig. 3-21**, the placement of diploid populations is the same, but the only difference was there was a large group of polyploid populations (SP1, SP2, SP3, SP4, SP5, and SP7) inserted between SD7 and SD8 and SP6 was placed as the sister branch to this large cluster. The combined data indicates that most of the polyploid populations might share a closer genomic relationship with SD7 and SD8 than with the other diploid populations.

However, the population SP6 was placed outside of the cluster formed by most of the other polyploid populations, and exhibited a somewhat higher genetic distance from other polyploids. The possible explanation could be that SP6 was isolated from other populations. Unfortunately, the sample size of this species was quite low when compared with *E. rotundifolium* because of the nature of the species, which was more scattered and isolated than *E. rotundifolium*. The pattern that clearly emerges from ISSR results is that there is more variation in diploid populations than in polyploids (range of polymorphism = 4.75 – 16.78%; **Fig. 3-18**), which supports the evidence of the essentially clonal reproduction of polyploid cytotypes by agamospermy.

Combining Eupatorium rotundifolium and E. sessilifolium ISSR data

To explore further aspects of the relationship between *Eupatorium rotundifolium* and *E. sessilifolium*, the two data sets of ISSR profiles were combined. The neighbor-joining tree (**Fig. 3-23**) constructed from the distance methods included a total of 206

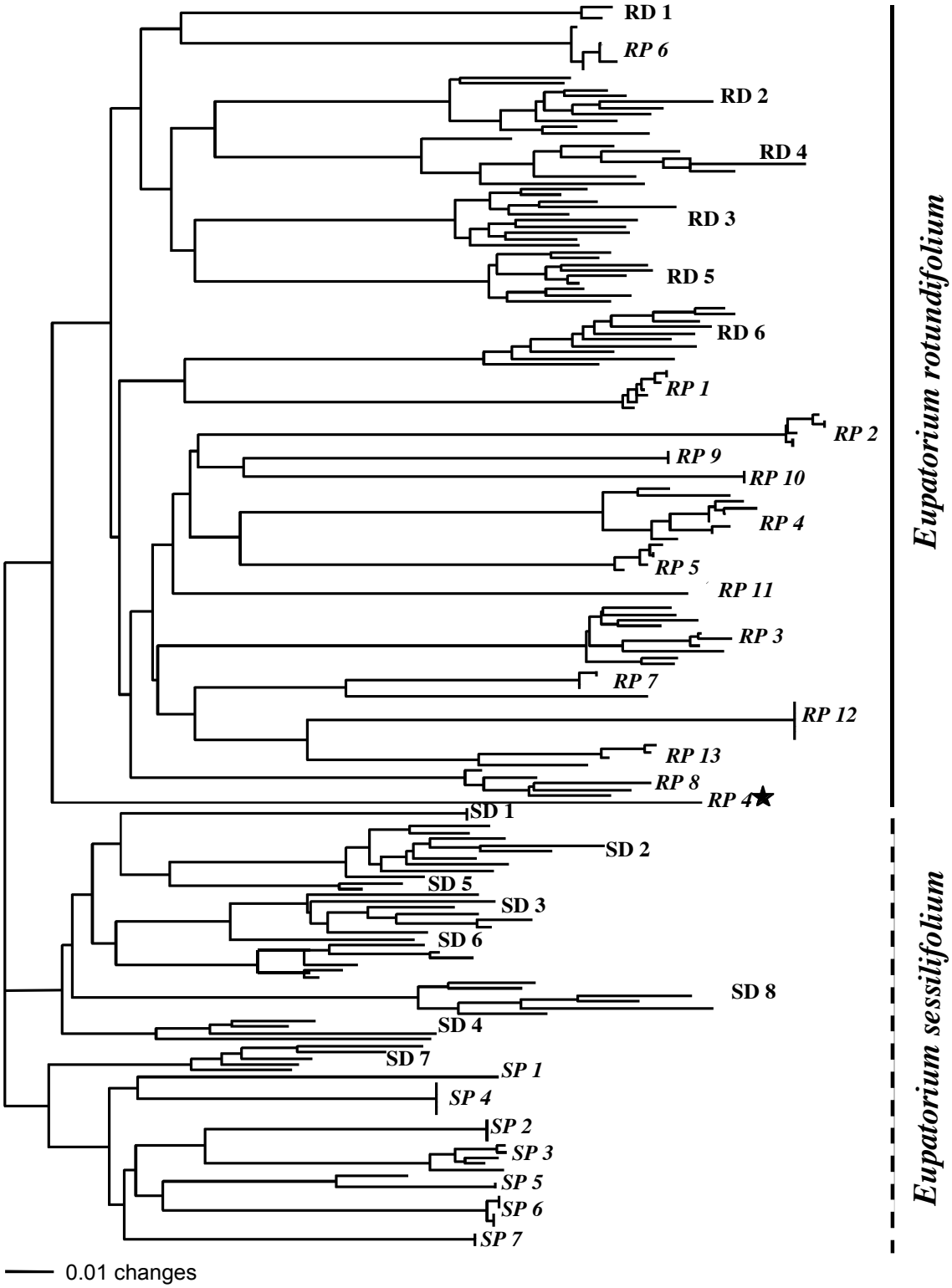


Figure 3-23. Neighbor-joining tree of ISSR band profiles of *Eupatorium rotundifolium* and *E. sessilifolium* combined together. The star represents the placement of one RP4 sample that formed at the bottom of *E. rotundifolium* cluster.

samples. In this tree, there are two distinct clusters: one cluster is composed of all *E. rotundifolium* populations and the other included all *E. sessilifolium* populations. The overall topology of this tree is similar to the combining of **Figs. 3 – 20** and **3 – 22** together, except the placement of RP4 has been changed.

The *Eupatorium rotundifolium* cluster could be divided into two small subsets: one that is mostly diploid and a second that is polyploid. The only exception within the diploid subset was the position of RP6, which shared the same branch with RD1. Similarly one of the diploid populations, RD6, shared the same branch with RP1.

The surprising outcome was the position of RP4_934 that was split from its group and was placed basally to the *E. rotundifolium* cluster. The evidence of the clusters between RD1 and RP6 or RD6 and RP1 might suggest that the formation of polyploidy in *E. rotundifolium* could originate from multiple origins.

In the *Eupatorium sessilifolium* cluster, all of the polyploid samples are placed in the same cluster with a single diploid population (SD7); this might be interpreted as evidence for a single origin for the polyploids.

Comparison of ITS, cpDNA and ISSR data

In general the ISSR (**Fig. 3-23**), ITS (**Fig. 3- 7**) and cpDNA (**Fig. 3-11**) data suggested similar patterns of genetic relationship of *E. rotundifolium* and *E. sessilifolium*. Even though there was low level of genetic diversity within *E. sessilifolium* in the ITS data and a low level of genetic diversity in the cpDNA of *E. rotundifolium*, the complementary ISSR data provided a better view in terms of revealing their relationships, especially at the intraspecific level. Overall, the ISSR and ITS trees were congruent with one another and were more similar to the ITS than to the cpDNA trees; for example,

SD7 always formed a cluster with other diploids such as SD2, SD5, and SD6 rather than with other polyploids. This was seen in both the ITS and ISSR trees and that relationship was relatively strong (bootstrap value = 60%). Similar results have been observed earlier in the population studies of genus *Hyobanche* (Wolfe and Randle, 2001) and in Alpine *Raoulia* (Smissen et al., 2003).

One reason that the ISSR data would provide more details about relationships is because the amplification is from the whole genome, unlike ITS (nuclear genome) or cpDNA: *trnC-psbM*. According to Wang et al. (1994), the most abundant dinucleotide sequence in plant genomes is (AT)n, followed by (A)n/(T)n and (AG)n/(CT)n, the four primers that were used in this study consisted mostly of AG, which made them easy to amplify. Another important parameter is to make the right decision of using suitable primers because the dinucleotide primers could yield better results than the trinucleotide primers. In this study the dinucleotide primer 811 gave higher polymorphism than a trinucleotide primer, MANNY; however, the trinucleotide primers would be useful if amplification conditions were optimized (Tsumura et al., 1996).

The most distinctive outcome of the ISSR data is that in polyploids of both species, each population was distinct in its overall banding pattern but individuals frequently were identical, especially in *E. sessilifolium*. A similar pattern was reported by Vis (1998) who showed that the gametophyte of freshwater red algae (*Batrachospermum*) was more uniform among the individuals within the same population. However, the low level of genetic diversity among polyploid populations could come from several possible factors such as, the sample size being too small (Wolfe and Randle, 2001), or it could be affected by the number of markers used and their

distribution in the genome (Kumar et al., 2001) or the species is very recent in origin such as in the case of *E. sessilifolium* polyploid populations.

As mentioned earlier, ISSR markers are inherited as dominant or codominant genetic markers (Gupta et al., 1994; Tsumura et al., 1996). The absence of the bands could imply that divergence has occurred at one or both of the primer sites (Wolfe and Liston, 1998). The possibilities that could cause the absence of the bands might come from the loss of the ISSR primer annealing sites, or a chromosomal rearrangement, or mutation in either of the primer sites, or from the insertion/deletion of large enough size to increase or decrease the band size sufficiently for it to be scored as a separate locus (Wolfe, 1998; Wolfe and Liston, 1998).

Using few primers, ISSR could reveal high levels of genetic diversity and could be a supplement to other molecular approaches. The advantages of ISSR methods are: (1) they require no prior knowledge of genomes; (2) only a small amount of DNA may be needed; (3) small reaction volumes and small amounts of enzyme are needed for the PCR; (4) the hypervariability of banding patterns and easy scoring; and (5) no specific machine is required other than that needed for standard PCR (Wolfe et al., 1998; Tsumura et al., 1996). However, the limitation of ISSR techniques are (1) clean DNA template and the optimal concentration are required; (2) optimization of the initial reaction is needed and (3) lack of co-dominance and the consequent resolution of effectively bi-allelic loci (Tsumura et al., 1996; Wolfe et al., 1998). To solve this problem, Ge and Sun (1999) suggested using larger number of polymorphic loci and sampling a large number of samples, which would enhance the resolution. To enhance the quality of the ISSR-PCR, adding formamide into the reaction mix critically reduces

the background and smears in the PCR product so it is easier to score the presence of the bands and also the number of PCR amplification cycles affect the banding patterns (Tsumura et al., 1996). Hence, the protocol optimization is essential for ISSR analysis such as the case of this study.

CONCLUSIONS

This study provides an example of the successful use of ITS, cpDNA sequence data, and ISSR methods to reconstruct reticulate evolution in plants and confirms that molecular data can be highly informative, especially when there is a limitation of morphological characters. The results provided evidence to suggest that polyploid populations within both *Eupatorium rotundifolium* and *E. sessilifolium* are allopolyploids (**Fig. 3-24**). The evidence of molecular approaches was consistent with the explanation that that these two species showed reticulate evolution. Even though nuclear and organellar genomes provided low variation at the intraspecific level, it was possible to extend the analysis by using the ISSR method to investigate the origin of polyploidy.

ITS and chloroplast DNA

The ITS data showed more variation in *E. rotundifolium* than *E. sessilifolium*, whereas cpDNA showed a greater variation in *E. sessilifolium*. The strict consensus tree that was generated from ITS sequence data with parsimony analysis indicated that *E. rotundifolium* and *E. sessilifolium* are monophyletic. One of *E. rotundifolium* polyploid samples (RP11) was placed in a basally diverging position within its species, and the evidence of expanded ITS data showed that this sample was more closely related to *E. sessilifolium* diploid population that was collected by Ito et al. (2000b).

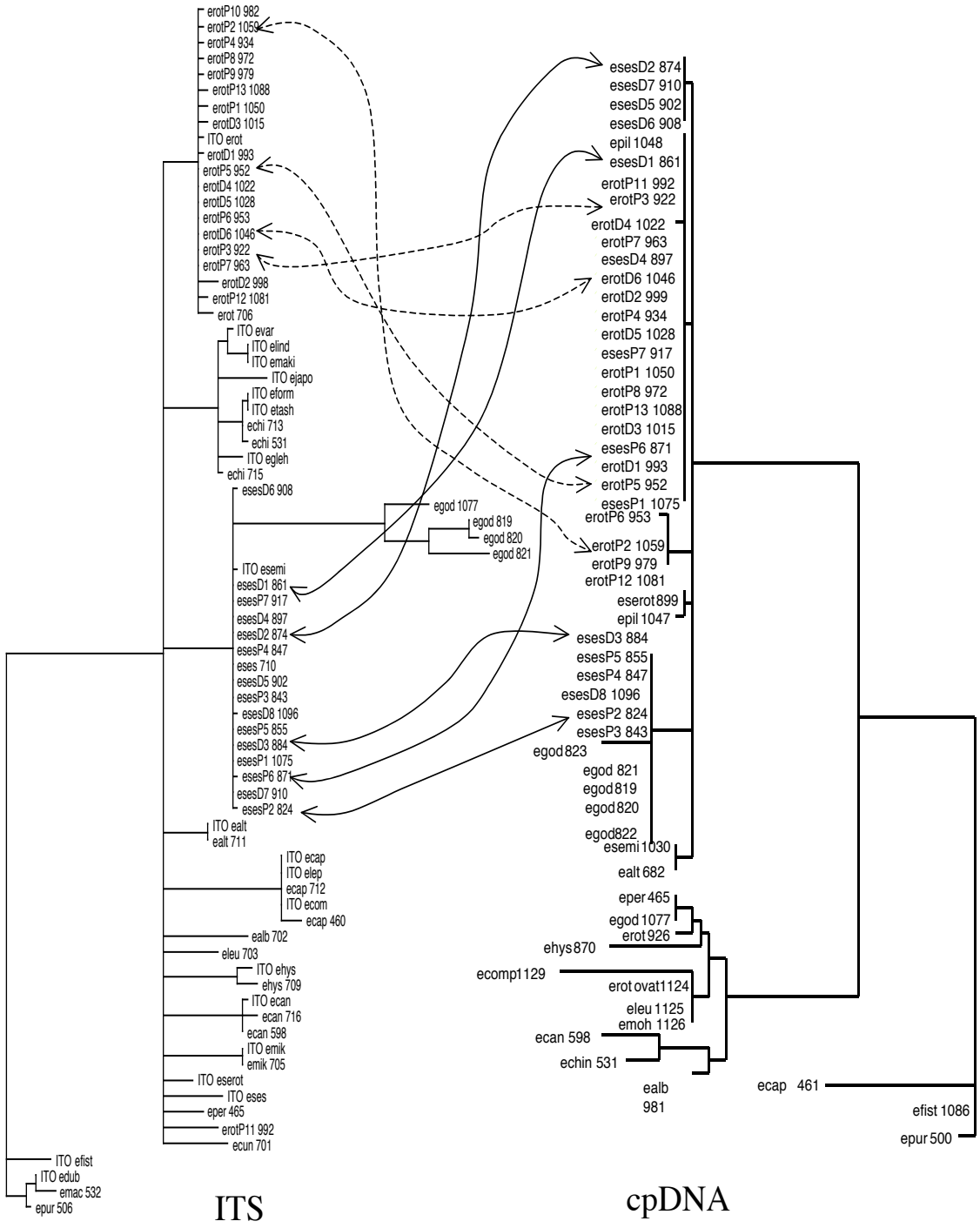


Figure 3-24. The comparison between the phylograms that were based on the ITS and cpDNA: *trnC-psbM*. The solid line shows the placement of *E. sessilifolium* and the dash line represents the placement of *E. rotundifolium* between the two data sets.

The low variability in ITS among *E. sessilifolium* samples may possibly reflect that it is from a recent origin. The strict consensus tree that was generated by the parsimony analysis using cpDNA: *trnC-psbM* showed that *E. sessilifolium* is paraphyletic, whereas all *E. rotundifolium* samples were placed in the same clade with a combination of a few samples of *E. sessilifolium*. Both ITS and cpDNA data indicated that *E. rotundifolium* and *E. sessilifolium* polyploid populations originated from multiple origins and probably involved reticulation. The evidence of cpDNA also suggests that *E. godfreyanum* has multiple origins; the Blue Ridge Parkway populations were from *E. sessilifolium*, while the one from TN (Cumberland Co.) was from *E. rotundifolium* and *E. perfoliatum*. However, the ITS sequences suggested that *E. godfreyanum* species is more closely related to *E. sessilifolium* than *E. rotundifolium*. The ITS data also suggested that a diploid sample of *E. rotundifolium* that was collected by Ito (Ito et al., 2000b), from St. Johns Co., FL, was more closely related to many polyploid populations that were used in this study, which were mainly distributed in TN, GA, and AL.

Inter Simple Sequence Repeat (ISSR)

ISSR data reveals genetic relationships between diploid and polyploid populations within and between both species. The highest number of bands that was produced in *E. rotundifolium* was generated from primer 808, whereas in *E. sessilifolium* the highest number of bands was generated by primer 811. The overall result showed that there was a greater number of bands that were produced in *E. rotundifolium* than from *E. sessilifolium* for all four primers that were used, which implies that there is more variation in *E. rotundifolium* than *E. sessilifolium*. It is noteworthy that the number of bands that was produced by diploid populations was higher than polyploid populations in

E. sessilifolium; in contrast the number of bands that was produced in polyploid populations was higher than diploid populations in *E. rotundifolium*. The percent of polymorphism in diploid populations of both species was more than in polyploid populations, and the average percent polymorphism between the diploid and the polyploid was significantly different in both species.

The banding pattern showed a greater variation among individuals in the diploids than the polyploids in both species. This result was not unexpected because the polyploid samples are expected to be originally from the same clone (asexual reproduction), whereas the diploids might be expected to show more variation because their seeds are produced via sexual reproduction. However, the variation of banding pattern of polyploid populations was increased when comparing among the polyploid populations.

The neighbor-joining trees constructed from ISSR data of both species showed similar patterns in that the samples of each population almost always were placed in the same cluster (with a few exceptions in *E. rotundifolium*). The diploid and polyploid clusters of *E. rotundifolium* were not completely separated because the placement of RP1, which is a polyploid, with RD6; this indicated that RP1 might have a greater genetical similarity to RD6 than the other polyploid populations do. After combining the ISSR data of both species together, samples of both *E. rotundifolium* and *E. sessilifolium* were placed in two separate clusters. In *Eupatorium rotundifolium* there were two sub-clusters: diploid and polyploid. The diploid cluster showed the similar pattern that RD1 was more closely related to RP1, whereas RD6 shared the same cluster with RP1 and so it was placed within the polyploid cluster. One of the RP4 samples was the basal most of all *E. rotundifolium* populations and perhaps was more closely related to *E. sessilifolium*

than the others. Interestingly, the strict consensus tree generated from cpDNA data showed that RP4 (#934) was placed in the same clade with SD1. *Eupatorium sessilifolium* polyploid populations were placed in a single cluster with a diploid population, SD7, and the other cluster included only diploid populations. It is likely that *Eupatorium rotundifolium* and *E. sessilifolium* polyploid populations have multiple origins (from the ITS and cpDNA data), because the polyploid populations showed high variation when comparing among them; however, the *E. sessilifolium* polyploid samples that were used in this study were all genetically similar to the diploid population, SD7.

The geographic distribution patterns of *Eupatorium rotundifolium* and *E. sessilifolium* can be interpreted in light of the molecular data. In both species, the diploid populations may be interpreted to inhabit regions that are glacial refugia (**Fig. 3-25**); in *E. sessilifolium*, this involves areas that may be interpreted as that avoided glaciation by being at high elevations. In *E. rotundifolium*, this involved a retreat from the glaciars to a more southerly area that has been shown to have numerous endemic species. Thus, glaciation may have resulted in constriction of *E. rotundifolium* diploid populations, which once might be common and widespread. Diploid populations of both species may produce diploid gametes via the failure of normal chromosome separation during meiosis (Otto and Whitton, 2000) so that there is high probability that the diploid gametes may hybridize with haploid gametes of other species and form an allopolyploid hybrid, which later on could become stable and established in new ecological niches further away from its original diploid parent. The ISSR results indicate that hybridization that occurred within these two species may have happened many times and from various origins, because none of the polyploid banding patterns was identical. Thus, in both species, the

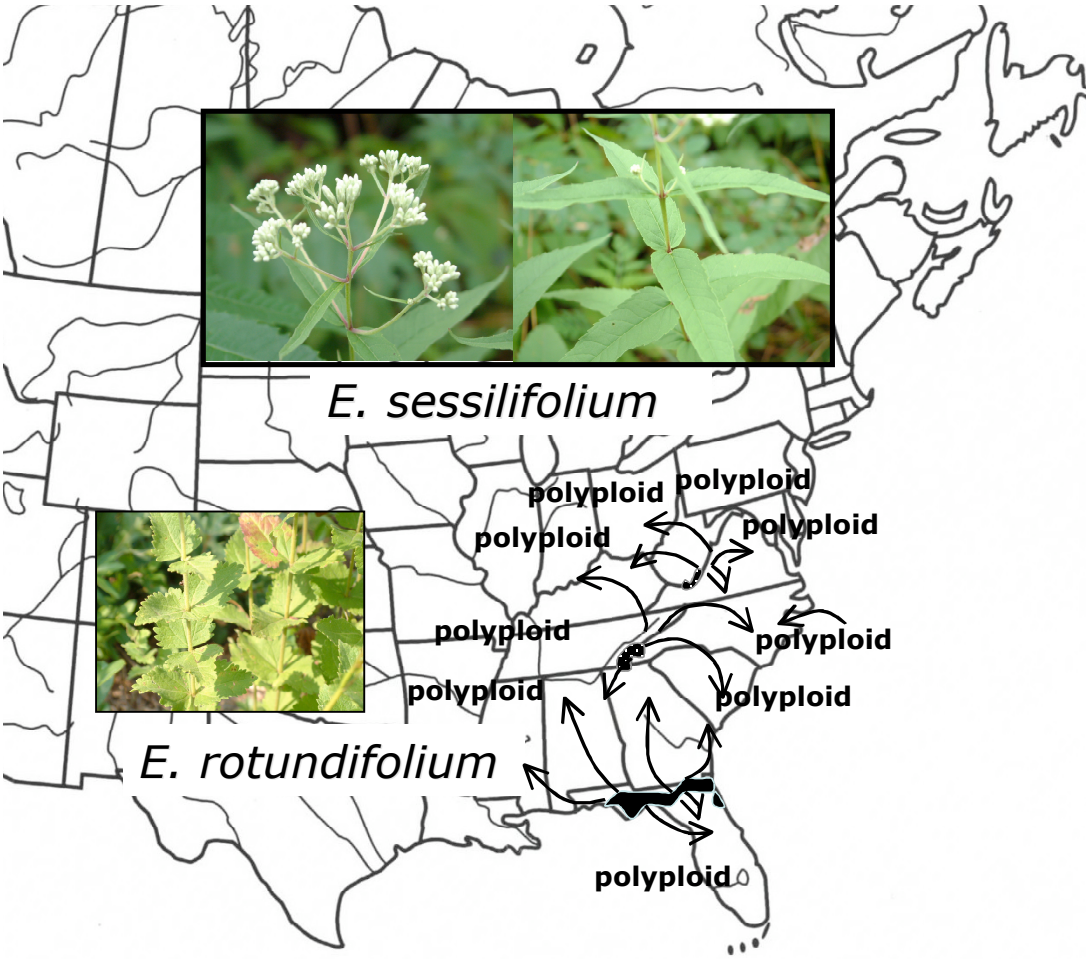


Figure 3-25. The possibility of polyploid distribution when the diploid populations are restricted in a small and narrow range, whereas the polyploid has wider range of distribution. The black shade represents the range *E. rotundifolium* diploid population and the area of a black background with white spots shows the range of *E. sessilifolium* diploid population. The arrows indicate the possible migration of their polyploid cytotypes.

polyploid populations appear to mimic the variation to be expected in a sexually reproducing species, not because of segregation and recombination, but rather because they have multiple origins from varying parental combinations. This pattern is even more easily interpreted in the apomictic species *E. godfreyanum*, where there is variation within the species even though it does not include any diploid, sexually reproducing species; in this case, the molecular data clearly suggest the involvement of similar but slightly different sets of diploid parents in the formation of different populations of this polyploid apomict.

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APPENDICES

APPENDIX A



Figure 2-7. *Eupatorium album* L. var. *album*



Figure 2-8. *Eupatorium album* L. var. *subvenosum* A. Gray



Figure 2-9. *Eupatorium album* L. var. *vaseyi* (Porter) Cronquist



Figure 2-10. *Eupatorium altissimum* L.



Figure 2-11. *Eupatorium anomalum* Nash.



Figure 2-12. *Eupatorium capillifolium* (Lamark) Small.



Figure 2-13. *Eupatorium compositifolium* Walter



Figure 2-14. *Eupatorium cuneifolium* Willd.



Figure 2-15. *Eupatorium godfreyanum* Cronquist



Figure 2-16. *Eupatorium hyssopifolium* L.



Figure 2-17. *Eupatorium leptophyllum* DC.



Figure 2-18. *Eupatorium leucolepis* (DC.) Torr. & Gray var. *leucolepis*



Figure 2-19. *Eupatorium leucolepis* (DC.) Torr. & A. Gray var. *novae-angliae* Fernald



Figure 2-20. *Eupatorium mikanioides* Chapm.



Figure 2-21. *Eupatorium mohrii* Greene



Figure 2-22. *Eupatorium perfoliatum* L.



Figure 2-23. *Eupatorium pilosum* Walter



Figure 2-24. *Eupatorium pinnatifidum* Elliott



Figure 2-25. *Eupatorium resinosum* Torr. Ex DC.



Figure 2-26. *Eupatorium rotundifolium* L. var. *rotundifolium*



Figure 2-27. *Eupatorium rotundifolium* L. var. *ovatum* (Bigelow) J. D. Montgom. & Fairbrothers



Figure 2-28. *Eupatorium rotundifolium* L. var. *scabridum* (Elliott) A. Gray

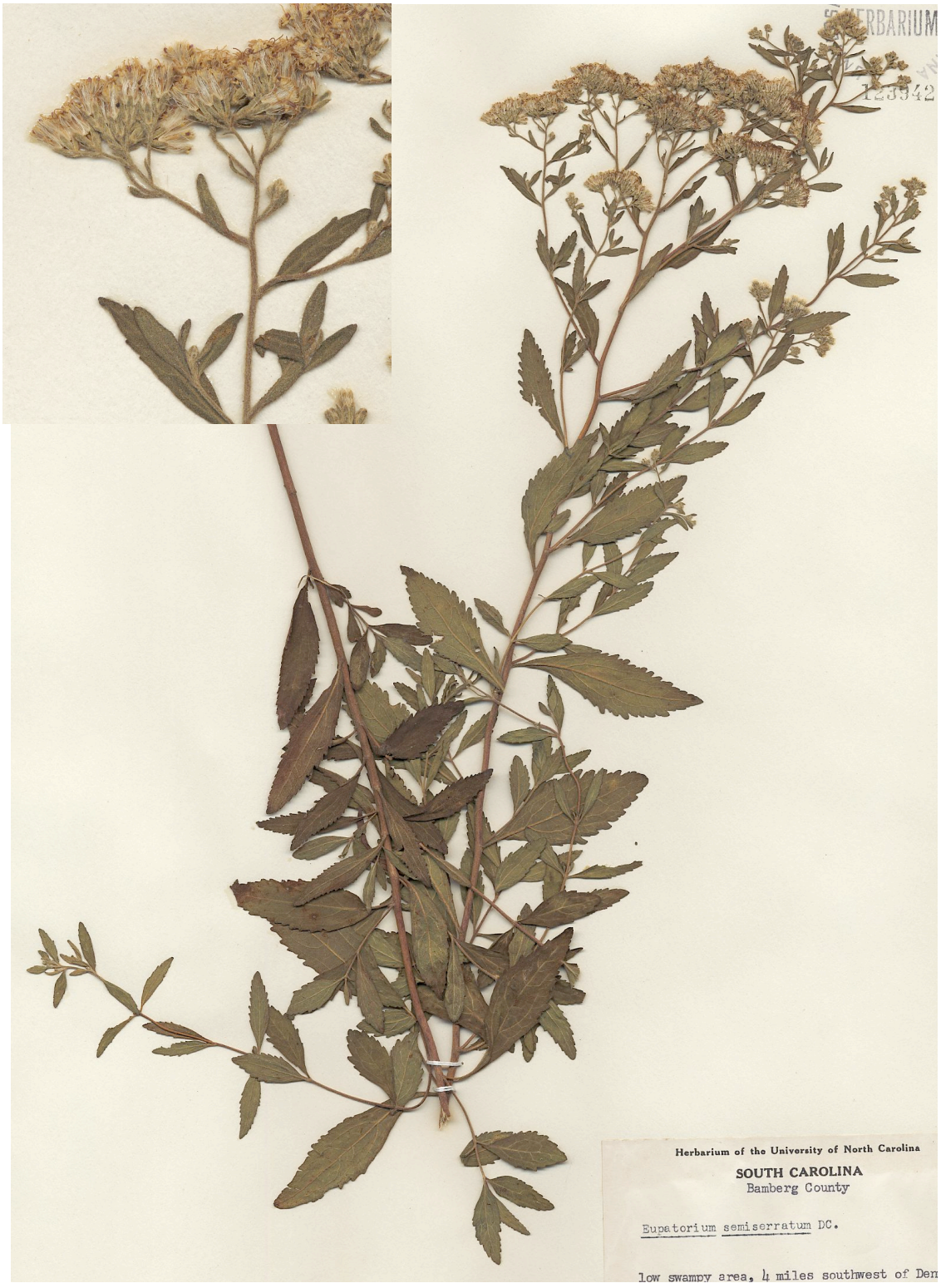


Figure 2-29. *Eupatorium semiserratum* DC.



Figure 2-30. *Eupatorium serotinum* Michx.



Figure 2-31. *Eupatorium sessilifolium* L.

APPENDIX B

APPENDIX B-1: Vouchers for DNA extraction

Species: *Eupatorium rotundifolium*

Diploid population

RD1: Florida, Jefferson Co. About 2.2 miles from Lamont N 30° 23.26′ W 83° 50.58′, Kunsiri Chaw Siripun, 09-21-2000, DNA #993-996

RD2: Florida, Calhoun Co. About 1 mile west of Blountstown along Fla. Rt. 20, N 30° 25.76′ W 85° 6.28′ Kunsiri Chaw Siripun, 08-05-2001, DNA #997-1006

RD3: Florida, Calhoun Co. About 7 miles from Trammell Bridge, west of Bloutstown along Fla. Rt. 20 or about 1.5 miles from Chipola River, N 30° 25.55′ W 85° 8.72′, Kunsiri Chaw Siripun, 08-05-2001, DNA #1007-1018

RD4: Florida, Calhoun Co. About 1.5 miles north of junction of Fla. 275 and Fla. 20, Along Rt. 71, N 30° 28.50′ W 85° 8.59′, Kunsiri Chaw Siripun, DNA #1019-1026

RD5: Florida, St. Johns Co. In front of S. J. C. Fairground, SR207 Elkton, N 29° 46.28′ W 81° 27.27′, Kunsiri Chaw Siripun, 08-06-2001, DNA #1027-1036

RD6: Florida, St. Johns Co. Southeast of Orangedale about 1.5 miles from the junction of Fla. Rt. 16A and Fla. 16, N 29° 59.72′ W 81° 32.29′, Kunsiri Chaw Siripun, 08-06-2001, DNA #1037-1046

Polyploid population

RP1: South Carolina, Colleton Co. Near Walterboro, along I-95 about 0.5 mile from exit 57, N 32° 55.38′ W 80° 41.62′, Kunsiri Chaw Siripun, 08-07-2001, DNA #1050-1056

RP2: Tennessee, Bledsoe Co. Fall Creek Falls State Park, Chris Flemming, 08-23-2001, DNA #1057-1066

RP3: North Carolina, Richmond Co. North of Rockingham on U. S. 1- site of subdivision being developed, east side of road, N 34° 58.55′ W 79° 38.22′, Dr. Edward E. Schilling, 09-13-2002, DNA #922-931

RP4: South Carolina, Berkeley Co. Steed Creek Rd. corner of Dog Swamp Rd., ca. 3.8 mile east of the turnoff from Hwy 41, Dr. Edward E. Schilling 09-13-2002, DNA #932-941

RP5: North Carolina, Orange Co. Along the roadside, close to the University of North Carolina, Chapel Hill, N 35° 56□5.3□□W 79° 3□31.0□, Kunsiri Chaw Siripun, 10-04-2002, DNA #948-952

RP6: North Carolina, Brunswick Co. Along the roadside by Prong Branch Rd. on Rt. 17, N 34° 0.05□48.7□□W 78° 7□33.8□, Kunsiri Chaw Siripun, 10-05-2002, DNA #953-962.

RP7: South Carolina, Georgetown Co. About 2 miles south of Georgetown, on the side of the road Rt. 17, N 33° 18□24.5□□W 79° 20□7.6□, Kunsiri Chaw Siripun, 10-06-2002, DNA #963-967

RP8: South Carolina, Berkeley Co. Collected from Francis Marion National Forest, close to Charleston, Kunsiri Chaw Siripun, 10-06-2002, DNA #968-977

RP9: Georgia, Dade Co. Cloundland Canyon State Park, next to the group lodge, Kunsiri Chaw Siripun, 10-19-2002, DNA #978-980

RP10: Alabama, Dekalb Co. Little River Canyon Reserve, Kunsiri Chaw Siripun, 10-19-2002, DNA #982-984

RP11: Alabama, Marshall Co. Along the roadside in the abandoned area, Kunsiri Chaw Siripun, 10-20-2002, DNA #988-992

RP12: Tennessee, Cumberland Co. Along Rte. 70 S to Mount Crest Glade, thin soil, abandoned area. Opened habitat, plants are scattered in the open field. N 35° 51□9.8□□W 85° 7□16.49□, Kunsiri Chaw Siripun, 07-26-2003, DNA # 1077-1085

RP13: Tennessee, Bledsoe Co. About 0.2 miles from the junction 285, on Rt. 30 Opened habitat, just recently burned. Plants are scattered. N 35° 43□34.8□□W 85° 13□41.6□, Kunsiri Chaw Siripun, 07-26-2003, DNA #1086-1093

Species: *Eupatorium sessilifolium*

Diploid population

SD1: Virginia, Montgomery Co. Brush Mountain Rd., about 1.5 miles from Rt. 460, edge of Jefferson National Forest, N 37° 16.68□W 80° 26.17□, Kunsiri Chaw Siripun, 07-28-2002, DNA #859-864

SD2: Tennessee, Blount Co. Chilhowee Dam, along Hwy 129, N 35° 32′ 49.4″ W 84° 2′ 51.9″, Dr. Edgar Lickey & Kunsiri Chaw Siripun, 08-13-2002, DNA #874-883

SD3: Tennessee, Blount Co. Calderwood, along the roadside, N 35° 29′ 56.3″ W 83° 59′ 25.7″, Dr. Edgar Lickey & Kunsiri Chaw Siripun, 08-13-2002, DNA #884-893

SD4: Tennessee, Blount Co. along Hwy 129, N 35° 30′ 12.5″ W 83° 58′ 34.4″, Dr. Edgar Lickey & Kunsiri Chaw Siripun, 08-13-2002, DNA #895-898

SD5: North Carolina, Graham Co. Near the entrance to Tapoco Lodge, on the cliff, N 35° 30′ 26″ W 83° 56′ 18.9″, Dr. Edgar Lickey & Kunsiri Chaw Siripun, 08-13-2002, DNA #902-903

SD6: North Carolina, Graham Co. along Hwy 129, about 4.5 miles from Tapoco Lodge, N 35° 24′ 55.5″ W 83° 53′ 19.9″, Dr. Edgar Lickey & Kunsiri Chaw Siripun, 08-13-2002, DNA #904-909

SD7: North Carolina, Swain Co. along Hwy 129, N 35° 26′ 56.7″ W 83° 56′ 8.5″, Dr. Edgar Lickey & Kunsiri Chaw Siripun, 08-13-2002, DNA #910-914

SD8: Tennessee, Bledsoe Co. Along Rte. 30. Just pass Pikeville or about 3 miles from Rhea county line. N 35° 35′ 26.3″ W 85° 9′ 10.8″, Kunsiri Chaw Siripun & Sam Grubbs, 07-26-2003, DNA #1094-1100

Polyploid population

SP1: Virginia, Craig Co. Along the roadside VA Rt. 311, under the power-line, N 37° 22.73′ W 80° 3.77′, Kunsiri Chaw Siripun & Sam Grubbs, 10-14-2001, DNA #1072-1075

SP2: Virginia, Floyd Co. The Blue Ridge Parkway, ca. Mile post 138.1, close to Adney Gap, N 37° 05′ 56.9″ W 80° 7′ 10.1″, Kunsiri Chaw Siripun & Sam Grubbs, 07-27-2002, DNA #824-828

SP3: Virginia, Franklin Co. The Blue Ridge Parkway, ca. Mile post 133.3, N 37° 09′ 2.3″ W 80° 5′ 38.1″, Kunsiri Chaw Siripun & Sam Grubbs, 07-27-2002, DNA #829-830, 843-846

SP4: Virginia, Botetourt Co. The Blue Ridge Parkway, ca. Mile post 103, N 37° 23′ 39.2″ W 79° 50′ 19.6″, Kunsiri Chaw Siripun & Sam Grubbs, 07-27-2002, DNA #847-853

SP5: Virginia, Bedford Co. The Blue Ridge Parkway, ca. Mile post 72.8, N 37° 32′ 30.3″ W 79° 28′ 6.7″, Kunsiri Chaw Siripun & Sam Grubbs, 07-27-2002, DNA #854-858

SP6: Virginia, Roanoke Co. Poor Mountain, on VA Rt. 916, N 37° 13′ 0.69″ W 80° 8′ 5.0″, Kunsiri Chaw Siripun & Sam Grubbs, 07-28-2002, DNA #865-868, 871-873

SP7: Tennessee, Jefferson Co., Hickory Rd., ca. 75 yards east of Barber Rd. N 36° 02′ 25.1″ W 83° 39′ 38.4″, Dr. Edgar Lickey, 08-19-2002, DNA #915-921.

Other species for chloroplast DNA analysis:

Eupatorium album: Alabama, Dekalb Co. Little River Canyon Reserve, Kunsiri Chaw Siripun, 10-19-2002, DNA #981, *Eupatorium altissimum*: TN, Knox Co., Kunsiri Chaw Siripun, 08-30-2000, DNA #682, *Eupatorium cannabinum*: Heronswood Nurseries, Kingston, WA, 1998 DNA #598, *Eupatorium capillifolium*: NC, Orange Co., Kunsiri Chaw Siripun, 10-04-2002, DNA #869, *Eupatorium chinense*: J. L. Panero 1995 DNA #531, *Eupatorium compositifolium*: FL, Levy Co., Manatee Spring State Park, Kunsiri Chaw Siripun, 10-2000, DNA #1029, *Eupatorium godfreyanum*: VA, Floyd Co., Kunsiri Chaw Siripun, 07-27-2002, DNA #819-823, *Eupatorium godfreyanum*: TN, Cumberland Co., Kunsiri Chaw Siripun and Sam Grubbs, 07-26-2003, DNA #1077, *Eupatorium hyssopifolium*: NC, Orange Co. Kunsiri Chaw Siripun,, 10-04-2002, DNA #870, *Eupatorium leucolepis*: NC, Camp Lejune, Kunsiri Chaw Siripun, 10-03-2002, DNA #1125, *Eupatorium mohrii*: NC, Camp Lejune, Kunsiri Chaw Siripun, 10-03-2002, DNA #1126, *Eupatorium perfoliatum*: DNA # 465, *Eupatorium pilosum*, TN Cumberland Co., Kunsiri Chaw Siripun, 07-26-2003, DNA #1047, *Eupatorium pilosum*, Kunsiri Chaw Siripun, FL, St. Johns Co., 08-06-2001, DNA #1048 , *Eupatorium semiserratum*: Florida Dr. Schilling collection 08-2003 DNA #1030, *Eupatoriadelphus fistulosus*: TN, Bledsoe Co., Kunsiri Chaw Siripun and Sam Grubbs, 07-26-2003, DNA #1086,

APPENDIX B-2: Solutions for molecular studies

Cotton Blue solution

Stock: 1 g Cotton blue dissolve in 100 ml of lactophenol

Working Solution:

5 ml of stock solution in 100 ml of lactophenol

Lactophenol

10 g Phenol in 10 ml distilled water

10 ml Glycerine

10 ml lactic acid

CTAB Extraction Buffer (Doyle and Doyle, 1987)

100 mM Tris-HCL, pH 8.0

1.4 M NaCl

20 mM EDTA

2% hexadecyltrimethylammonium bromide (CTAB)

0.2% 2-mercaptoethanol (add fresh per use)

For 500 ml:

50 ml Tris (1M stock)

140 ml NaCl (5M stock)

30 ml EDTA

10 g CTAB

Add ddH_2O to make 500 ml

Millipore filter and aliquot into 50 ml falcon tubes and store at -20°C

Add 2-mercaptoethanol just before using (100 L/50 ml)

EDTA 0.5 M, pH 7.5

For 100 ml add 18.61 g EDTA

-Heat in beaker with 50 ml ddH_2O

pH to 7.5 with NaOH (start by adding 2 g NaOH to dissolve EDTA)

adjust to 1L with dd H_2O

Ethidium Bromide

For 10 mg/ml

Add 1.0 g EtBr to 100 ml ddH_2O

Stir several hours to dissolve

Wrap container in aluminum foil (light sensitive)

76% ETOH/10 mM NH_4OAc

For 200 ml

152 mL EtOH

48 ml ddH_2O

0.152 g NH_4OAc

100 bp LADDER

Where stock is 1 mg/ml

5 ml 100 bp ladder stock

145 ml TE buffer, pH 8

50 ml loading dye

LOADING DYE (6□)

50% Glycerin (w/v)

Add 1X TAE to make 100 ml

Slightly add Bromophenol Blue until the color turns dark blue

NaCl 5M

For 500 ml

146.1 g adjust to 500 ml with ddH_2O

TAE

For 1 liter of 50X stock

242 g Tris

57.1 ml Acetic acid

100 ml 0.5 M EDTA

For 1 liter of 1X TAE

20 ml 50X TAE

980 ml ddH_2O

TE Buffer

10 mM Tris-HCl; pH 8.0 (autoclave)

1 mM EDTA (autoclave)

Per 100 ml: add

1 ml 1M Tris

0.2 ml 0.5 M EDTA

Adjust to 100 ml with ddH_2O

TRIS

For 1 liter

121.1 g Tris

Add to 500 ml ddH_2O

Adjust pH to 7.5 with conc. HCl (~65 ml to start)

Adjust to 1 L with ddH_2O

Use milli-pore and autoclave

APPENDIX B-3: DNA Extraction protocols (Applied from Doyle and Doyle, 1987)

Protocol for Liquid Nitrogen DNA Extraction (for 1.5 gram fresh weight or more)

A. Turn on 60° C water bath. Get CTAB from freezer and shake in bath.
B. Weight out 1.5 g fresh weight (or 0.3 g dried material)
C. In the fume hood, add 100 μL of beta-mercaptoethanol to the CTAB (100 μL beta-mercaptoethanol to 50 mL CTAB). Return CTAB to water bath and wait for 5 min prior use.
D. Place material in large mortar. Fill mortar approximately halfway with liquid Nitrogen.
E. Grind well until nitrogen has almost totally boiled away.
F. Fill mortar approximately a quarter full with liquid nitrogen and grind until nitrogen has almost totally boiled away.
G. When a small amount of liquid nitrogen remains, transfer grindate to new, labeled falcon tube.
H. Carefully add 15 ml of CTAB to tube (excessive bubbles will form if you add the CTAB before the nitrogen boils away completely.
I. Place tube in the water bath and shake for 60 min.
J. Add a small amount of dish soap to mortar and grind it until it freezes (this will help prevent staining of the mortar).
K. In the fume hood, add an equal amount of chloroform-isoamylalcohol to tube.
L. Tighten cap well and gently invert the tube a few times.
M. Place tube in centrifuge at highest setting for 30 min.

N. Carefully remove the tubes from centrifuge. Carefully draw off (with sterile glass pipette or P1000) the upper aqueous layer and place in clean, labeled falcon tube. Do not disturb the middle precipitate layer.

O. Discard the middle and bottom layers in organic waste bottle in fume hood.

P. To the aqueous layer (in new, clean tube), add two-thirds volume of -20°C isopropanol.

Q. Invert gently to precipitate DNA. Place in freezer for 20-30 min.

R. Centrifuge for 15 min at highest setting.

S. Carefully pour of liquid into flask, leaving DNA pellet in bottom of tube. If DNA is still suspended, the liquid can be poured back into the tube and recentrifuged.

T. Turn falcon tube upside down over a paper towel and allow alcohol to drip off.

U. Wash with 20 ml 76% EtOH-10 mM NH_4OAc. Leave for 20 min to 2 days.

V. Dislodge pellet and centrifuge for 5 min at highest setting.

W. Pour liquid off as in Step T and put in vacuum for approximately 30 min.

X. Resuspend pellet in 1 ml TE (if pellet is small, you may wish to use only 0.5 ml).

Y. Let sit overnight (or until DNA is resuspended) and transfer to clean, labeled 1.5 Eppendorf tube. Place in freezer.

Protocol for Liquid Nitrogen DNA Extraction (for small amount of plant material; 0.1- 0.2 gram fresh weight)

A. Turn on 60° C water bath. Get CTAB from freezer and shake in bath.

B. Weight out 0.1-0.2 g fresh weight

C. In the fume hood, add 100 µL of beta-mercaptoethanol to the CTAB (100 µL beta-mercaptoethanol to 50 mL CTAB). Return CTAB to water bath and wait for 5 min prior use.

D. Place material in 1.5 mL microfuge tube and cap.

E. Flash-freeze by dropping tube into liquid nitrogen.

F. Carefully open tube without touching the sides and without breaking off the cap

G. Add liquid N_2 to tube and grind sample with a plastic pestle

H. Add 1 mL CTAB solution, mix well and incubate 60 min. in 60° C water bath, frequently mix by inversion

I. Add 500 µL Chloroform:Isoamyl alcohol (24:1) to tube, and invert to mix

J. Centrifuge at maximum speed for 1 min.

K. Remove aqueous supernatant (top layer) to a new labeled tube, discard organic layer in the fume hood

L. Add 500 µL Chloroform:Isoamyl alcohol (24:1) to tube, and invert to mix

M. Centrifuge at maximum speed for 1 min.

N. Remove aqueous supernatant (top layer) to a new labeled tube, discard organic layer in the fume hood

O. Add 0.8 volumes cold isopropanol (2-propanol), mix gently to precipitate DNA

P. Centrifuge at maximum speed for 5 min to pellet DNA; pour off supernatant

Q. Add 200 µL 70% Ethanol, invert to mix several times, and centrifuge at maximum speed for 2 min

R. Aspirate supernatant and allow pellet to air-dry for at least an hour

S. Dissolve pellet in 50 – 100 µL TE

T. Check DNA concentration via fluorometry and record on tube

APPENDIX B-4: DNA Purification protocol

Wizard® PCR Preps DNA Purification Systems (Promega)

Purification with a vacuum manifold

A. Aliquot 100 µl of Direct PCR Purification Buffer into a tube
B. Add 30-100 µl of the DNA extraction, vortex briefly to mix
C. Add 1 ml of resin and vortex briefly 3 times over a 1-minute interval
D. Prepare a Wizard® Minicolumn for each sample, attach the Syringe Barrel to the Minicolumn and insert the Minicolumn/Syringe Barrel into the Vacuum Manifoild
E. Add the Resin/DNA mix to the Syringe/Barrel
F. Apply the vacuum to pull liquid through the Minicolumn, release vacuum when all the liquid has pass through the Minicolumn
G. Wash by adding 2 ml of 80% isopropanol to the Syringe Barerel, apply vacuum to pull solution through the Minicolumn
H. Dry the resin by continuing to apply the vacuum for 30 seconds
I. Remove the Syringe Barrel and transfer the Minicolumn to a 1.5 ml microcentrifuge tube
J. Centrifuge at 10,000×g for 2 minutes
K. Elute by transferring the Minicolumn to a clean 1.5 microcentrifuge tube, add 50 µl of ddH_2O or TE buffer and wait 1 minute
L. Centrifuge at 10,000×g for 20 seconds at room temperature
M. Remove and discard Minicolumn, store DNA at~20°C or below

APPENDIX B-5: Centri-Sep protocol

Materials

1. Sephadex = Sigma G 50-80 fine for filtration of globular proteins
2. Deionized H_2O
3. Sterile ddH_2O

Methods

1. Prepare sephadex G50 ahead of time in 250 ml batches and keep in the refrigerator (20 g G50/250 ml deionized H_2O or 10 g G50/125 ml deionized H_2O), let that hydrate for at least 30 min before its first use. After that, you will need to swirl it around as you are loading your columns

2. Load reusable column with 800 µl of 50G solution as we need them. This will save a lot of time waiting for it to hydrate. It keeps several weeks with good results.

3. Clean columns by rinsing out the sephadex several times with water then add 500 µl milliQ or sterile ddH_2O and centrifuge through at low speed (3000 rpm) about 2 mins.

Protocol

- Load 800 µl 50G sephadex solution into the column, and put the column into the wash tube
- Spin the column + wash tube at 3000 rpm (750 µg) 2 min
- Approximately 300 µl of fluid will be removed, discard the liquid part
- Process the sample within the next few minutes, bring sample to 20 µl with sterile H_2O and hold the column up to the light, transfer 20 µl of the reaction mixture to the top of the gel
- Carefully dispense the sample directly onto the center of the gel bed at the top of the column, without disturbing the gel surface
- Prepare the column into the sample collection tube*, and spin the column and the collection tube at 3000 rpm for 2 min
- The purified sample will collect in the bottom of the sample collection tube

- Dry sample in the vacuum centrifuge, discard the spin column and rinse with water few times, add 500 µl sterile dd H_2O and centrifuge through at low speed (3000 rpm) about 2 mins.

APPENDIX B-6: ISSR primers & protocol (Dr. Randall Small, University of Tennessee, personal communication)

PCR reaction conditions; 15 µl total reaction volume

1 µl clean, diluted DNA (~10 – 100 ng)

1X Taq buffer

2.5 mM $MgCl_2$ final concentration

200 µM dNTP final concentration

2% v:v Formamide (e.g. 0.3 µl Formamide in a 15 µl reaction)

0.33 µM primer (e.g. 0.5 µl of 10 µM primer solution in a 15 µl reaction)

0.5 units *Taq* polymerase

Primers:

808	$(AG)_8$-C
810	$(GA)_8$-T
811	$(GA)_8$-C
815	$(CT)_8$-G
818	$(CA)_8$-G
820	$(GT)_8$-C
826	$(AC)_8$-C
835	$(AG)_8$-YC
841	$(GA)_8$-YC
842	$(GA)_8$-YG
844	$(CT)_8$-RC
845	$(CT)_8$-RG
848	$(CA)_8$-RG
851	$(GT)_8$-YG
857	$(AC)_8$-YG
868	$(GAA)_6$
871	$(TAT)_6$

MANNY	$(CAC)_4$-RC
MAO	$(CTC)_4$-RC
OMAR	$(GAG)_4$-RC
TERRY	$(GTG)_4$-RC

Single letter abbreviations for mixed base positions

R = (A,G)

Y = (C,T)

primers in the "800" series are from the University of British Colombia primer set #9

primers in the "name" series are from Dr. Andrea Wolfe's ISSR web page (http://www.biosci.ohio-state.edu/~awolfe/ISSR.html)

APPENDIX B-7: Electrophoresis agarose gel

For small gel (13□17 cm):
(E-C Classic□ CSSU911; E-C Apparatus, Corp)

50 ml 1X TAE

0.5 g agarose (1% Gel); Fisher Agarose Low EED for regular reaction

1.0 g agarose (2% Gel); Fisher Agarose Low EED for ISSRs

A. Heating plate to boiling

B. Cool with stirrer to ~50°C

C. Add 2.5 □l of ethidium bromide

D. Pour gel and set ~30 min

E. Cover with 1X TAE and then remove comb

F. Mix each sample with 3.0 □l 6X loading dye

H. Load 15 □l 100 bp ladder in the first well, then follow by samples respectively

I. Leave the very last wells for negative control and 100 bp ladder

J. Let the gel run 1 hr 45 min, 96-98 volts (ca. distance 8.0 cm) by EC-105, EC

Apparatus Corporation

K. Cool the gel with tap water, a picture is taken under the UV light with KODAK DC

120 (KODAK Digital Science 1D)

L. Data scoring will be taken from the present/absent of the bands

APPENDIX B-8: Cloning protocol

I. Ligation

You want a 1:1 ratio of plasmid to insert. The plasmid is ~3kb long. To determine the amount of insert you need, divide the length of the plasmid by the length of the insert (e.g. ITS is ~700bp so 3000/700 = 4.3). Take this number and divide it into 50 (the amount of plasmid used) to get the ng of insert needed (e.g. 50/4.3 = 11.6). If the template is very clean, single band, well-characterized, etc., you may use only 25 ng of plasmid (adjust your calculations appropriately).

For 50 ng of plasmid:

5.0 µl of 2X Ligation buffer
1.0 µl of T vector*
1.0 µl of DNA ligase*
x µl of insert (determined by above calculations)
y µl of sdH_2O

10.0 µl total volume
* divide by 2 if using only 25 ng of plasmid
Mix reagents in 0.5 ml tube and place in refrigerator (4°C) overnight to incubate.

II. Transformation

- Prepare 2 LB/ampicillin/IPTG/X-Gal plates per ligation reaction. Bring plates to room temperature before plating. If not already in agar, spread 10 µl of IPTG and 40 µl of X-Gal on the plate before using.
- Transfer ligation reaction to a 1.5 ml tube.
- Remove cells from the -70°C freezer, thaw in ice bath, and mix by gently fliking tube.
- Carefully transfer 50 µl of cells into tube with ligation reaction.
- Gently flick tubes to mix.
- Place on ice for 20 min.
- Heat shock the cells for 45-50 sec in a 42°C water bath.
- Immediately return to ice for 2 min.
- Add 950 µl of room temperature SOC medium to cells.
- Incubate for 1.5 hours at 37°C with shaking (~150 rpm).
- Spread 100 µl of transformation reaction onto a plate.
- Spin the tube for 30 sec to concentrate the remaining cells.
- Remove all but 100 µl of the supernatant (remove ~800 µl).
- Re-suspend the pellet and plate onto a second plate.
- Invert the plates and incubate overnight at 37°C.

III. <u>Screening</u>

- Using a pipette tip, carefully scrape off a single colony and place in 10 µl sdH_2O in a 0.5 ml tube.
- Touch a pipette tip to a new plate (marked with reference grid).
- Repeat until 10-20 colonies have been sampled (each into its own tube).
- Incubate the plate at 37°C for several hours.
- Boil the tubes for 5 min to lyse cells.
- Spin the tubes for 30 sec to pellet the cells.
- Use 1-2 µl of the supernatant as the template for 10 µl PCR reaction.
- Run an agarose gel to check that proper fragment was cloned.
- Pick a sample(s) and scrape off part of the colony from the replicate plate.
- Add it to 5 µl Terrific Broth (or similar growth medium) and 62.5 µl ampicillin.
- Incubate 20 hrs at 37°C with shaking (~250 rpm).

IV. <u>Plasmid Miniprep Procedure</u>

*Before starting (1) turn heat block on to 37°C and (2) get RNAse out of the –20°C

- Cool overnight bacterial culture to 4°C and transfer 1-3 ml to a clean microfuge tube. Centrifuge 30s to pellet cells; remove supernatant by decanting; centrifuge an additional 5s and remove supernatant by pipetting.
- Add 200 µl Cell Resuspension Buffer (GTE) and resuspend cells by pipetting up and down.
- Add 200 µl Cell Lysis Buffer and immediately vortex to mix. Incubate at RT for 2 min.
- Add 300 µl Protein Removal Buffer and immediately vortex to mix. Incubate on ice for 5 min.
- Centrifuge for 5 min at RT. Transfer supernatant to clean microfuge tube.
- Add 5 µl RNAse A; incubate at 37°C for 5-10 min.
- Add 500 µl Plasmid Precipitation Reagent and vortex to mix.
- Centrifuge 5 min at RT; remove supernatant by pipetting or aspirating; centrifuge additional 5 sec and remove additional supernatant by pipetting or aspiration.
- Add 400 µl 70% ethanol, vortex and centrifuge 2 min at RT.
- Decant supernatant, invert tube on Kimwipe and dry pellet on bench for ca. 0.5 hr.
- Resuspend pellet in 50-100 µl 10 mM Tris HCl pH 8.0.

Reagents:

<u>Cell Resuspension Buffer</u> (GTE)
50 mM glucose (sterile)
25 mM Tris
10 mM EDTA
pH to 8.0

Cell Lysis Buffer
0.2 M NaOH
1% SDS

Protein Removal Buffer
3 M Potassium
5 M Acetate
for 100 ml: 60 ml 5M potassium acetate
11.5 ml glacial acetic acid
28.5 ml of H_2O

Plasmid Precipitation Reagent
20% PEG 8000
2.5 M NaCl

APPENDIX B-9: ITS sequence alignment for the genus *Eupatorium*

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CLUSTAL X (1.81) multiple sequence alignment


ITO_emaki          TCGAACCCTGGATGGCAAAGCAACCTGTGAACGTGTATCAACAATG-TGGCTTGGCGGGT
ITO_elind          TCGAACCCTGGATGGCAAAGCAACCTGTGAACGTGTATCAACAATG-TGGCTTGGCGGGT
erotP2_1059        TCGAACCCTGGATGGCAAAACAACCCGTGAACGTGTATCAACAAAGATGGCTTGGCGGGT
erotP13_1088       TCGAACCCTGGATGGCAAAACAACCCGTGAACGTGTATCAACAAAGATGGCTTGGCGGGT
erotP9_979         TCGAACCCTGGATGGCAAAACAACCCGTGAACGTGTATCAACAAAGATGGCTTGGCGGGT
erotP8_972         TCGAACCCTGGATGGCAAAACAACCCGTGAACGTGTATCAACAAAGATGGCTTGGCGGGT
erotP10_982        TCGAACCCTGGATGGCAAAACAACCCGTGAACGTGTATCAACAAAGATGGCTTGGCGGGT
emac_532           TCGAACC-TGGATGGCAAAACAACCTGTGAACGTGTATCAACAA-GATGGNNNGGGGGGN
erotD2_998         TCGAACCCTGGATGGCAAAACAACCCGTGAACGTGTATCAACAAAGACGGCTTGGCGGGT
emik_705           TCGAACC-TGGATGGCAAAACAACCCGCGAACATGTACCAACAA-GATGGCTTGGCGGGT
ITO_eform          TCGAACCCTGGATGGCAAAACAACCCGTGAACGTGTATCAACAA-TATGGCTTGGCGGGT
ITO_ecom           TCGAACCCTGGATGGTAAAACAACCCGTGAACGTGTATCAACAA-GATGGCTTGGCGGGT
ITO_etash          TCGAACCCTGGATGGCAAAACAACCCGTGAACGTGTATCAACAA-TATGGCTTGGCGGGT
egod_822           TCGAACACTGGATGGCAAAACAAGGGGTGAACGTGTATCAACAA-GATGGCTTGGCGGGT
erotD3_1015        TCGAACCCTGGATGGCAAAACAACCCGTGAACTTGTATCAACAAAGATGGCTTGGCGGGT
echi713            TCGAAC-CTGGATGGCAAAACAACCCGTGAACGTGTATCAACAA-TATGGCTTGGCGGGT
ITO_egleh          TCGAACCCTGGATGGCAAAACAACCCGTGAACGTGTATCAACAAT-ATGGCTTGGCGGGT
erotP11_992        TCGAACCCTGGATGGCAAAACAACCCGCGAACGTGTATCAACAAAGATGGCTTGGCGGGT
erotP4_934         TCGAACCCTGGATGGCAAAACAACCCGTGAACGTGTATCAACAAAGATGGCTTGGCGGGT
echi531            TCGAACCCTGGATGGCAAAACAACCCGTGAACGTGTATCAACAAATTGGCTTGGGCGGGT
ecap_460           TCGAACCCTGGATGGTAAAACAACCCGTGAACGTGTATCAACAAG-ATGGCTTGGCGGGT
esesD8_1096        TCGAACCCTGGATGGCAAAACAACCGGTGAACGTGTATCAACAAG-ATGGCTTGGCGGGT
eleu_703           TCGAACC-TGGATGGCAAAACAACCCGTGAACGTGTATCAACAAAAATGGCTNGGCGGGT
erot_706           TCGAACCCTGGATGGCAAAACAACCCGTGAACGTGTATCAACAAAGATGGCTTGGCGGGT
ITO_emik           TCGAACCCTGGATGGCAAAACAACCCGCGAACATGTACCAACAA-GATGGCTTGGCGGGT
esesD6_908         TCGAACCCTGGATGGCAAAACAACCGGTGAACGTGTATCAACAA-GATGGCTTGGCGGGT
eses_710           TCGAACCCTGGATGGCAAAACAACCGGTGAACGTGTATCAACAAGA-TGGCTTGGCGGGT
ehys_709           TCGAACC-TGGATGGCAAAACAACCCGTGAACGTGTATCAACAAAAACGGCTTGGCGGGT
esesP7_917         TCGAACCCTGGATGGCAAAACAACCGGTGAACGTGTATCAACAAGA-TGGCTTGGCGGGT
esesP2_824         TCGAACCCTGGATGGCAAAACAACCGGTGAACGTGTATCAACAA-GATGGCTTGGCGGGT
esesD1_861         TCGAACCCTGGATGGCAAAACAACCGGTGAACGTGTATCAACAA-GATGGCTTGGCGGGT
erotP12_1081       TCGAACCCTGGATGGCAAAACAACCCGTGAACGTGTATCAACAAAGATGGCTTGGCGGGT
esesD2_874         TCGAACCCTGGATGGCAAAACAACCGGTGAACGTGTATCAACAA-GATGGCTTGGCGGGT
ITO_elep           TCGAACC-TGGATGGTAAA-CAACCCGTGAACGTGTATCAACAA-GATGGCTTGGCGGGT
esesP1_1075        TCGAACCCTGGATGGCAAAACAACCGGTGAACGTGTATCAACAA-GATGGCTTGGCGGGT
esesD3_884         TCGAACCCTGGATGGCAAAACAACCGGTGAACGTGTATCAACAA-GATGGCTTGGCGGGT
esesP6_871         TCGAACCCTGGATGGCAAAACAACCGGTGAACGTGTATCAACAAG-ATGGCTTGGCGGGT
ITO_ejapo          TCGAACCCTGGATGGCAAAACAACCCGTGAACTTGTAACAACAAT-GTGGCTTGGCGGGT
esesP4_847         TCGAACCCTGGATGGCAAAACAACCGGTGAACGTGTATCAACAAG-ATGGCTTGGCGGGT
ITO_erot           TCGA-CCCTGGATGGCAAAACAACCCGTGAACGTGTATCAACAAAGATGGCTTGGCGGGT
ITO_evar           TCGAACCCTGGATGGCAAAACAACCCGTGAACGTGTATCAACAAT-ACGGCTTGGCGGGT
esesD7_910         TCGAACCCTGGATGGCAAAACAACCGGTGAACGTGTATCAACAAG-ATGGCTTGGCGGGT
ITO_ehys           TCGA-CCCTGGATGGCAAA-CAACCG-TGAACGTGTAGCAACAAAGACGGCTTGGCGGGT
esesP3_843         TCGAACCCTGGATGGCAAAACAACCGGTGAACGTGTATCAACAA-GATGGCTTGGCGGGT
esesD4_897         TCGAACCCTGGATGGCAAAACAACCGGTGAACGTGTATCAACAA-GATGGCTTGGCGGGT
egod_819           TCGAACCCTGGATGGCAAAACAACCGGTGAACGTGTATCAACAA-GATGGCTTGGCGGGT
ecan_716           TCGAACC-TGGATGGCAAAACAACCCGTGAACGTGTATCAACAA-TATGGCTTGGCGGGT
esesD5_902         TCGAACCCTGGATGGCAAAACAACCGGTGAACGTGTATCAACAA-GATGGCTTGGCGGGT
ITO_ealt           TCGA-CCCTGGATGGCAAA-CAACCGT--GACGTGTATCA-CAA-GATG-CTTGGCGGGT
egod_821           TCGAACCCTGGATGGCAAAACAACCGGTGAACGTGTATCAACAA-GATGGCTTGGCGGGT
egod_820           TCGAACCCTGGATGGCAAAACAACCGGTGAACGTGTATCAACAA-GATGGCTTGGCGGGT
epur_506           TCGAACC-TGGATGGCAAAACAACCTGTGAACGTGTATCAACAA-GATGGCTTGGCGGGT
ITO_eserot         TCGAACCCTGGATGGCAAAACAACCCGTGAACGTGTATCAACAAAGATGGCTTGGCGGGT
ITO_esemi          TCGA--CCTGGATGGCAAA-CAACCGGTGAACGTGTATCAACAA-GATGGCTTGGCGGGT
ITO_eses           TCGAACCCTGGATGGCAAAACAACCCGCGAACGTGTATCAACAAAGATGGCTTGGCGGGT
ITO_ecap           TCGAACCCTGGATGGTAAAACAACCCGTGAACGTGTATCAACAA-GATGGCTTGGCGGGT
ecan_598           TCGAACCCTGGATGGCAAAACAACCCGTGAACGTGTATCAACAA-TATGGCTTGGCGGGT
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egod_1077          TCGAACCCTGGATGGCAAAACAACCGGTGAACGTGTATCAACAA-GATGGCTTGGCGGGT
esesP5_855         TCGAACCCTGGATGGCAAAACAACCGGTGAACGTGTATCAACAA-GATGGCTTGGCGGGT
ealb_702           TCGAACCCTGGATGG-AAAACAACCCGTGAACGTGTATCAACAAAGATGGCTTGGCGGGT
ITO_efist          TCGA-CCCTGGATGC-AAA-CAACCTGTGAACGTGTATCAACAA-GATGGCTTGGCGGGT
ecap_712           TCGAACC-TGGATGGTAAAACAACCCGTGAACGTGTATCAACAA-GATGGCTTGGCGGGT
eper_465           TCGAANCCTGGATGGCAAAACAACCCGTGAACGTGTATCAACAA-GATGGCTTGGCGGGT
erotD1_993         TCGAACCCTGGATGGCAAAACAACCCGTGAACGTGTATCAACAAAGATGGCTTGGCGGGT
ealt_711           TCGAACCCTGGATGGCAAAACAACCCGTGAACGTGTATCAACAAAGATGGCTTGGCGGGT
echi_715           TCGAACC-TGGATGGCAAAACAACCCGTGAACGTGTATCAACAAT-ATGGCTTGGCGGGT
erotP1_1050        TCGAACCCTGGATGGCAAAACAACCCGTGAACGTGTATCAACAAAGATGGCTTGGCGGGT
erotP5_952         TCGAACCCTGGATGGCAAAACAACCCGTGAACGTGTATCAACAAAGATGGCTTGGCGGGT
ecun_701           TCGAATCCTGGATGGCAAAACAACCCGTGAACNTGTNTCAACAAAGATGGCTTGGCGGGT
erotP6_953         TCGAACCCTGGATGGCAAAACAACCCGTGAACGTGTATCAACAAAGATGGCTTGGCGGGT
erotD4_1022        TCGAACCCTGGATGGCAAAACAACCCGTGAACGTGTATCAACAAAGATGGCTTGGCGGGT
erotP7_963         TCGAACCCTGGATGGCAAAACAACCCGTGAACGTGTATCAACAAAGATGGCTTGGCGGGT
erotD5_1028        TCGAACCCTGGATGGCAAAACAACCCGTGAACGTGTATCAACAAAGATGGCTTGGCGGGT
erotD6_1046        TCGAACCCTGGATGGCAAAACAACCCGTGAACGTGTATCAACAAAGATGGCTTGGCGGGT
erotP3_922         TCGAACCCTGGATGGCAAAACAACCCGTGAACGTGTATCAACAAAGATGGCTTGGCGGGT
ITO_ecan           TCGAACCCTGGATGGCAAAACAACCCGTGAACGTGTATCAACAAT-ATGGCTTGGCGGGT
ITO_edub           TCGAACCCTGGATGGCAAA-CAACCTGTGAACGTGTATCAACAA-GATGGCTTGGCGGGT
                   ****    ******  *** ***       ** ***  ** ***    *    ** ***

ITO_emaki          ACT-GACGCTTCTTGTTTCAATGCCC-GTGAAGCCCTGTCGACGTGCGC--TCGTGGTGT
ITO_elind          ACT-GACGCTTCTTGTTTCAATGCCC-GTGAAGCCCTGTCGACGTGCGC--TCGTGGTGT
erotP2_1059        ACT-GATGCTTCTTGTTTCAATCCCC-GCGAAGCCCTGTCGACGTGCGC--TTGTGGCGT
erotP13_1088       ACT-GATGCTTCTTGTTTCAATCCCC-GCGAAGCCCTGTCGACGTGCGC--TTGTGGCGT
erotP9_979         ACT-GATGCTTCTTGTTTCAATCCCC-GCGAAGCCCTGTCGACGTGCGC--TTGTGGCGT
erotP8_972         ACT-GATGCTTCTTGTTTCAATCCCC-GCGAAGCCCTGTCGACGTGCGC--TTGTGGCGT
erotP10_982        ACT-GATGCTTCTTGTTTCAATCCCC-GCGAAGCCCTGTCGACGTGCGC--TTGTGGCGT
emac_532           GTN-GANACTTTTTGTTTCAAACCTC-GNGAAGCCCTGTCNACGTGTGTGTTTGTGNNGT
erotD2_998         ACT-GATGCTTCTTGTTTCAATCCCC-GCGAAGCCCCGTCGACGTGCGC--TTGTGGCGT
emik_705           ACT-GATGCTTCTTGTTTCAATCCCC-GCGAGGCCCTGTCNACGTGCGC--CTGTGGCGT
ITO_eform          ACT-GACGCTTCTTGTTTCATTGCCC-GTGAAGCCCCGTCGACGTGCGC--TCGTGGTGT
ITO_ecom           ACTTGATGCTTCTTGTTTCAATCCCC-GTGAAGCCCCGTCGACGTGTGT--TTGTGGTGT
ITO_etash          ACT-GACGCTTCTTGTTTCATTGCCC-GTGAAGCCCCGTCGACGTGCGC--TCGTGGTGT
egod_822           ACT-GATGCTTCTCGTTTCAATCCCC-GCGAAGCCCTGTCGACGTGCGC--TTGTGGTGT
erotD3_1015        ACT-GATGCTTCTTGTTTCAATCCCC-GCGAAGCCCCGTCGACGTGCGC--TTGTGGCGT
echi713            ACT-GACGCTTCTTGTTTCAATGCCC-GTGAAGCCCCGTCNACGTGCGC--TCGTGGTGT
ITO_egleh          ACT-GACGCTTCTTGTTTCAATGCCC-GTGAAGCCCTGTCGACGTGCGC--TCGTGGTGT
erotP11_992        ACT-GACGCTTCTTGTTTCAATCCCC-GCGAAGCCCTGTCGGCGTGCGC--ATGTGGTGT
erotP4_934         ACT-GATGCTTCTTGTTTCAATCCCC-GCGAAGCCCCGTCGACGTGCG--CTTGTGGCGT
echi531            ACT-GACGCTTCTTGTTTCAATGCCC-GTGAAGCCCCGTCGACGTGCG--CTCGTGGTGT
ecap_460           ACTTGATGCTTCTTGTTTCAATCCCC-GTGAAGCCCCGTCNACGTGTG--TTTGTGGTGT
esesD8_1096        ACT-GATGCTTCTCGTTTCAATCCCC-GCGAAGCCCTGTCGACGTGCG--CTTGTGGTGT
eleu_703           ACT-GATGCTGCTTGTTNCAANCCCCCGCGAAGCCCTGTCGACGTGCG--CTTGTGGTGT
erot_706           ACT-GATGCTTCNGGTTTCAANCCCC-GNGAAGCCCCGTCNACATGCG--CTTGTGGNGT
ITO_emik           ACT-GATGCTTCTTGTTTCAATCCCC-GCGAGGCCCTGTCGACGTGCG--CCTGTGGCGT
esesD6_908         ACT-GATGCTTCTCGTTTCAATCCCC-GCGAAGCCCTGTCGACGTGCG--CTTGTGGTGT
eses_710           ACT-GATGCTTCTCGTTTCAATCCCC-GCGAAGCCCTGTCGACGTGCG--CTTGTGGTGT
ehys_709           ACT-GATGCTNCATGTTTCAANCCCC-GCAAANCCCTGTCNGCGTGCG--CTTGTGGCNT
esesP7_917         ACT-GATGCTTCTCGTTTCAATCCCC-GCGAAGCCCTGTCGACGTGCG--CTTGTGGTGT
esesP2_824         ACT-GATGCTTCTCGTTTCAATCCCC-GCGAAGCCCTGTCGACGTGCG--CTTGTGGTGT
esesD1_861         ACT-GATGCTTCTCGTTTCAATCCCC-GCGAAGCCCTGTCGACGTGCG--CTTGTGGTGT
erotP12_1081       ACT-GACGCTTCTTGTTTCAATCCCC-GCGAAGCCCTGTCGACGTGCG--CTTGTGGCGT
esesD2_874         ACT-GATGCTTCTCGTTTCAATCCCC-GCGAAGCCCTGTCGACGTGCG--CTTGTGGTGT
ITO_elep           ACTTGATGCTTCTTGTTTCAATCCCC-GTGAAGCCCCGTCGACGTGTG--TTTGTGGTGT
esesP1_1075        ACT-GATGCTTCTCGTTTCAATCCCC-GCGAAGCCCTGTCGACGTGCG--CTTGTGGTGT
esesD3_884         ACT-GATGCTTCTCGTTTCAATCCCC-GCGAAGCCCTGTCGACGTGCG--CTTGTGGTGT
esesP6_871         ACT-GATGCTTCTCGTTTCAATCCCC-GCGAAGCCCTGTCGACGTGCG--CTTGTGGTGT
ITO_ejapo          ACT-GACGCTTCTTGTTTCAATGCCC-GTGAAGCCCTGTCGACGTGCG--CTCGTGGTGT
esesP4_847         ACT-GATGCTTCTCGTTTCAATCCCC-GCGAAGCCCTGTCGACGTGCG--CTTGTGGTGT
ITO_erot           ACT-GATGCTTCTTGTTTCAATCCCC-GCGAAGCCCTGTCGACGTGCG--CTTGTGGCGT
ITO_evar           ACT-GACGCTTCTTGTTTCAATGCCC-GTGAAGCCCTGTCGACGTGCG--CTCGTGGTGT
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esesD7_910        ACT-GATGCTTCTCGTTTCAATCCCC-GCGAAGCCCTGTCGACGTGCG--CTTGTGGTGT
ITO_ehys          ACT-GATGCTTCATGTTTCAATCCCC-GCGAAGCC-TGTCGGCGTGCG--CTTGTGGCGT
esesP3_843        ACT-GATGCTTCTCGTTTCAATCCCC-GCGAAGCCCTGTCGACGTGCG--CTTGTGGTGT
esesD4_897        ACT-GATGCTTCTCGTTTCAATCCCC-GCGAAGCCCTGTCGACGTGCG--CTTGTGGTGT
egod_819          ACT-GATGCTTCTCGTTTCAATCCCC-GCGAAGCCCTGTCGACGTGCG--CTTGTGGTGT
ecan_716          ACC-GAAGCTTCTTGTTTCAATCCCT-GTGAAGCCCTGTCGACGTGCG--CTTGTGGCGT
esesD5_902        ACT-GATGCTTCTCGTTTCAATCCCC-GCGAAGCCCTGTCGACGTGCG--CTTGTGGTGT
ITO_ealt          ACT-GATGCTTCTTGTTTCAATCCCC-GCGAAGCCTTGTCGACGTGCG--CTTGTGGTGT
egod_821          ACT-GATGCTTCTCGTTTCAATCCCC-GCGAAGCCCTGTCGACGTGCG--CTTGTGGTGT
egod_820          ACT-GATGCTTCTCGTTTCAATCCCC-GCGAAGCCCTGTCGACGTGCG--CTTGTGGTGT
epur_506          ATT-GATGCTTTTTGTTTCAAACCTC-GTGAAGCCCTGTCGACGTGTGTGTTTGTGGTGT
ITO_eserot        ACT-GATGCTTCTCGTTTCAATCCCC-GCGAAGCC-TGTCGACGTGCG--CTTGTGGTGT
ITO_esemi         ACT-GATGCTTCTCGTTTCAATCCCC-GCGAAGCC-TGTCGACGTGCG--CTTGTGGTGT
ITO_eses          ACT-GACGCTTCTTGTTTCAATCCCC-GCGAAGCCCTGTCGGCGTGCG--CATGTGGTGT
ITO_ecap          ACTTGATGCTTCTTGTTTCAATCCCC-GTGAAGCCCCGTCGACGTGTG--TTTGTGGTGT
ecan_598          ACC-GAANCTTCTTGTTTCAATCCCT-GTGAAGCCCTGTCGACGTGCG--CTTGTGGCGT
egod_1077         ACT-GATGCTTCTCGTTTCAATCCCC-GCGAAGCCCTGTCGACGTGCG--CTTGTGGTGT
esesP5_855        ACT-GATGCTTCTCGTTTCAATCCCC-GCGAAGCCCTGTCGACGTGCG--CTTGTGGTGT
ealb_702          ACC-GATGCTTCTTGTTTCAATCCCC-GCGAAGCCCTGTCNACGTGCGC--TTGTGGTGT
ITO_efist         ATT-GATGCTTTTGGTTTCAAACCTC-GTGAAGCCCTGTCGACGTGTGTGTTTGTGGTGT
ecap_712          ACTTGATGCTTCTTGTTTCAATCCCC-GTGAAGCCCCGTCNACGTGTG--TTTGTGGTGT
eper_465          ACT-GATGCTTCTTGTTTCAATCCCC-GCNAAGCCCTGTCNACGTGCG--CATGTGTTGT
erotD1_993        ACT-GATGCTTCTTGTTTCAATCCCC-GCGAAGCCCCGTCGACGTGCG--CTTGTGGCGT
ealt_711          ACT-GATGCTTCTTGTTTCAATCCCC-GCGAAGCCTTGTCGACGTGCG--CTTGTGGTGT
echi_715          ACT-GACGCTTCTTGTTTCAATGCCC-GTGAAGCCCTGTCGACGTGCG--CTCGTGGTGT
erotP1_1050       ACT-GATGCTTCTTGTTTCAATCCCC-GCGAAGCCCCGTCGACGTGCG--CTTGTGGCGT
erotP5_952        ACT-GATGCTTCTTGTTTCAATCCCC-GCGAAGCCCCGTCGACGTGCG--CTTGTGGCGT
ecun_701          GCT-GATNCTTCTTGTNTCAAACCCC-GCGAAGCCNTGTCAACGTGCG--CTTGT---GT
erotP6_953        ACT-GATGCTTCTTGTTTCAATCCCC-GCGAAGCCCCGTCGACGTGCG--CTTGTGGCGT
erotD4_1022       ACT-GATGCTTCTTGTTTCAATCCCC-GCGAAGCCCCGTCGACGTGCG--CTTGTGGCGT
erotP7_963        ACT-GATGCTTCTTGTTTCAATCCCC-GCGAAGCCCCGTCGACGTGCG--CTTGTGGCGT
erotD5_1028       ACT-GATGCTTCTTGTTTCAATCCCC-GCGAAGCCCCGTCGACGTGCG--CTTGTGGCGT
erotD6_1046       ACT-GATGCTTCTTGTTTCAATCCCC-GCGAAGCCCCGTCGACGTGCG--CTTGTGGCGT
erotP3_922        ACT-GATGCTTCTTGTTTCAATCCCC-GCGAAGCCCCGTCGACGTGCG--CTTGTGGCGT
ITO_ecan          ACC-GAAGCTTCTTGTTTCAATCCCT-GTGAAGCCCTGTCGACGTGCG--CTTGTGGCGT
ITO_edub          ATT-GATGCTTTTTGTTTCAAACCTC-GTGAAGCCCTGTCGACGTGTG--TGTTTGTGGT
                      **  **    **  **   *   *  *  **  ***  * ** *      *    *

ITO_emaki         GTCTT--T-CGGCACTCACGGCATCACGTT-GACGCAACAACAACCCCCGGCACAGCACG
ITO_elind         GTCTT--T-CGGCACTCACGGCATCACGTT-GACGCAACAACAACCCCCGGCACAGCACG
erotP2_1059       GTCTTTTT-CGGCACTCACGGCATCATGTT-GACGCAACAACAACCCCCGGCACAGCACG
erotP13_1088      GTCTTTTT-CGGCACTCACGGCATCATGTT-GACGCAACAACAACCCCCGGCACAGCACG
erotP9_979        GTCTTTTT-CGGCACTCACGGCATCATGTT-GACGCAACAACAACCCCCGGCACAGCACG
erotP8_972        GTCTTTTT-CGGCACTCACGGCATCATGTT-GACGCAACAACAACCCCCGGCACAGCACG
erotP10_982       GTCTTTTT-CGGCACTCACGGCATCATGTT-GACGCAACAACAACCCCCGGCACAGCACG
emac_532          CTCTNC-T-NGGNACTCACGGCATCACGTA-NACACAACAACAACCCCCGGCACAGCACG
erotD2_998        GCCTTTTT-CGGCACTCACGGCATCATGTT-GACGCAACAACAACCCCCGGCACAGCACG
emik_705          GTCTTTT--CGGCACTCACGGCATCACGGT-GACGCAACAACAACCCCCGGCACAGCACG
ITO_eform         GTCTTT---CGGCACTCACGGCATCACGTT-GACGCAACAACAACCCCCGGCACAGCACG
ITO_ecom          GTCTTTT--CGGCACTCGTGGCATTACGTT-GACGCAACAACCACCCCCGGCACAGCACG
ITO_etash         GTCTTT---CGGCACTCACGGCATCACGTT-GACGCAACAACAACCCCCGGCACAGCACG
egod_822          GTCTTTT--CGGCACTCACGGCATCAGGTT-GACGCAACAACAACCCCCGGCACAGCACG
erotD3_1015       GTCTTTTT-CGGCACTCACGGCATCATGTT-GACGCAACAACAACCCCCGGCACAGCACG
echi713           GTCTTT---CGGCACTCACGGCNTCACGTT-GACGCAACAACAACCCCCGGCACAGCACG
ITO_egleh         GTCTTT---CGGCACTCACGGCATCACGTT-GACGCAACAACAACCCCCGGCACAGCACG
erotP11_992       GTCTTTTT-CGGCACTCACGGCATAACGTT-GACGCAACAACAACCCCCGGCACAGCACG
erotP4_934        GCCTTTTT-CGGCACTCACGGCATCATGTT-GACGCAACAACAACCCCCGGCACAGCACG
echi531           GTCTT--T-CGGCACTCACGGCATCACGTT-GACGCAACAACAACCCCCGGCACAGCACG
ecap_460          GTCTTT-T-CGGCACTCGTGGCATTACGTTTGACGCAACAACCACCCCCGGCACAGCACG
esesD8_1096       GTCTTT-T-CGGCACTCACGGCATCAGGTT-GACGCAACAACAACCCCCGGCACAGCACG
eleu_703          GTCTTTTN-CGGCNCTCNCGGCGTCACGGA-GACNCAACAACNNCCCCCGGCACAGCNCG
erot_706          GTCTTTTT-CGGCACTCACGGCATCNTGTT-NACGCAACAACNNCCCCCGGCACAGCACG
ITO_emik          GTCTTTT--CGGCACTCACGGCATCACGGT-GACGCAACAACAACCCCCGGCACAGCACG
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esesD6_908        GTCTTTT--CGGCACTCACGGCATCAGGTT-GACGCAACAACAACCCCCGGCACAGCACG
eses_710          GTCTTTTC-GG-CACTCACGGCATCAGGTT-GACGCAACAACAACCCCCGGCACAGCACG
ehys_709          NTCTTTTT-GGGCACTCACGGCATCNCNTN-GACNCAACAACAACCCCCGGCACAGCAGG
esesP7_917        GTCTTTTC-GG-CACTCACGGCATCAGGTT-GACGCAACAACAACCCCCGGCACAGCACG
esesP2_824        GTCTTTT--CGGCACTCACGGCATCAGGTT-GACGCAACAACAACCCCCGGCACAGCACG
esesD1_861        GTCTTTT--CGGCACTCACGGCATCAGGTT-GACGCAACAACAACCCCCGGCACAGCACG
erotP12_1081      GTCTTTTT-CGGCACTCACGGCATCATGTT-GACGCAACAACAACCCCCGGCACAGCACG
esesD2_874        GTCTTTT--CGGCACTCACGGCATCAGGTT-GACGCAACAACAACCCCCGGCACAGCACG
ITO_elep          GTCTTTT--CGGCACTCGTGGCATTACGTT-GACGCAACAACCACCCCCGGCACAGCACG
esesP1_1075       GTCTTTT--CGGCACTCACGGCATCAGGTT-GACGCAACAACAACCCCCGGCACAGCACG
esesD3_884        GTCTTTT--CGGCACTCACGGCATCAGGTT-GACGCAACAACAACCCCCGGCACAGCACG
esesP6_871        GTCTTTT--CGGCACTCACGGCATCAGGTT-GACGCAACAACAACCCCCGGCACAGCACG
ITO_ejapo         GTCTTT---CGGCACTCACGGAATCACGTT-GACGCCACAACAACCCCCGGCACAGCACG
esesP4_847        GTCTTTT--CGGCACTCACGGCATCAGGTT-GACGCAACAACAACCCCCGGCACAGCACG
ITO_erot          GTCTTTTT-CGGCACTCACGGCATCATGTT-GACGCAACAACAACCCCCGGCACAGCACG
ITO_evar          GTCTTT---CGGCACTCACGGCATCACGTT-GACGCAACAACAACCCCCGGCACAGCACG
esesD7_910        GTCTTTT--CGGCACTCACGGCATCAGGTT-GACGCAACAACAACCCCCGGCACAGCACG
ITO_ehys          GTCTTTTTTGGGCACTCACGGCATCACGTT-GACGCAACAACAACCCCCGGCACAGCACG
esesP3_843        GTCTTTTC--GGCACTCACGGCATCAGGTT-GACGCAACAACAACCCCCGGCACAGCACG
esesD4_897        GTCTTTTC--GGCACTCACGGCATCAGGTT-GACGCAACAACAACCCCCGGCACAGCACG
egod_819          GTCTTTTT--CGGCACTCACGGCATCAGGTT-GACGCAACAACAACCCCCGGCACAGCACG
ecan_716          GTCTTTT--CGGCACTCACGGCATCACGTT-GACGCAACAACAACCCCCGGCACAGCACG
esesD5_902        GTCTTTT--CGGCACTCACGGCATCAGGTT-GACGCAACAACAACCCCCGGCACAGCACG
ITO_ealt          GTCTTTTC--GGCGCTCACGGCGTCACGTT-GACGCAACAACAACCCCCGGCACAGCACG
egod_821          GTCTTTTC--GGCACTCACGGCATCAGGTT-GACGCAACAACAACCCCCGGCACAGCACG
egod_820          GTCTTTTC--GGCACTCACGGCATCAGGTT-GACGCAACAACAACCCCCGGCACAGCACG
epur_506          CTCTTCTT--GGCACTCACGGCATCACGTA-AACGCAACAACAACCCCCGGCACAGCACG
ITO_eserot        GTCTTTTT-CGGCACTCACGGCATCACGTT-GACGCAACAACAACCCCCGGCACAGCACG
ITO_esemi         GTCTTTTC--GGCACTCACGGCATCAGGTT-GACGCAACAACAACCCCCGGCACAGCACG
ITO_eses          GTCTTTTC--GGCACTCACGGCATAACGTT-GACGCAACAACAACCCCCGGCACAGCACG
ITO_ecap          GTCTTT-C--GGCACTCGTGGCATTACGTT-GACGCAACAACCACCCCCGGCACAGCACG
ecan_598          GTCTTTTC--GGCACTCACGGCATCACGTT-GACGCAACAACAACCCCCGGCACAGCACG
egod_1077         GTCTTTTC--GGCACTCACGGCATCAGGTT-GACGCAACAACAACCCCCGGCACAGCACG
esesP5_855        GTCTTTTC--GGCACTCACGGCATCAGGTT-GACGCAACAACAACCCCCGGCACAGCACG
ealb_702          GTCTTTTC--GGCACTCACGGCATCACGTT-GACGCAACAACCACCCCCGGCACAGCACG
ITO_efist         CTCTTCTT--GGCACTCACGGCATCACGTA-GACGCAACAACAACCCCCGGCACAGCACG
ecap_712          GTCTTTT--CGGCACTCGTGGCATTACGTT-GACGCAACAACCACCCCCGGCACAGCACG
eper_465          GTCTTTT--CGGCACTCACGGCATCACGTT-GACGCAACAACAACCCCCGGCACAGCACG
erotD1_993        GTCTTTT-TCGGCACTCACGGCATCATGTT-GACGCAACAACAACCCCCGGCACAGCACG
ealt_711          GTCTTTT--CGGCGCTCACGGCGTCACGTT-GACGCAACAACAACCCCCGGCACAGCACG
echi_715          GTCTTT---CGGCACTCACGGCATCACGTT-GACGCAACAACAACCCCCGGCACAGCACG
erotP1_1050       GCCTTTT-TCGGCACTCACGGCATCATGTT-GACGCAACAACAACCCCCGGCACAGCACG
erotP5_952        GTCTTTT-TCGGCACTCACGGCATCATGTT-GACGCAACAACAACCCCCGGCACAGCACG
ecun_701          NTCTNTT-NCGGCNCTCANGGNATCNCGTT-GACNCAACAACAACCCCCGGCACAGCACG
erotP6_953        GTCTTTT-TCGGCACTCACGGCATCATGTT-GACGCAACAACAACCCCCGGCACAGCACG
erotD4_1022       GTCTTTT-TCGGCACTCACGGCATCATGTT-GACGCAACAACAACCCCCGGCACAGCACG
erotP7_963        GTCTTTT-TCGGCACTCACGGCATCATGTT-GACGCAACAACAACCCCCGGCACAGCACG
erotD5_1028       GTCTTTT-TCGGCACTCACGGCATCATGTT-GACGCAACAACAACCCCCGGCACAGCACG
erotD6_1046       GTCTTTT-TCGGCACTCACGGCATCATGTT-GACGCAACAACAACCCCCGGCACAGCACG
erotP3_922        GTCTTTT-TCGGCACTCACGGCATCATGTT-GACGCAACAACAACCCCCGGCACAGCACG
ITO_ecan          GTCTT---TCGGCACTCACGGCATCACGTT-GACGCAACAACAACCCCCGGCACAGCACG
ITO_edub          GTCTCTTCTTGGCACTCACGGCATCACGTA-GACGCAACAACAACCCCCGGCACAGCACG
                    **      *   ***  **  *        ** * *****  *************  *

ITO_emaki         TGCCAAGGAAAACCAAACATAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
ITO_elind         TGCCAAGGAAAACCAAACATAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
erotP2_1059       TGCCAAGGAAAACCAAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
erotP13_1088      TGCCAAGGAAAACCAAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
erotP9_979        TGCCAAGGAAAACCAAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
erotP8_972        TGCCAAGGAAAACCAAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
erotP10_982       TGCCAAGGAAAACCAAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
emac_532          TGCCAAGGAAAACCAAACTTAAGAGTGCCTGTGCCATGATG-CCCTCGTATTAGGTGTGT
erotD2_998        TGCCAAGGAAAACCAAACTTAAGAGCGCCCGTGCCAAGACG-CCC-CGTGCTAGGTGTGT
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emik_705          TGCCAAGGAAAACCAAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGCGTGT
ITO_eform         TGCCAAGGAAAACCAAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
ITO_ecom          TGCCAAGGAAAACCAAACTTAAGAGCGTCCGTGCCATGAGG-CCC-CGTGCTAGGTGTGT
ITO_etash         TGCCAAGGAAAACCAAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
egod_822          TGCCAAGGAAAACCGAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
erotD3_1015       TGCCAAGGAAAACCAAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
echi713           TGCCAAGGAAAACCAAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
ITO_egleh         TGCCAAGGAAAACCAAACTTAAGAGCGCCCGTGCCATGACG-CCT-CGTGCTAGGTGTGT
erotP11_992       TGCCAAGGAAAACCAAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
erotP4_934        TGCCAAGGAAAACCAAACTTAAGAGCGCCCGTGCCATGACG--CCCCGTGCTAGGTGTGT
echi531           TGCCAAGGAAAACCAAACTTAAGAGCGCCCGTGCCATGACG--CCCCGTGCTAGGTGTGT
ecap_460          TGCCAAGGAAAACCAAACTTAAAA-CGTCCGTGCCATGAAGGCCCCCGTGCTAGGTNTGT
esesD8_1096       TGCCAAGGAAAACCGAACTTAAGAGCGCCCGTGCCATGACG--CCCCGTGCTAGGTGTGT
eleu_703          TGCCGAGGAAAACNAAACTTAAGANCGCCCGTGCCATGACC-CCC-CGTGCNAGGCGTGT
erot_706          TGCCAAGGAAAACCAAACTTAAGAGCGCCCGTGCCATGACN-CCC-CGTGCTAGGTGTGT
ITO_emik          TGCCAAGGAAAACCAAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGCGTGT
esesD6_908        TGCCAAGGAAAACCGAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
eses_710          TGCCAAGGAAAACCGAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
ehys_709          TGCCAGGGAAAACAAAACTTAAGAGCGCCCGTGCCANGACN-CCC-CGTGCTNNGCGTGT
esesP7_917        TGCCAAGGAAAACCGAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
esesP2_824        TGCCAAGGAAAACCGAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
esesD1_861        TGCCAAGGAAAACCGAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
erotP12_1081      TGCCAAGGAAAACCAAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
esesD2_874        TGCCAAGGAAAACCGAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
ITO_elep          TGCCAAGGAAAACCAAACTTAAGAGCGTCCGTGCCATGAGG-CCC-CGTGCTAGGTGTGT
esesP1_1075       TGCCAAGGAAAACCGAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
esesD3_884        TGCCAAGGAAAACCGAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
esesP6_871        TGCCAAGGAAAACCGAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
ITO_ejapo         TGTCAAGGAAAAGCAAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
esesP4_847        TGCCAAGGAAAACCGAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
ITO_erot          TGCCAAGGAAAACCAAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
ITO_evar          TGCCAAGGAAAACCAAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
esesD7_910        TGCCAAGGAAAACCGAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
ITO_ehys          TGCCAAGGAAAACAAAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGCGTGT
esesP3_843        TGCCAAGGAAAACCGAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
esesD4_897        TGCCAAGGAAAACCGAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
egod_819          TGCCAAGGAAAACCGAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
ecan_716          TGCCAAGGAAAACCAAACTTAAAGACGTCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
esesD5_902        TGCCAAGGAAAACCGAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
ITO_ealt          TGCCAAGGAAAACTAAACTTAAGAGCGCCGGTGCCATGACG-CCC-CGTGCTAGGTGTGT
egod_821          TGCCAAGGAAAACCGAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
egod_820          TGCCAAGGAAAACCGAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
epur_506          TGCCAAGGAAAACCAAACTTAAGAGTGCCTGTGCCATGATG-CCCTCGTATTAGGTGTGT
ITO_eserot        TGCCAAGGAAAACCTAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
ITO_esemi         TGCCAAGGAAAACCGAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
ITO_eses          TGCCAAGGAAAACCAAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
ITO_ecap          TGCCAAGGAAAACCAAACTTAAGAGCGTCCGTGCCATGAGG-CCC-CGTGCTAGGTGTGT
ecan_598          TGCCAAGGAAAACCAAACTTAAGAGCGTCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
egod_1077         TGCCAAGGAAAACCGAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
esesP5_855        TGCCAAGGAAAACCGAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
ealb_702          TGCCAAGGAAAACCAAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
ITO_efist         TGCCAAGGAAAACCAAACTTAAGAGTGCCTGTGCCATGATG-CCCTCGTATTAGGTGTGT
ecap_712          TGCCAAGGAAAACCAAACTTAAGAGCGTCCGTGCCATGAGG-CCC-CGTGCTAGGTGTGT
eper_465          TGCCAAGGAAAACCAAACTTAAGAGCGCCCGTGCCANGACG-CCC-CGTGCTAGGCGTGT
erotD1_993        TGCCAAGGAAAACCAAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
ealt_711          TGCCAAGGAAAACTAAACTTAAGAGCGCCGGTGCCATGACN-CCC-CGTGCTAGGTGTGT
echi_715          TGCCAAGGAAAACCAAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
erotP1_1050       TGCCAAGGAAAACCAAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
erotP5_952        TGCCAAGGAAAACCAAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
ecun_701          TGCCNAGGAAAACCAAACTTAAGAGCGCCCGTGCCATGACN-CCC-CGTGCTAGGTGTGT
erotP6_953        TGCCAAGGAAAACCAAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
erotD4_1022       TGCCAAGGAAAACCAAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
erotP7_963        TGCCAAGGAAAACCAAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
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erotD5_1028       TGCCAAGGAAAACCAAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
erotD6_1046       TGCCAAGGAAAACCAAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
erotP3_922        TGCCAAGGAAAACCAAACTTAAGAGCGCCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
ITO_ecan          TGCCAAGGAAAACCAAACTTAAGAGCGTCCGTGCCATGACG-CCC-CGTGCTAGGTGTGT
ITO_edub          TGCCAAGGAAAACCAAACTTAAGAGTGCCTGTGCCATGATG-CCCTCGTATTAGGTGTGT
                  ** *  ******   *** ***    * * ****** **    *  ***     *  ***

ITO_emaki         TCATGGTACGCGGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
ITO_elind         TCATGGTACGCGGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
erotP2_1059       TCATGGTATGCGGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
erotP13_1088      TCATGGTATGCGGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
erotP9_979        TCATGGTATGCGGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
erotP8_972        TCATGGTATGCGGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
erotP10_982       TCATGGTATGCGGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
emac_532          TCATTGTATGTGGCTTCTTTGTAATTCTNATCACG-------TCGCCCACACCAAACA--
erotD2_998        TCATGGTATGCGGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
emik_705          TCATGGTACGCGGCTTCTTTGTAATTCTNATCACG-------TCGCCCACATCAAACA--
ITO_eform         TCATGGTATGCGGCTTCTTTGTAATTCTAATCACG-------TCGCCCACACCAAACA--
ITO_ecom          TCATGGTATGCGGCTTCTTATTAATTCTAATCACG-------TCGCCCACATCAAACA--
ITO_etash         TCATGGTATGCGGCTTCTTTGTAATTCTAATCACG-------TCGCCCACACCAAACA--
egod_822          TCATGGTACGCTGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
erotD3_1015       TCATGGTATGCGGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
echi713           TCATGGTATGCGGCTTCTTTGTAATTCTNATCACG-------TCGCCCACACCAAACA--
ITO_egleh         TCATGGTATGCGGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
erotP11_992       TCATGGTATGCGGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
erotP4_934        TCATGGTATGCGGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
echi531           TCATGGTATGCGGCTTCTTTGTAATTCTNATCACG-------TCGCCCACACCAAACA--
ecap_460          TTCATGTATGCGGCTNCTTATTAATTCTNATCACG-------TCGCCCACATCAAACA--
esesD8_1096       TCATGGTACGCTGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
eleu_703          TCATGGGATGCGGCTTCTTTTTAATTCTNATCACG-------TCGCCCACATCAAACA--
erot_706          NCATGGTATGCGGCTTCTTTGTAATTCTNATCACG-------TCGCCCACATCNAACA--
ITO_emik          TCATGGTACGCGGCTTCTTTGTAATTCTAATCACGCATCACGTCGCCCACATCAAACA--
esesD6_908        TCATGGTACGCTGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
eses_710          TCATGGTACGCTGCTTCTTTGTAATTCTNATCACG-------TCGCCCACATCAAACA--
ehys_709          TCATGGGACGCGGCTTCTTTTTAATTCTNATCACG-------TCGCCCACATCAAACA--
esesP7_917        TCATGGTACGCTGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
esesP2_824        TCATGGTACGCTGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
esesD1_861        TCATGGTACGCTGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
erotP12_1081      TCATGGTATGCGGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
esesD2_874        TCATGGTACGCTGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
ITO_elep          TCATGGTATGCGGCTTCTTATTAATTCTAATCACG-------TCGCCCACATCAAACA--
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esesP6_871        TCATGGTACGCTGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
ITO_ejapo         TCATGGTATGCGGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
esesP4_847        TCATGGTACGCTGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
ITO_erot          TCATGGTATGCGGCTTCTTTGTAATTCTAATCACG-------TCGCCCA-ATCAAACA--
ITO_evar          TCATGGTATGCGGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
esesD7_910        TCATGGTACGCTGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
ITO_ehys          TCATGGGACGCGGCTTCTTTTTAATTCTAATCACGCATCACGTCGCCCACATCAAACA--
esesP3_843        TCATGGTACGCTGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
esesD4_897        TCATGGTACGCTGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
egod_819          TCATGGTACGCTGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
ecan_716          TCGTGGTANGCGGCTTCTTTGTAATTCTNATCACG-------TCGCCCACATCAAACA--
esesD5_902        TCATGGTACGCTGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
ITO_ealt          TCATGGTATGCGGCTTCTATGTAATTCTGATCACGCATCACGTCGCCCACATCAAACA--
egod_821          TCATGGTACGCTGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
egod_820          TCATGGTACGCTGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
epur_506          TCATTGTATGTGGCTTCTTTGTAATTCTNATCACG-------TCGCCCACATCAAACA--
ITO_eserot        TCATGGTATGCGGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
ITO_esemi         TCATGGTACGCTGCTTCTTTGTAATTCTAATCACGCATCACGTCGCCCACATCAAACA--
ITO_eses          TCATGGTATGCGGCTTCTTTGCAATTCTAATCACG-------TCGCCCACATCAAACAAC
ITO_ecap          TCATGGTATGCGGCTTCTTATTAATTCTAATCACG-------TCGCCCACATCAAACA--
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ecan_598          TCGTGGTATGCGGCTTCTTTGTAATTCTNATCACG-------TCGCCCACATCAAACA--
egod_1077         TCATGGTACGCTGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
esesP5_855        TCATGGTACGCTGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
ealb_702          TCGTGGTATGCGGCTTCTTTGTAATTCTNATCACG-------TCGCCCACATCAAACA--
ITO_efist         TCATTGTATGTGGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
ecap_712          TCATGGTATGCGGCTTCTTATTAATTCTNATCACG-------TCGCCCACATCAAACA--
eper_465          TCATGGTACGCGGCTTCTTTGTAATTCTNATCANG-------TCGCCCACATCAAACA--
erotD1_993        TCATGGTATGCGGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
ealt_711          TCATGGTATGCGGCTTCTATGTAATTCTNATCACG-------TCGCCCACATCAAACA--
echi_715          TCATGGTATGCGGCTTCTTTGTAATTCTNATCACG-------TCGCCCACATCAAACA--
erotP1_1050       TCATGGTATGCGGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
erotP5_952        TCATGGTATGCGGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
ecun_701          TCATGGTATGCGGCTTCTTTGTAATTCTNATCACG-------TCGCCCACATCACACA--
erotP6_953        TCATGGTATGCGGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
erotD4_1022       TCATGGTATGCGGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
erotP7_963        TCATGGTATGCGGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
erotD5_1028       TCATGGTATGCGGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
erotD6_1046       TCATGGTATGCGGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
erotP3_922        TCATGGTATGCGGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
ITO_ecan          TCGTGGTATGCGGCTTCTTTGTAATTCTAATCACG-------TCGCCCACATCAAACA--
ITO_edub          TCATTGTATGTGGCTTCTTTGTAATTCTAATCACG-------TCGCCCACACCAAACA--
                       * * *  *** **    ****** ***          ******* * *  ***

ITO_emaki         -TCCATG-----------ACGTATGTGGGCGGAGACTGGTCTCCCGTGCCCAT--GGTG-
ITO_elind         -TCCATG-----------ACGTATGTGGGCGGAGACTGGTCTCCCGTGCCCAT--GGTG-
erotP2_1059       -TCCATG-----------ATGTATGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
erotP13_1088      -TCCATG-----------ATGTATGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
erotP9_979        -TCCATG-----------ATGTATGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
erotP8_972        -TCCATG-----------ATGTATGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
erotP10_982       -TCCATG-----------ATGTATGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
emac_532          -TCCTTGTTTGGANTCCAATGTGTGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTGG
erotD2_998        -TCCATG-----------ATGTATGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
emik_705          -TCCATG-----------ATGCATGTGGGCGGAGNCTGGTCTCCTGTGCCCGT--GGCG-
ITO_eform         -TCCATG-----------ATGTATGTGGGCGGAGACTGGTCTCCCGTGCCCAT--GGCG-
ITO_ecom          -TCCACG-----------TTGTATGTGGGCGGAGACTGGTCTCCTGTGCCCTT--GGCG-
ITO_etash         -TCCATG-----------ATGTATGTGGGCGGAGACTGGTCTCCCGTGCCCAT--GGCG-
egod_822          -TCCATG-----------ATGTGTGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
erotD3_1015       -TCCATG-----------ATGTATGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
echi713           -TCCATG-----------ATGTATGTGGGCGGAGACTGGTCTCCCGTGCCCAT--GGCG-
ITO_egleh         -TCCATG-----------ATGTATGTGGGCGGAGACTGGTCTCCCGTGCCCAT--GGTG-
erotP11_992       -TCCATG-----------ATGTATGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
erotP4_934        -TCCATG-----------ATGTATGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
echi531           -TCCATG-----------ATGTATGTGGGCGGAGACTGGTCTCCCGTGCCCAT--GGCG-
ecap_460          -TCCACG-----------TTGTATGTGGGCGGAGACTGGTCTCCTGTGCCCTT--GGCG-
esesD8_1096       -TCCATG-----------ATGTGTGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
eleu_703          -TCCATG-----------ATGTATGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
erot_706          -TCCATG-----------ATGTATGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
ITO_emik          -TCCATG-----------ATGCATGTGGGCGGAGGCTGGTCTCCTGTGCCCGT--GGCG-
esesD6_908        -TCCATG-----------ATGTGTGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
eses_710          -TCCATG-----------ATGTGTGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
ehys_709          -TCCATG-----------ATGTGTGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
esesP7_917        -TCCATG-----------ATGTGTGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
esesP2_824        -TCCATG-----------ATGTGTGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
esesD1_861        -TCCATG-----------ATGTGTGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
erotP12_1081      -TCCATG-----------ATGTATGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
esesD2_874        -TCCATG-----------ATGTGTGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
ITO_elep          -TCCACG-----------TTGTATGTGGGCGGAGACTGGTCTCCTGTGCCCTT--GGCG-
esesP1_1075       -TCCATG-----------ATGTGTGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
esesD3_884        -TCCATG-----------ATGTGTGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
esesP6_871        -TCCATG-----------ATGTGTGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
ITO_ejapo         -TCCATG-----------ATGCATGTGGGCGGAGACTGGTCTCCCGTGCCCAT--GGCG-
esesP4_847        -TCCATG-----------ATGTGTGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
ITO_erot          -TCCATG-----------ATGTATGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
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ITO_evar          -TCCATG-----------ACGTATGTGGGCGGAGACTGGTCTCCCGTGCCCAT--GGTG-
esesD7_910        -TCCATG-----------ATGTGTGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
ITO_ehys          -TCCATG-----------ATGTGTGTGGGCGGAGACTGGTCTCCCGTGCCCAT--GGTG-
esesP3_843        -TCCATG-----------ATGTGTGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
esesD4_897        -TCCATG-----------ATGTGTGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
egod_819          -TCCATG-----------ATGTGTGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
ecan_716          -TCCATG-----------ATGTATGTGGGCGGAGACTGGTCTCCCGTGCCCAT--GGTG-
esesD5_902        -TCCATG-----------ATGTGTGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
ITO_ealt          -TCCATG-----------ATGTATGTGGGCGGAGACTGGTCTCCCGTGCCCATACGGTG-
egod_821          -TCCATG-----------ATGTGTGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
egod_820          -TCCATG-----------ATGTGTGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
epur_506          -TCCTTGTTTGGATTCCAATGTGTGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTGG
ITO_eserot        -TCCATG-----------ATGTATGTGGGCGGAGACTGGTCTCCTGTGCCCAC--GGTG-
ITO_esemi         -TCCATG-----------ATGTGTGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
ITO_eses          ATCCATG-----------ATGTATGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
ITO_ecap          -TCCACG-----------TTGTATGTGGGCGGAGACTGGTCTCCTGTGCCCTT--GGCG-
ecan_598          -TCCATG-----------ATGTATGTGGGCGGAGACTGGTCTCCCGTGCCCAT--GGTG-
egod_1077         -TCCATG-----------ATGTGTGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
esesP5_855        -TCCATG-----------ATGTGTGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
ealb_702          -TCCATG-----------ATGTATGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
ITO_efist         -TCCTTGTTTGGATTCCAATGTGTGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTGG
ecap_712          -TCCACG-----------TTGTATGTGGGCGGAGACTGGTCTCCTGTGCCCTT--GGCG-
eper_465          -TCGATG-----------ATGTATGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
erotD1_993        -TCCATG-----------ATGTATGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
ealt_711          -TCCATG-----------ATGTATGTGGGCGGAGACTGGTCTCCCGTGCCCATACGGTG-
echi_715          -TCCATG-----------ATGTATGTGGGCGGAGACTGGTCTCCCGTGCCCAT--GGTG-
erotP1_1050       -TCCATG-----------ATGTATGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
erotP5_952        -TCCATG-----------ATGTATGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
ecun_701          -TCCATG-----------TTGTATGTGGGCGGNGACTGGTCTCCNGNGCCCAT--GNTG-
erotP6_953        -TCCATG-----------ATGTATGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
erotD4_1022       -TCCATG-----------ATGTATGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
erotP7_963        -TCCATG-----------ATGTATGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
erotD5_1028       -TCCATG-----------ATGTATGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
erotD6_1046       -TCCATG-----------ATGTATGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
erotP3_922        -TCCATG-----------ATGTATGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTG-
ITO_ecan          -TCCATG-----------ATGTATGTGGGCGGAGACTGGTCTCCCGTGCCCAT--GGTG-
ITO_edub          -TCCTTGTTTGGATTCCAATGTGTGTGGGCGGAGACTGGTCTCCTGTGCCCAT--GGTGG
                   **   *             *  ********* * ********* * ****    *  *

ITO_emaki         --CGGTTGGCCCAAATACGAGTCCGCTTAAGAGTGACGCACGACTGGTGGTGGTTGATTA
ITO_elind         --CGGTTGGCCCAAATACGAGTCCGCTTAAGAGTGACGCACGACTGGTGGTGGTTGATTA
erotP2_1059       --CGGTTGGCCCAAATACGAGTCCGCTTAAGAGCGACGCACGACTGGTGGTGGTTGATTA
erotP13_1088      --CGGTTGGCCCAAATACGAGTCCGCTTAAGAGCGACGCACGACTGGTGGTGGTTGATTA
erotP9_979        --CGGTTGGCCCAAATACGAGTCCGCTTAAGAGCGACGCACGACTGGTGGTGGTTGATTA
erotP8_972        --CGGTTGGCCCAAATACGAGTCCGCTTAAGAGCGACGCACGACTGGTGGTGGTTGATTA
erotP10_982       --CGGTTGGCCCAAATACGAGTCCGCTTAAGAGCGACGCACGACTGGTGGTGGTTGATTA
emac_532          TGCGGTTGGCCCAAATATGAGTCCGCTTAAGGGTGACGCACGACTGGTGGTGGTTGATTA
erotD2_998        --CGGTTGGCCCAAATACGAGTCCGCTTAAGAGCGACGCACGACTGGTGGTGGTTGATTA
emik_705          --CGGTTGGCCCAAATAAGAGTCCGCTTAAGAGTGACGCACGACTGGTGGTGGTTGATTA
ITO_eform         --CGGTTGGCCCAAATACGAGTCCGCTTAAGAGTGACGCACGACTGGTGGTGGTTGATTA
ITO_ecom          --CGGTTGGCCCAAATATGAGTCCGCTTAAGAGTGACGCACGACTGGTGGTGGTTGATTA
ITO_etash         --CGGTTGGCCCAAATACGAGTCCGCTTAAGAGTGACGCACGACTGGTGGTGGTTGATTA
egod_822          --CGGTTGGCCCAAATAAGAGTCCGCTTAAGAGTGACGCACGGCTGGTGGTGGTTGATTA
erotD3_1015       --CGGTTGGCCCAAATACGAGTCCGCTTAAGAGCGACGCACGACTGGTGGTGGTTGATTA
echi713           --CGGTTGGCCCAAATACGAGTCCGCTTAAGAGTGACGCACGACTGGTGGTGGTTGATTA
ITO_egleh         --CGGTTGGCCCAAATACGAGTCCGCTTAAGAGTGACGCACGACTGGTGGTGGTTGATTA
erotP11_992       --CGGTTGGCCCAAATACGAGCCCGCTTAAGAGCGACGCACGACTGGTGGTGGTTGATTA
erotP4_934        --CGGTTGGCCCAAATACGAGTCCGCTTAAGAGCGACGCACGACTGGTGGTGGTTGATTA
echi531           --CGGTTGGCCCAAATACGAGTCCGCTTAAGAGTGACGCACGACTGGTGGTGGTTGATTA
ecap_460          --CGGTTGGCCCAAATATGAGTCCGCTTAAGAGTGACGCACGACTGGTGGTGGTTGATTA
esesD8_1096       --CGGTTGGCCCAAATAAGAGTCCGCTTAAGAGTGACGCACGGCTGGTGGTGGTTGATTA
eleu_703          --CGGTTGGCCCAAATACGAGTCCGCTTAAGAGTGACGCACGACTGGTGGTGGTTGATTA
erot_706          --CGGTTGGCCCAAATACGAGTCCGCNNAAGAGCGACGCACGACTGGTGGTGGTTGATTA
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ITO_emik          --CGGTTGGCCCAAATAAGAGTCCGCTTAAGAGTGACGCACGACTGGTGGTGGTTGATTA
esesD6_908        --CGGTTGGCCCAAATAAGAGTCCGCTTAAGAGTGACGCACGGCTGGTGGTGGTTGATTA
eses_710          --CGGTTGGCCCAAATAAGAGTCCGCTTAAGAGTGACGCACGGCTGGTGGTGGTTGATTA
ehys_709          --CGGTTGGCCCAAATATGAGTCTGCTTAAGAGTGACGCACGACTGGTGGTGGTTGATTA
esesP7_917        --CGGTTGGCCCAAATAAGAGTCCGCTTAAGAGTGACGCACGGCTGGTGGTGGTTGATTA
esesP2_824        --CGGTTGGCCCAAATAAGAGTCCGCTTAAGAGTGACGCACGGCTGGTGGTGGTTGATTA
esesD1_861        --CGGTTGGCCCAAATAAGAGTCCGCTTAAGAGTGACGCACGGCTGGTGGTGGTTGATTA
erotP12_1081      --CGGTTGGCCCAAATACGAGTCCGCTTAAGAGCGACGCACGACTGGTGGTGGTTGATTA
esesD2_874        --CGGTTGGCCCAAATAAGAGTCCGCTTAAGAGTGACGCACGGCTGGTGGTGGTTGATTA
ITO_elep          --CGGTTGGCCCAAATATGAGTCCGCTTAAGAGTGACGCACGACTGGTGGTGGTTGATTA
esesP1_1075       --CGGTTGGCCCAAATAAGAGTCCGCTTAAGAGTGACGCACGGCTGGTGGTGGTTGATTA
esesD3_884        --CGGTTGGCCCAAATAAGAGTCCGCTTAAGAGTGACGCACGGCTGGTGGTGGTTGATTA
esesP6_871        --CGGTTGGCCCAAATAAGAGTCCGCTTAAGAGTGACGCACGGCTGGTGGTGGTTGATTA
ITO_ejapo         --CGGTTGGCCCAAATACGAGTCCGCTTAAGAGTGACGCACGACTGGTGGTGGTTGATTA
esesP4_847        --CGGTTGGCCCAAATAAGAGTCCGCTTAAGAGTGACGCACGGCTGGTGGTGGTTGATTA
ITO_erot          --CGGTTGGCCCAAATACGAGTCCGCTTAAGAGCGACGCACGACTGGTGGTGGTTGATTA
ITO_evar          --CGGTTGGCCCAAATACGAGTCCGCTTAAGAGTGACGCACGACTGGTGGTGGTTGATTA
esesD7_910        --CGGTTGGCCCAAATAAGAGTCCGCTTAAGAGTGACGCACGGCTGGTGGTGGTTGATTA
ITO_ehys          --CGGTTGGCCCAAATATGAGTCTGCTTAAGAGTGACGCACGACTGGTGGTGGTTGATTA
esesP3_843        --CGGTTGGCCCAAATAAGAGTCCGCTTAAGAGTGACGCACGGCTGGTGGTGGTTGATTA
esesD4_897        --CGGTTGGCCCAAATAAGAGTCCGCTTAAGAGTGACGCACGGCTGGTGGTGGTTGATTA
egod_819          --CGGTTGGCCCAAATAAGAGTCCGCTTAAGAGTGACGCACGGCTGGTGGTGGTTGATTA
ecan_716          --CGGTTGGCCCAAATACGAGTCCGTTTAAGGGTGACGCACGACTGGTGGTGGTTGATTA
esesD5_902        --CGGTTGGCCCAAATAAGAGTCCGCTTAAGAGTGACGCACGGCTGGTGGTGGTTGATTA
ITO_ealt          --CGGTTGGCCCAAATACGAGTCCGCTTAAGAGTGACGCACGACTGGTGGTGGTTGATTA
egod_821          --CGGTTGGCCCAAATAAGAGTCCGCTTAAGAGTGACGCACGGCTGGTGGTGGTTGATTA
egod_820          --CGGTTGGCCCAAATAAGAGTCCGCTTAAGAGTGACGCACGGCTGGTGGTGGTTGATTA
epur_506          TGCGGTTGGCCCAAATANGAGTCCGCTTAAGAGTGACGCACGACTGGTGGTGGTTGATTA
ITO_eserot        --CGGTTGGCCCAAATANGAGTCCGCTTAAGAGTGACGCACGACTGGTGGTGGTTGCTTA
ITO_esemi         --CGGTTGGCCCAAATAAGAGTCCGCTTAAGAGTGACGCACGGCTGGTGGTGGTTGATTA
ITO_eses          --CGGTTGGCCCAAATATGAGTCCGCTCAAGAGTGACGCACGACTGGTGGTGGTTGATTA
ITO_ecap          --CGGTTGGCCCAAATATGAGTCCGCTTAAGAGTGACGCACGACTGGTGGTGGTTGATTA
ecan_598          --CGGTTGGCCCAAATACGAGTCCGTTTAAGGGTGACGCACGACTGGTGGTGGTTGATTA
egod_1077         --CGGTTGGCCCAAATAAGAGTCCGCTTAAGAGTGACGCACGGCTGGTGGTGGTTGATTA
esesP5_855        --CGGTTGGCCCAAATAAGAGTCCGCTTAAGAGTGACGCACGGCTGGTGGTGGTTGATTA
ealb_702          --CGGTTGGCCCAAATATGAGTCCGCTTAAGAGTGACGCACGACTGGTGGTGGTTGATTA
ITO_efist         TGCGGTTGGCCCAAATACGAGTCCGCTTAAGAGTGACGCACGACTGGTGGTGGTTGATTA
ecap_712          --CGGTTGGCCCAAATATGAGTCCGCTTAAGAGTGACGCACGACTGGTGGTGGTTGATTA
eper_465          --CGGTTGGCCCAAATATGAGTCCGCTTAAGAGTGACGCACGACTGGTGGTGGTTGANNA
erotD1_993        --CGGTTGGCCCAAATACGAGTCCGCTTAAGAGCGACGCACGACTGGTGGTGGTTGATTA
ealt_711          --CGGTTGGCCCAAATACGAGTCCGCTTAAGAGTGACGCACGACTGGTGGTGGTTGATTA
echi_715          --CGGTTGGCCCAAATACGAGTCCGCTTAAGAGTGACGCACGACTGGTGGTGGTTGATTA
erotP1_1050       --CGGTTGGCCCAAATACGAGTCCGCTTAAGAGCGACGCACGACTGGTGGTGGTTGATTA
erotP5_952        --CGGTTGGCCCAAATACGAGTCCGCTTAAGAGCGACGCACGACTGGTGGTGGTTGATTA
ecun_701          --CGGNCGGCCCAAATANGAGTCCGTTCAAGANNGACGCACGACTGGTGGTGGNNGATTA
erotP6_953        --CGGTTGGCCCAAATACGAGTCCGCTTAAGAGCGACGCACGACTGGTGGTGGTTGATTA
erotD4_1022       --CGGTTGGCCCAAATACGAGTCCGCTTAAGAGCGACGCACGACTGGTGGTGGTTGATTA
erotP7_963        --CGGTTGGCCCAAATACGAGTCCGCTTAAGAGCGACGCACGACTGGTGGTGGTTGATTA
erotD5_1028       --CGGTTGGCCCAAATACGAGTCCGCTTAAGAGCGACGCACGACTGGTGGTGGTTGATTA
erotD6_1046       --CGGTTGGCCCAAATACGAGTCCGCTTAAGAGCGACGCACGACTGGTGGTGGTTGATTA
erotP3_922        --CGGTTGGCCCAAATACGAGTCCGCTTAAGAGCGACGCACGACTGGTGGTGGTTGATTA
ITO_ecan          --CGGTTGGCCCAAATACGAGTCCGTTTAAGGGTGACGCACGACTGGTGGTGGTTGATTA
ITO_edub          TGCGGTTGGCCCAAATATGAGTCCGCTTAAGGGTGACGCACGACTGGTGGTGGTTGATTA
                    ***  ********** *** * *   ***   ******** **********  *   *

ITO_emaki         CACAGTCGTCTCGTGTCGTGCGTCTCG-ATTCTTG---ACGGTATTAGCT-CTTGTAGTA
ITO_elind         CACAGTCGTCTCGTGTCGTGCGTCTCG-ATTCTTG---ACGGTATTAGCT-CTTGTAGTA
erotP2_1059       CACAGTCGTCTCGTGTCGCGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTTTTAGTA
erotP13_1088      CACAGTCGTCTCGTGTCGCGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTTTTAGTA
erotP9_979        CACAGTCGTCTCGTGTCGCGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTTTTAGTA
erotP8_972        CACAGTCGTCTCGTGTCGCGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTTTTAGTA
erotP10_982       CACAGTCGTCTCGTGTCGCGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTTTTAGTA
emac_532          CACAGTCGTCTCGTGTCGTGTGTTT-G-ATTCTTG---ACGGGAA-AACT-CTTAAAGTA
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erotD2_998        CACAGTCGTCTCGTGTCGCGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTTTTAGTA
emik_705          CGCAGTCGTCTCGTGTCGCGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTTGTAGTA
ITO_eform         CACAGTCGTCTCGTGTCGTGCGTCTCG-ATTCTTG---ACGGTATTAGCT-CTTGTAGCA
ITO_ecom          CACAGTCGTCTCGTGTCGTGCGTCTCG-ATTCTTG---ACGGTAC-AGCT-CTTGTAGAA
ITO_etash         CACAGTCGTCTCGTGTCGTGCGTCTCG-ATTCTTG---ACGGTATTAGCT-CTTGTAGCA
egod_822          CACAGTCGTCTCGTGTCGAGCGTCTCG-ATTCTTG---ACGGCAT-AACT-TTTCTCGTA
erotD3_1015       CACAGTCGTCTCGTGTCGCGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTTTTAGTA
echi713           CACAGTCGTCTCGTGTCGTGCGTCTCG-ATTCTTG---ACGGTATTAGCT-CTTGTAGCA
ITO_egleh         CACAGTCGTCTCGTGTCGTGCGTCTCG-ATTCTTG---ACGGTATTAGCT-CTTGTAGTA
erotP11_992       CACAGTCGTCTCGTGTCGCGCGTCTCG-ATTCTTG---ACGGT-TTAGCT-CTTTTAGTA
erotP4_934        CACAGTCGTCTCGTGTCGCGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTTTTAGTA
echi531           CACAGTCGTCTCGTGTCGTGCGTCTCG-ATTCTTG---ACGGTATTAGCT-CTTGTAGCA
ecap_460          CACAGTCGTCTCGTGTCGTGCGTCTCG-ATTCTTG---ACGGTAC-AGCT-CTTGTAGAA
esesD8_1096       CACAGTCGTCTCGTGTCGTGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTCGCGGTA
eleu_703          CACAGTCGTCTCGTGCCGTGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTTGTAGTA
erot_706          CACAGTCGTNTCGTGTCGCGCGTATCG-ATTCTTG---ACGGTAT-AGCT-CTTTTAGTA
ITO_emik          CGCAGTCGTCTCGTGTCGCGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTTGTAGTA
esesD6_908        CACAGTCGTCTCGTGTCGTGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTCGCAGTA
eses_710          CACAGTCGTCTCGTGTCGTGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTCGCAGTA
ehys_709          CACAGTCGTCTCGTGCCGTGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTCGTAGTA
esesP7_917        CACAGTCGTCTCGTGTCGTGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTCGCAGTA
esesP2_824        CACAGTCGTCTCGTGTCGTGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTCGCAGTA
esesD1_861        CACAGTCGTCTCGTGTCGTGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTCGCAGTA
erotP12_1081      CACAGTCGTCTCGTGTCGCGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTTTTAGTA
esesD2_874        CACAGTCGTCTCGTGTCGTGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTCGCAGTA
ITO_elep          CACAGTCGTCTCGTGTCGTGCGTCTCG-ATTCTTG---ACGGTAC-AGCT-CTTGTAGAA
esesP1_1075       CACAGTCGTCTCGTGTCGTGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTCGCAGTA
esesD3_884        CACAGTCGTCTCGTGTCGTGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTCGCAGTA
esesP6_871        CACAGTCGTCTCGTGTCGTGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTCGCAGTA
ITO_ejapo         CACAGTCGTCTCGTGTCGTGCGTCTCG-ATTCTTG---ACGGTAT-CGCT-CTTGTAGTA
esesP4_847        CACAGTCGTCTCGTGTCGTGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTCGCAGTA
ITO_erot          CACAGTCGTCTCGTGTCGCGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTTTTAGTA
ITO_evar          CACAGTCGTCTCGTGTCGTGCGTCTCG-ATTCTTG---ACGGTATTAGCT-CTTGTAGTA
esesD7_910        CACAGTCGTCTCGTGTCGTGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTCGCAGTA
ITO_ehys          CACAGTCGTCTCGTGCCGTGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTCGTAGTA
esesP3_843        CACAGTCGTCTCGTGTCGTGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTCGCAGTA
esesD4_897        CACAGTCGTCTCGTGTCGTGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTCGCAGTA
egod_819          CACAGTCGTGCTACGTAGTGGGTAGCGTATTTATGTGAACGGTAT-A-TT-CTTCTCATA
ecan_716          CACAGTCGTCTCGTGTCGTGGGTCTCG-ATTCTTG---ACGGTAT-GGCT-CTTGTAGTA
esesD5_902        CACAGTCGTCTCGTGTCGTGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTCGCAGTA
ITO_ealt          CACAGTCGTCTCGTGCCGTGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTTGTAGTA
egod_821          CACAGTCGTGCTACGTAGTGGGTAGCGTATTTATGTGAACGGTAT-A-TT-CTTCTCATA
egod_820          CACAGTCGTCTCGTGTAGCGTGCTACGTATTTATGTGAACGGTAT-A-TT-CTTCTCATA
epur_506          CACAGTCGTCTCGTGTCGTGTGTTTTG-ATTCTTG---ACGGGAA-AACTTCTTAAAGTA
ITO_eserot        CACAGTCGTCTCGTGCCGTGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTTGTAGTA
ITO_esemi         CACAGTCGTCTCGTGTCGTGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTCGCAGTA
ITO_eses          CACAGTCGTCTCGTGTCGTGCGTCT-G-ATTCTTG---ACGGTAC-GGCT-CTTGTAGTA
ITO_ecap          CACAGTCGTCTCGTGTCGTGCGTCTCG-ATTCTTG---ACGGTAC-AGCT-CTTGTAGAA
ecan_598          CACAGTCGTCTCGTGTCGTGGGTCTCG-ATTNTTG---ACGGTAT-GGCT-CTTGTAGTA
egod_1077         CACAGTCGTGCTACGTAGTGGGTCTCG-ATTGTGA---ACGGTAT-A-TT-CTTCTCATA
esesP5_855        CACAGTCGTCTCGTGTCGTGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTCGCAGTA
ealb_702          CACTGTCGTCTCGTGTCGCGCGTCTCT-ATTCTCG---ACGGTAC-AGCT-CTTGTAGTA
ITO_efist         CACAGTCGTCTCGTGTCGTGTGTTTTG-ATTCTTG---ACGGGAA-AACT-CTTAAAGTA
ecap_712          CACAGTCGTCTCGTGTCGTGCGTCTNG-ATTCTTG---ACGGTAC-AGCT-CTTGTAGAA
eper_465          CACAGTCGTCTCGTGTCGTGCGTCTCG-ATTCTTG---ACGGTGT-AGCT-NNTGTAGTA
erotD1_993        CACAGTCGTCTCGTGTCGCGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTTTTAGTA
ealt_711          CACAGTCGTCTCGTGCCGTGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTTGTAGTA
echi_715          CACAGTCGTCTCGTGTCGTGCGTCTCG-ATTCTTG---NCGGTATTAGCT-CTTGTAGTA
erotP1_1050       CACAGTCGTCTCGTGTCGCGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTTTTAGTA
erotP5_952        CACAGTCGTCTCGTGTCGCGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTTTTAGTA
ecun_701          CACAGTNGTNTCGNGTCGTGCGTC-CG-ATTCTTG---ACGGTNG----------TAGTA
erotP6_953        CACAGTCGTCTCGTGTCGCGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTTTTAGTA
erotD4_1022       CACAGTCGTCTCGTGTCGCGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTTTTAGTA
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erotP7_963          CACAGTCGTCTCGTGTCGCGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTTTTAGTA
erotD5_1028         CACAGTCGTCTCGTGTCGCGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTTTTAGTA
erotD6_1046         CACAGTCGTCTCGTGTCGCGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTTTTAGTA
erotP3_922          CACAGTCGTCTCGTGTCGCGCGTCTCG-ATTCTTG---ACGGTAT-AGCT-CTTTTAGTA
ITO_ecan            CACAGTCGTCTCGTGTCGTGGGTCTCG-ATTCTTG---ACGGTAT-GGCT-CTTGTAGTA
ITO_edub            CACAGTCGTCTCGTGTCGTGTGTTTTG-ATTCTTG---ACGGGAA-AACT-CTTAAAGTA
                    * * ** **     *  * * *      ***        ***                 *

ITO_emaki           CCCTAACGCGCCGTC-TTGTGATGGCTCTTCGATC
ITO_elind           CCCTAACGCGCCGTC-TTGTGATGGCTCTTCGATC
erotP2_1059         CCCTAATGCGTCGTC-TTGTGACGGCTCTTCGATC
erotP13_1088        CCCTAATGCGTCGTC-TTGTGACGGCCCTTCGATC
erotP9_979          CCCTAATGCGTCGTC-TTGTGACGGCTCTTCGATC
erotP8_972          CCCTAATGCGTCGTC-TTGTGACGGCTCTTCGATC
erotP10_982         CCCTAATGCGTCGTC-TTGTGACGGCTCTTCGATC
emac_532            CCCTGATGTGCTGTC-TTGTGATGGCTCTTCGATC
erotD2_998          CCCTAACGCGTCGTC-TTGTGACGGCTCTTCGATC
emik_705            CCCTAACGCGTCGTC-TTGTGACGGCTCTTCGATC
ITO_eform           CCCTAACGCGCCGTC-TTGTGATGGCCCTTCGATC
ITO_ecom            CCCTAATGCGTCGTC-CTGTGATGGCTCTTCGATC
ITO_etash           CCCTAACGCGCCGTC-TTGTGATGGCCCTTCGATC
egod_822            CCCTAAAGGGAAGTGGTTGTGACGGCTCTTCAATC
erotD3_1015         CCCTAACGCGTCGTC-TTGTGACGGCTCTTCGATC
echi713             CCCTAACGCGCCGTC-TTGTGATGGCCCTTCGATC
ITO_egleh           CCCTAACGCGTCGTC-CTGTGACGGCCCTTCGATC
erotP11_992         CCCTAACGGGTCGTC-TTGTGACGGCTCTTCGATC
erotP4_934          CCCTAATGCGTCGTCT-TGTGACGGCTCTTCGATC
echi531             CCCTAACGCGCCGTCT-TGTGATGGCCCTTCGATC
ecap_460            CCCTAATGCGTCGTCCCTGTGATGGNCCTTCGATC
esesD8_1096         CCCTAACGCGTCGTCT-TGTGACGGCTCTTCGATC
eleu_703            CCCTAACGCGTCGTCTTG-TGACGGCTCTTCGATC
erot_706            CCCTAANGCGTNGTCTTG-TGACGGCTCTTCGATC
ITO_emik            CCCTAACGCGTCGTCTTG-TGACGGCTCTTCGATC
esesD6_908          CCCTAACGCGTCGTCTTG-TGACGGCTCTTCGATC
eses_710            CCCTAACGCGTCGTCTTG-TGACGGCTCTTCGATC
ehys_709            CCCTAACGCGTCGTCTTG-TGACGGCTCTTCGATC
esesP7_917          CCCTAACGCGTCGTCTTG-TGACGGCTCTTCGATC
esesP2_824          CCCTAACGCGTCGTCTTG-TGACGGCTCTTCGATC
esesD1_861          CCCTAACGCGTCGTCTTG-TGACGGCTCTTCGATC
erotP12_1081        CCCTAATGCGTCGTCTTG-TGACGGCTCTTCGATC
esesD2_874          CCCTAACGCGTCGTCTTG-TGACGGCTCTTCGATC
ITO_elep            CCCTAATGCGTCGTCCTG-TGATGGCTCTTCGATC
esesP1_1075         CCCTAACGCGTCGTCTTG-TGACGGCTCTTCGATC
esesD3_884          CCCTAACGCGTCGTCTTG-TGACGGCTCTTCGATC
esesP6_871          CCCTAACGCGTCGTCTTG-TGACGGCTCTTCGATC
ITO_ejapo           CCCTAACGCGCCGTCTTG-TGATGGCTCTTCGATC
esesP4_847          CCCTAACGCGTCGTCTTG-TGACGGCTCTTCGATC
ITO_erot            CCCTAATGCGTCGTCTTG-TGACGGCTCTTCGATC
ITO_evar            CCCTAACGCGCCGTCTTG-TGATGGCTCTTCGATC
esesD7_910          CCCTAACGCGTCGTCTTG-TGACGGCTCTTCGATC
ITO_ehys            CCCTAACGCGTCGTCTTG-TGACGGCTCTTCGATC
esesP3_843          CCCTAACGCGTCGTCTTG-TGACGGCTCTTCGATC
esesD4_897          CCCTAACGCGTCGTCTTG-TGACGGCTCTTCGATC
egod_819            TCAGAACGCACGGGAGGGGTGATGAATGTAGGT--
ecan_716            CCCTAATGCGTCGTCTTG-TGATGGCTCTTCGATC
esesD5_902          CCCTAACGCGTCGTCTTG-TGACGGCTCTTCGATC
ITO_ealt            CCCTAACGCGTCGTCTTG-TGACGGCTCTTCGATC
egod_821            TCAGTACGCACGGGAGGG-AAGTGTATTGTAGGT-
egod_820            TCAGAGCGCACGGGAGGG-AAGTGTATTGTAGGTC
epur_506            CCCTGATGTGCTGTCTTG-TGATGGCTCTTCGATC
ITO_eserot          CCCTAACGGGTCGTCTTG-TGACGGCTCTTCGATC
ITO_esemi           CCCTAACGCGTCGTCTTG-TGACGGCT-TTCGATC
ITO_eses            CCCTAACGCGTCNTCCTG-TGATGGCTCTTCGATC
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ITO_ecap          CCCTAATGCGTCGTCCTG-TGATGGCTCTTCGATC
ecan_598          CCCTAATGCGNCGTCTTG-TGATGGCTCTTCGANC
egod_1077         TCAGACCGCACGGGAGGG-AAGTGTATTGTAGGTC
esesP5_855        CCCTAACGCGTCGTCTTG-TGACGGCTCTTCGATC
ealb_702          CCCTAATGCGTCGTCTT-GNGACGGCTCTTCGATC
ITO_efist         CCCTGATGNGCTGTCTTCGTGATGGCTCTTCGATC
ecap_712          CCCTAATGCGTCGTCCTG-TGATGGCTCTTCGATC
eper_465          CCCTAATGCGTCGTCTTG-TGACGGCTCTTCGATC
erotD1_993        CCCTAACGCGTCGTCTTG-TGACGGCTCTTCGATC
ealt_711          CCCTAACGCGTCGTCTTG-TGACGGCTCTTCGATC
echi_715          CCCTAACGCGCCGTCTTG-TGATGGCTCTTCGATC
erotP1_1050       CCCTAACGCGTCGTCTTG-TGACGGCTCTTCGATC
erotP5_952        CCCTAATGCGTCGTCTTG-TGACGGCTCTTCGATC
ecun_701          CCCTAATGCGTNGTCNTG-NGATGGCTTTTCGATC
erotP6_953        CCCTAATGCGTCGTCTTG-TGACGGCTCTTCGATC
erotD4_1022       CCCTAATGCGTCGTCTTG-TGACGGCTCTTCGATC
erotP7_963        CCCTAATGCGTCGTCTTG-TGACGGCTCTTCGATC
erotD5_1028       CCCTAATGCGTCGTCTTG-TGACGGCTCTTCGATC
erotD6_1046       CCCTAATGCGTCGTCTTG-TGACGGCTCTTCGATC
erotP3_922        CCCTAATGCGTCGTCTTG-TGACGGCTCTTCGATC
ITO_ecan          CCCTAATGCGTCGTCTTG-TGATGGCTCTTCGATC
ITO_edub          CCCTGATGTGCTGTCTTG-TGATGGCTCTTCGATC
                   *     *              *
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APPENDIX B-10: **A List of the additional ITS sequences of *Eupatorium* that were downloaded from Gen-Bank.**

Schmidt, G. J. and E. E. Schilling. 2000. Phylogeny and biogeography of *Eupatorium* (Asteraceae: Eupatorieae) based on nuclear ITS sequence data. American Journal of Botany 87: 716-726.

Species	Region	Accession number
Eupatorium mikanioides	ITS1/ITS2	AF177818/AF177858
Eupatorium leucolepis	ITS1/ITS2	AF177817/AF177857
Eupatorium album	ITS1/ITS2	AF177816/AF177856
Eupatorium rotundifolium	ITS1/ITS2	AF177815/AF177855
Eupatorium perfoliatum	ITS1/ITS2	AF177814/AF177854
Eupatorium sessilifolium	ITS1/ITS2	AF177813/AF177853
Eupatorium hyssopifolium	ITS1/ITS2	AF177812/AF177852
Eupatorium altissimum	ITS1/ITS2	AF177811/AF177851
Eupatorium cuneifolium	ITS1/ITS2	AF177810/AF177850
Eupatorium chinense	ITS1/ITS2	AF1777807/AF177847
Eupatorium cannabinum	ITS1/ITS2	AF177805/AF177845
Eupatorium cannabinum	ITS1/ITS2	AF177806/AF177846
Eupatorium capillifolium	ITS1/ITS2	AF177804/AF177844
Eupatorium capillifolium	ITS1/ITS2	AF177803/Schmidt's thesis
Eupatoriadelphus purpureus	ITS1/ITS2	AF177802/AF177842
Eupatoriadelphus maculatus	ITS1/ITS2	AF177798/AF177838

Ito, M., K. Watanabe, Y. Kita, T. Kawahara, and T. Yahara. 2000. Phylogeny of *Eupatorium*: insight from sequence of nrDNA ITS regions. Published only in database. Regions: ITS1, ITS2 and 5.8S nrDNA.

Species	Accession number
Eupatorium sessilifolium	AB032051
Eupatorium serotinum	AB032050
Eupatorium semiserratum	AB032049
Eupatorium rotundifolium	AB032048
Eupatorium mikanioides	AB032047
Eupatorium hyssopifolium	AB032046
Eupatorium altissimum	AB032045
Eupatorium leptophyllum	AB032044
Eupatorium compositifolium	AB032043
Eupatorium capillifolium	AB032042
Eupatorium fistulosum	AB032041
Eupatorium dubium	AB032040
Eupatorium cannabinum	AB032039
Eupatorium variabile	AB032038
Eupatorium tashiroi	AB032037
Eupatorium makinoi	AB032036
Eupatorium lindleyanum	AB032035
Eupatorium japonicum	AB032034
Eupatorium glehnii	AB032033
Eupatorium formosanum	AB032032

APPENDIX B-11: Chloroplast gene: *trnC-psbM* sequence alignment of the genus *Eupatorium*

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CLUSTAL X (1.81) multiple sequence alignment


esesD2_874          CCCACGAATCGAATCTCTTATTCTGTTCTGCTGGAGCAGAACAGAATAAGGGGACTGTTA
efist_1086          CCCACGAATCGAATCTCTTATTCTGTTCTGCTGAAGCAGAACAGAATAAGGGGACTGTTA
ecan_598            CCCACGAATCGAATCTCTTATTCTGTTCTGCTGAAGCAGAACAGAATAAGGGGACTTTTA
eleu_1125           CCCACGAATCGAATCTCTTATTCTGTTCTGCTGAAGCAGAACAGAATAAGGGGACTGTTA
esesD1_861          CCCACGAATCGAATCTCTTATTCTGTTCTGCTGGAGCAGAACAGAATAAGGGGACTGTTA
esesP2_824          CCCACGAATCGAATCTCTTATTCTGTTCTGCTGGAGCAGAACAGAATAAGGGGACTGTTA
erotP8_972          CCCACGAATCGAATCTCTTATTCTGTTCTGCTGGAGCAGAACAGAATAAGGGGACTGTTA
ecomp_1129          CCCACGAATCGAATCTCTTATTCTGTTCTGCTGAAGCAGAACAGAATAAGGGGACTGTTA
erotP9_979          CCCACGAATCGAATCTCTTATTCTGTTCTGCTGGAGCAGAACAGAATAAGGGGACTGTTA
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esesP7_917          CCCACGAATCGAATCTCTTATTCTGTTCTGCTGGAGCAGAACAGAATAAGGGGACTGTTA
esesP3_843          CCCACGAATCGAATCTCTTATTCTGTTCTGCTGGAGCAGAACAGAATAAGGGGACTGTTA
erotP13_1088        CCCACGAATCGAATCTCTTATTCTGTTCTGCTGGAGCAGAACAGAATAAGGGGACTGTTA
esesD8_1096         CCCACGAATCGAATCTCTTATTCTGTTCTGCTGGAGCAGAACAGAATAAGGGGACTGTTA
erotD5_1028         CCCACGAATCGAATCTCTTATTCTGTTCTGCTGGAGCAGAACAGAATAAGGGGACTGTTA
echin_531           CCCACGAATCGAATCTCTTATTCTGTTCTGCTGAAGCAGAACAGAATAAGGGGACTTTTA
emoh_1126           CCCACGAATCGAATCTCTTATTCTGTTCTGCTGAAGCAGAACAGAATAAGGGGACTGTTA
erotD1_993          CCCACGAATCGAATCTCTTATTCTGTTCTGCTGGAGCAGAACAGAATAAGGGGACTGTTA
esesP1_1075         CCCACGAATCGAATCTCTTATTCTGTTCTGCTGGAGCAGAACAGAATAAGGGGACTGTTA
esesD6_908          CCCACGAATCGAATCTCTTATTCTGTTCTGCTGGAGCAGAACAGAATAAGGGGACTGTTA
esesD7_910          CCCACGAATCGAATCTCTTATTCTGTTCTGCTGGAGCAGAACAGAATAAGGGGACTGTTA
epur_500            CCCACGAATCGAATCTCTTATTCTGTTCTGCTGAAGCAGAACAGAATAAGGGGACTGTTA
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erotP10_982         CCCACGAATCGAATCTCTTATTCTGTTCTGCTGGAGCAGAACAGAATAAGGGGACTGTTA
erotP6_953          CCCACGAATCGAATCTCTTATTCTGTTCTGCTGGAGCAGAACAGAATAAGGGGACTGTTA
erot_ovat1124       CCCACGAATCGAATCTCTTATTCTGTTCTGCTGAAGCAGAACAGAATAAGGGGACTGTTA
eper_465            CCCACGAATCGAATCTCTTATTCTGTTCTGCTGAAGCAGAACAGAATAAGGGGAATGTTA
esesD5_902          CCCACGAATCGAATCTCTTATTCTGTTCTGCTGGAGCAGAACAGAATAAGGGGACTGTTA
erotP2_1059         CCCACGAATCGAATCTCTTATTCTGTTCTGCTGGAGCAGAACAGAATAAGGGGACTGTTA
erotP11_992         CCCACGAATCGAATCTCTTATTCTGTTCTGCTGGAGCAGAACAGAATAAGGGGACTGTTA
erotD2_999          CCCACGAATCGAATCTCTTATTCTGTTCTGCTGGAGCAGAACAGAATAAGGGGACTGTTA
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eserot_899          CCCACGAATCGAATCTCTTATTCTGTTCTGCTGGAGCAGAACAGAATAAGGGGACTGTTA
egod_1077           CCCACGAATCGAATCTCTTATTCTGTTCTGCTGAAGCAGAACAGAATAAGGGGAATGTTA
erotP7_963          CCCACGAATCGAATCTCTTATTCTGTTCTGCTGGAGCAGAACAGAATAAGGGGACTGTTA
erotP12_1081        CCCACGAATCGAATCTCTTATTCTGTTCTGCTGGAGCAGAACAGAATAAGGGGACTGTTA
epil_1047           CCCACGAATCGAATCTCTTATTCTGTTCTGCTGGAGCAGAACAGAATAAGGGGACTGTTA
ehys_870            CCCACGAATCGAATCTCTTATTCTGTTCTGCTGAAGCAGAACAGAATAAGGGGACTGTTA
erotP5_952          CCCACGAATCGAATCTCTTATTCTGTTCTGCTGGAGCAGAACAGAATAAGGGGACTGTTA
egod_821            CCCACGAATCGAATCTCTTATTCTGTTCTGCTGGAGCAGAACAGAATAAGGGGACTGTTA
esesD4_897          CCCACGAATCGAATCTCTTATTCTGTTCTGCTGGAGCAGAACAGAATAAGGGGACTGTTA
erotP3_922          CCCACGAATCGAATCTCTTATTCTGTTCTGCTGGAGCAGAACAGAATAAGGGGACTGTTA
egod_823            CCCACGAATCGAATCTCTTATTCTGTTCTGCTGGAGCAGAACAGAATAAGGGGACTGTTA
erotD3_1015         CCCACGAATCGAATCTCTTATTCTGTTCTGCTGGAGCAGAACAGAATAAGGGGACTGTTA
ealb_981            CCCACGAATCGAATCTCTTATTCTGTTCTGCTGAAGCAGAACAGAATAAGGGGACTGTTA
egod_819            CCCACGAATCGAATCTCTTATTCTGTTCTGCTGGAGCAGAACAGAATAAGGGGACTGTTA
erotD4_1022         CCCACGAATCGAATCTCTTATTCTGTTCTGCTGGAGCAGAACAGAATAAGGGGACTGTTA
erotD6_1046         CCCACGAATCGAATCTCTTATTCTGTTCTGCTGGAGCAGAACAGAATAAGGGGACTGTTA
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esesD3_884          CCCACGAATCGAATCTCTTATTCTGTTCTGCTGGAGCAGAACAGAATAAGGGGACTGTTA
erotP4_934          CCCACGAATCGAATCTCTTATTCTGTTCTGCTGGAGCAGAACAGAATAAGGGGACTGTTA
ealt_682            CCCACGAATCGAATCTCTTATTCTGTTCTGCTGGAGCAGAACAGAATAAGGGGACTGTTA
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erotP1_1050       CCCACGAATCGAATCTCTTATTCTGTTCTGCTGGAGCAGAACAGAATAAGGGGACTGTTA
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ecan_598          ATACTTGGTTGAATCTAAACATCCGTTCTGGGGATTTTCTCTTTGAATCCAAAATTAAAA
eleu_1125         ATACTTGGTTGAATCTAAACATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
esesD1_861        ATACTTGGTTGAATCTAAATATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
esesP2_824        ATACTTGGTTGAATCTAAATATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
erotP8_972        ATACTTGGTTGAATCTAAATATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
ecomp_1129        ATACTTGGTTGAATCTAAACATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
erotP9_979        ATACTTGGTTGAATCTAAATATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
esemi_1030        ATACTTGGTTGAATCTAAATATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
esesP7_917        ATACTTGGTTGAATCTAAATATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
esesP3_843        ATACTTGGTTGAATCTAAATATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
erotP13_1088      ATACTTGGTTGAATCTAAATATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
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erotD5_1028       ATACTTGGTTGAATCTAAATATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
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erotD1_993        ATACTTGGTTGAATCTAAATATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
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epur_500          ATACTTGGTTGAATCTAAACATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
epil_1048         ATACTTGGTTGAATCTAAATATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
esesP5_855        ATACTTGGTTGAATCTAAATATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
erotP10_982       ATACTTGGTTGAATCTAAATATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
erotP6_953        ATACTTGGTTGAATCTAAATATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
erot_ovat1124     ATACTTGGTTGAATCTAAACATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
eper_465          ATACTTGGTTGAATCTAAACATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
esesD5_902        ATACTTGGTTGAATCTAAATATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
erotP2_1059       ATACTTGGTTGAATCTAAATATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
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erotD2_999        ATACTTGGTTGAATCTAAATATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
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eserot_899        ATACTTGGTTGAATCTAAATATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
egod_1077         ATACTTGGTTGAATCTAAACATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
erotP7_963        ATACTTGGTTGAATCTAAATATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
erotP12_1081      ATACTTGGTTGAATCTAAATATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
epil_1047         ATACTTGGTTGAATCTAAATATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
ehys_870          ATACTTGGTTGAATCTAAACATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
erotP5_952        ATACTTGGTTGAATCTAAATATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
egod_821          ATACTTGGTTGAATCTAAATATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
esesD4_897        ATACTTGGTTGAATCTAAATATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
erotP3_922        ATACTTGGTTGAATCTAAATATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
egod_823          ATACTTGGTTGAATCTAAATATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
erotD3_1015       ATACTTGGTTGAATCTAAATATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
ealb_981          ATACTTGGTTGAATCTAAACATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
egod_819          ATACTTGGTTGAATCTAAATATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
erotD4_1022       ATACTTGGTTGAATCTAAATATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
erotD6_1046       ATACTTGGTTGAATCTAAATATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
erot_926          ATACTTGGTTGAATCTAAACATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
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erotP4_934        ATACTTGGTTGAATCTAAATATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
ealt_682          ATACTTGGTTGAATCTAAATATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTAAAA
esesP6_871        ATACTTGGTTGAATCTAAATATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
egod_820          ATACTTGGTTGAATCTAAATATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
erotP1_1050       ATACTTGGTTGAATCTAAATATCCGTTCTGGGGATTTTATCTTTGAATCCAAAATTCAAA
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esesD2_874          GAAAGATTCTGATGGATAATTTACTTTTTGATAGAGAATGAAACCCCTCGTCAATAGTAT
efist_1086          GAAAGATTCTGATGGATAATTTACTTTTTTATAGA----GAAACCCCTCGTCAATAGTAT
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eleu_1125           GAAAGATTCTGATGGATAATTTACTTTTTGATAGAGAATGAAACCCCTCGTCAATAGTAT
esesD1_861          GAAAGATTCTGATGGATAATTTACTTTTTGATAGAGAATGAAACCCCTCGTCAATAGTAT
esesP2_824          GAAAGATTCTGATGGATAATTTACTTTTTTATAGAGAATGAAACCCCTCGTCAATAGTAT
erotP8_972          GAAAGATTCTGATGGATAATTTACTTTTTGATAGAGAATGAAACCCCTCGTCAATAGTAT
ecomp_1129          GAAAGATTCTGATGAATAATTTACTTTTTGATAGAGAA----ACCCCTCGTCAATAGTAT
erotP9_979          GAAAGATTCTGATGGATAATTTACTTTTTGATAGAGAATGAAACCCCTCGTCAATAGTAT
esemi_1030          GAAAGATTCTGATGGATAATTTACTTTTTGATAGAGAATGAAACCCCTCGTCAATAGTAT
esesP7_917          GAAAGATTCTGATGGATAATTTACTTTTTGATAGAGAATGAAACCCCTCGTCAATAGTAT
esesP3_843          GAAAGATTCTGATGGATAATTTACTTTTTTATAGAGAATGAAACCCCTCGTCAATAGTAT
erotP13_1088        GAAAGATTCTGATGGATAATTTACTTTTTGATAGAGAATGAAACCCCTCGTCAATAGTAT
esesD8_1096         GAAAGATTCTGATGGATAATTTACTTTTTTATAGAGAATGAAACCCCTCGTCAATAGTAT
erotD5_1028         GAAAGATTCTGATGGATAATTTACTTTTTGATAGAGAATGAAACCCCTCGTCAATAGTAT
echin_531           GAAAGATTCTGATGAATAATTTACTTTTTGATAGAAAATGAAACCCCTCGTCAATAGTAT
emoh_1126           GAAAGATTCTGATGGATAATTTACTTTTTGATAGAGAATGAAACCCCTCGTCAATAGTAT
erotD1_993          GAAAGATTCTGATGGATAATTTACTTTTTGATAGAGAATGAAACCCCTCGTCAATAGTAT
esesP1_1075         GAAAGATTCTGATGGATAATTTACTTTTTGATAGAGAATGAAACCCCTCGTCAATAGTAT
esesD6_908          GAAAGATTCTGATGGATAATTTACTTTTTGATAGAGAATGAAACCCCTCGTCAATAGTAT
esesD7_910          GAAAGATTCTGATGGATAATTTACTTTTTGATAGAGAATGAAACCCCTCGTCAATAGTAT
epur_500            GAAAGATTCTGATGGATAATTTACTTTTTTATAGA----GAAACCCCTCGTCAATAGTAT
epil_1048           GAAAGATTCTGATGGATAATTTACTTTTTGATAGAGAATGAAACCCCTCGTCAATAGTAT
esesP5_855          GAAAGATTCTGATGGATAATTTACTTTTTTATAGAGAATGAAACCCCTCGTCAATAGTAT
erotP10_982         GAAAGATTCTGATGGATAATTTACTTTTTGATAGAGAATGAAACCCCTCGTCAATAGTAT
erotP6_953          GAAAGATTCTGATGGATAATTTACTTTTTGATAGAGAATGAAACCCCTCGTCAATAGTAT
erot_ovat1124       GAAAGATTCTGATGGATAATTTACTTTTTGATAGAGAATGAAACCCCTCGTCAATAGTAT
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erotP2_1059         GAAAGATTCTGATGGATAATTTACTTTTTGATAGAGAATGAAACCCCTCGTCAATAGTAT
erotP11_992         GAAAGATTCTGATGGATAATTTACTTTTTGATAGAGAATGAAACCCCTCGTCAATAGTAT
erotD2_999          GAAAGATTCTGATGGATAATTTACTTTTTGATAGAGAATGAAACCCCTCGTCAATAGTAT
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eserot_899          GAAAGATTCTGATGGATAATTTACTTTTTGATAGAGAATGAAACCCCTCGTCAATAGTAT
egod_1077           GAAAGATTCTGATGGATAATTTACTTTTTGATAGAGAATGAAACCCCTCGTCAATAGTAT
erotP7_963          GAAAGATTCTGATGGATAATTTACTTTTTGATAGAGAATGAAACCCCTCGTCAATAGTAT
erotP12_1081        GAAAGATTCTGATGGATAATTTACTTTTTGATAGAGAATGAAACCCCTCGTCAATAGTAT
epil_1047           GAAAGATTCTGATGGATAATTTACTTTTTGATAGAGAATGAAACCCCTCGTCAATAGTAT
ehys_870            GAAAGATTCTGATGGATAATTTACTTTTTTATAGAGAA----ACCCCTCGTCAATAGTAT
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egod_821            GAAAGATTCTGATGGATAATTTACTTTTTTATAGAGAATGAAACCCCTCGTCAATAGTAT
esesD4_897          GAAAGATTCTGATGGATAATTTACTTTTTGATAGAGAATGAAACCCCTCGTCAATAGTAT
erotP3_922          GAAAGATTCTGATGGATAATTTACTTTTTGATAGAGAATGAAACCCCTCGTCAATAGTAT
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ealb_981            GAAAGATTCTGATGGATAATTTACTTTTTGATAGAAAATGAAACCCCTCGTCAATAGTAT
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erotD4_1022         GAAAGATTCTGATGGATAATTTACTTTTTGATAGAGAATGAAACCCCTCGTCAATAGTAT
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erot_926            GAAAGATTCTGATGGATAATTTACTTTTTGATAGAGAATGAAACCCCTCGTCAATAGTAT
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erotP4_934          GAAAGATTCTGATGGATAATTTACTTTTTGATAGAGAATGAAACCCCTCGTCAATAGTAT
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esesP6_871          GAAAGATTCTGATGGATAATTTACTTTTTGATAGAGAATGAAACCCCTCGTCAATAGTAT
egod_820            GAAAGATTCTGATGGATAATTTACTTTTTTATAGAGAATGAAACCCCTCGTCAATAGTAT
erotP1_1050         GAAAGATTCTGATGGATAATTTACTTTTTGATAGAGAATGAAACCCCTCGTCAATAGTAT
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esesD1_861        AATATACTCTCTCTCAACTCCTTCAGAGAGACAATCGGGAAAGGGTACGTATTAGATCAA
esesP2_824        AATATACTATCTCTCAACTCCTTCAGAGAGACAATCGGGAAAGGGTACGTATTAGATCAA
erotP8_972        AATATACTCTCTCTCAACTCCTTCAGAGAGACAATCGGGAAAGGGTACGTATTAGATCAA
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echin_531         AATATACTATCTCTCAACTCCTTCAGAGAGACAATCGGGAAAGGGTACGTATTAGATCAA
emoh_1126         AATATACTATCTCTCAACTCCTTCAGAGAGACAATCGGGAAAGGGTACGTATTAGATCAA
erotD1_993        AATATACTCTCTCTCAACTCCTTCAGAGAGACAATCGGGAAAGGGTACGTATTAGATCAA
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esesD6_908        AATATACTATCTCTCAACTCCTTCAGAGAGACAATCGGGAAAGGGTACGTATTAGATCAA
esesD7_910        AATATACTATCTCTCAACTCCTTCAGAGAGACAATCGGGAAAGGGTACGTATTAGATCAA
epur_500          AATATACTATCTCTCAACTCCTTCAGAGAGACAATCGGGAAAGGGTACGTATTAGATCAA
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erotP6_953        AATATACTCTCTCTCAACTCCTTCAGAGAGACAATCGGGAAAGGGTACGTATTAGATCAA
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erotP2_1059       AATATACTATCTCTCAACTCCTTCAGAGAGACAATCGGGAAAGGGTACGTATTAGATCAA
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erotD2_999        AATATACTCTCTCTCAACTCCTTCAGAGAGACAATCGGGAAAGGGTACGTATTAGATCAA
egod_822          AATATACTATCTCTCAACTCCTTCAGAGAGACAATCGGGAAAGGGTACGTATTAGATCAA
eserot_899        AATATACTATCTCTCAACTCCTTCAGAGAGACAATCGGGAAAGGGTACGTATTAGATCAA
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erotP7_963        AATATACTCTCTCTCAACTCCTTCAGAGAGACAATCGGGAAAGGGTACGTATTAGATCAA
erotP12_1081      AATATACTATCTCTCAACTCCTTCAGAGAGACAATCGGGAAAGGGTACGTATTAGATCAA
epil_1047         AATATACTCTCTCTCAACTCCTTCAGAGAGACAATCGGGAAAGGGTACGTATTAGATCAA
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esesD4_897        AATATACTCTCTCTCAACTCCTTCAGAGAGACAATCGGGAAAGGGTACGTATTAGATCAA
erotP3_922        AATATACTCTCTCTCAACTCCTTCAGAGAGACAATCGGGAAAGGGTACGTATTAGATCAA
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erotD3_1015       AATATACTCTCTCTCAACTCCTTCAGAGAGACAATCGGGAAAGGGTACGTATTAGATCAA
ealb_981          AATATACTATCTCTCAACTCCTTCAGAGAGACAATCGGGAAAGGGTACGTATTAGATCAA
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erotD4_1022       AATATACTCTCTCTCAACTCCTTCAGAGAGACAATCGGGAAAGGGTACGTATTAGATCAA
erotD6_1046       AATATACTCTCTCTCAACTCCTTCAGAGAGACAATCGGGAAAGGGTACGTATTAGATCAA
erot_926          AATATACTATCTCTCAACTCCTTCAGAGAGACAATCGGGAAAGGGTACGTATTAGATCAA
esesD3_884        AATATACTATCTCTCAACTCCTTCAGAGAGACAATCGGGAAAGGGTACGTATTAGATCAA
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erotP1_1050       AATATACTCTCTCTCAACTCCTTCAGAGAGACAATCGGGAAAGGGTACGTATTAGATCAA
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efist_1086        ATAGGAAAAAGTTTGTATATAGATAGCAATTGATCTATTTTTACTTTGAAGGGCAAGAAA
ecan_598          ATAGGAAAAAGTTTGTATATAGATAGCAATTGATCTATTTTTACTTTGAAGGGCAAGAAA
eleu_1125         ATAGGAAAAAGTTTGTATATAGATAGCAATTGATCTATTTTTACTTTGAAGGGCAAGAAA
esesD1_861        ATAGGAAAAAGTTTGTATATAGATAGCAATTGATCTATTTTTACTTTGAAGGGCAAGAAA
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ecomp_1129          ATAGGAAAAAGTTTGTATATAGATAGCAATTGATCTATTTTTACTTTGAAGGGCAAGAAA
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erotP12_1081        ATAGGAAAAAGTTTGTATATAGATAGCAATTGATCTATTTTTACTTTGAAGGGCAAGAAA
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erotP3_922          ATAGGAAAAAGTTTGTATATAGATAGCAATTGATCTATTTTTACTTTGAAGGGCAAGAAA
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egod_820            ATAGGAAAAAGTTTGTATATAGATAGCAATTGATCTATTTTTACTTTGAAGGGCAAGAAA
erotP1_1050         ATAGGAAAAAGTTTGTATATAGATAGCAATTGATCTATTTTTACTTTGAAGGGCAAGAAA
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efist_1086          AAAGGAATGGACCCTCTTCTTTTATTACTAGATGTTCCATTAGTAACATTCCTTTGTAGT
ecan_598            AAAGGAATGGACCCTCTGATTTTATTACTAGATGTTCCATTAGTAACATTCCTTTGTAGT
eleu_1125           AAAGGAATGGACCCTCTGATTTTATTACTAGATGTTCCATTAGTAACATTCCTTTGTAGT
esesD1_861          AAAGGAATGGACCCTCTGATTTTATTACTAGATGTTCCATTAGTAACATTCCTTTGTAGT
esesP2_824          AAAGGAATGGACCCTCTGATTTTATTACTAGATGTTCCATTAGTAACATTCCTTTGTAGT
erotP8_972          AAAGGAATGGACCCTCTGATTTTATTACTAGATGTTCCATTAGTAACATTCCTTTGTAGT
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esesP7_917          AAAGGAATGGACCCTCTGATTTTATTACTAGATGTTCCATTAGTAACATTCCTTTGTAGT
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erotD1_993          AAAGGAATGGACCCTCTGATTTTATTACTAGATGTTCCATTAGTAACATTCCTTTGTAGT
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eper_465            AAAGGAATGGACCCTCTGATTTTATTACTAGATGTTCCATTAGTAACATTCCTTTGTAGT
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erotP2_1059         AAAGGAATGGACCCTCTGATTTTATTACTAGATGTTCCATTAGTAACATTCCTTTGTAGT
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erotD2_999          AAAGGAATGGACCCTCTGATTTTATTACTAGATGTTCCATTAGTAACATTCCTTTGTAGT
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eserot_899          AAAGGAATGGACCCTCTGATTTTATTACTAGATGTTCCATTAGTAACATTCCTTTGTAGT
egod_1077           AAAGGAATGGACCCTCTGATTTTATTACTAGATGTTCCATTAGTAACATTCCTTTGTAGT
erotP7_963          AAAGGAATGGACCCTCTGATTTTATTACTAGATGTTCCATTAGTAACATTCCTTTGTAGT
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ehys_870            AAAGGAATGGACCCTCTTCTTTTATTACTAGATGTTCCATTAGTAACATTCCTTTGTAGT
erotP5_952          AAAGGAATGGACCCTCTGATTTTATTACTAGATGTTCCATTAGTAACATTCCTTTGTAGT
egod_821            AAAGGAATGGACCCTCTGATTTTATTACTAGATGTTCCATTAGTAACATTCCTTTGTAGT
esesD4_897          AAAGGAATGGACCCTCTGATTTTATTACTAGATGTTCCATTAGTAACATTCCTTTGTAGT
erotP3_922          AAAGGAATGGACCCTCTGATTTTATTACTAGATGTTCCATTAGTAACATTCCTTTGTAGT
egod_823            AAAGGAATGGACCCTCTGATTTTATTACTAGATGTTCCATTAGTAACATTCCTTTGTAGT
erotD3_1015         AAAGGAATGGACCCTCTGATTTTATTACTAGATGTTCCATTAGTAACATTCCTTTGTAGT
ealb_981            AAAGGAATGGACCCTCTGATTTTATTACTAGATGTTCCATTAGTAACATTCCTTTGTAGT
egod_819            AAAGGAATGGACCCTCTGATTTTATTACTAGATGTTCCATTAGTAACATTCCTTTGTAGT
erotD4_1022         AAAGGAATGGACCCTCTGATTTTATTACTAGATGTTCCATTAGTAACATTCCTTTGTAGT
erotD6_1046         AAAGGAATGGACCCTCTGATTTTATTACTAGATGTTCCATTAGTAACATTCCTTTGTAGT
erot_926            AAAGGAATGGACCCTCTGATTTTATTACTAGATGTTCCATTAGTAACATTCCTTTGTAGT
esesD3_884          AAAGGAATGGACCCTCTGATTTTATTACTAGATGTTCCATTAGTAACATTCCTTTGTAGT
erotP4_934          AAAGGAATGGACCCTCTGATTTTATTACTAGATGTTCCATTAGTAACATTCCTTTGTAGT
ealt_682            AAAGGAATGGACCCTCTGATTTTATTACTAGATGTTCCATTAGTAACATTCCTTTGTAGT
esesP6_871          AAAGGAATGGACCCTCTGATTTTATTACTAGATGTTCCATTAGTAACATTCCTTTGTAGT
egod_820            AAAGGAATGGACCCTCTGATTTTATTACTAGATGTTCCATTAGTAACATTCCTTTGTAGT
erotP1_1050         AAAGGAATGGACCCTCTGATTTTATTACTAGATGTTCCATTAGTAACATTCCTTTGTAGT
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esesD2_874          GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTCATTTT
efist_1086          GTGATTGTTTATTTTACTTGTTTTTAGTCTTTCCCGATTAGAAATAGGAAT------TTT
ecan_598            GTGATTTTTTATTTTACTTTTGTTTAGTCTTTACCGATTAGAATTAGAAATAGGAATTTT
eleu_1125           GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGGAATTTT
esesD1_861          GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
esesP2_824          GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
erotP8_972          GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
ecomp_1129          GTGATTTTTTATTTTACTTTTGTTTAGTTTTTCCCGATTAGAATGATAAATAGGAATTTT
erotP9_979          GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
esemi_1030          GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
esesP7_917          GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
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esesP3_843          GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
erotP13_1088        GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
esesD8_1096         GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
erotD5_1028         GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
echin_531           GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGGAATTTT
emoh_1126           GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGGAATTTT
erotD1_993          GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
esesP1_1075         GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
esesD6_908          GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTCATTTT
esesD7_910          GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTCATTTT
epur_500            GTGATTGTTTATTTTACTTGTTTTTAGTCTTTCCCGATTAGAAATAGGAAT------TTT
epil_1048           GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
esesP5_855          GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
erotP10_982         GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
erotP6_953          GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
erot_ovat1124       GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGGAATTTT
eper_465            GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGGAATTTT
esesD5_902          GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTCATTTT
erotP2_1059         GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
erotP11_992         GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
erotD2_999          GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
egod_822            GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
eserot_899          GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
egod_1077           GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGGAATTTT
erotP7_963          GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
erotP12_1081        GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
epil_1047           GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
ehys_870            GTGATTGTTTATTTTACTTGTTTTTAGTCTTTCCCGATTAGAA------ATAGGAATTTT
erotP5_952          GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
egod_821            GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
esesD4_897          GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
erotP3_922          GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
egod_823            GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
erotD3_1015         GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
ealb_981            GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAA------ATAGGAATTTT
egod_819            GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
erotD4_1022         GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
erotD6_1046         GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
erot_926            GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGGAATTTT
esesD3_884          GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
erotP4_934          GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
ealt_682            GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
esesP6_871          GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
egod_820            GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
erotP1_1050         GTGATTTTTTATTTTACTTTTGTTTAGTCTTTCCCGATTAGAATTAGAAATAGTAATTTT
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esesD2_874          TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
efist_1086          TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
ecan_598            TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCAGACAGAATCCCCTTTTGACTC
eleu_1125           TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
esesD1_861          TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
esesP2_824          TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
erotP8_972          TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
ecomp_1129          TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
erotP9_979          TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
esemi_1030          TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
esesP7_917          TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
esesP3_843          TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
erotP13_1088        TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
esesD8_1096         TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
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erotD5_1028         TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
echin_531           TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
emoh_1126           TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
erotD1_993          TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
esesP1_1075         TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
esesD6_908          TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
esesD7_910          TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
epur_500            TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
epil_1048           TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
esesP5_855          TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
erotP10_982         TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
erotP6_953          TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
erot_ovat1124       TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
eper_465            TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
esesD5_902          TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
erotP2_1059         TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
erotP11_992         TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
erotD2_999          TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
egod_822            TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
eserot_899          TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
egod_1077           TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
erotP7_963          TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
erotP12_1081        TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
epil_1047           TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
ehys_870            TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
erotP5_952          TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
egod_821            TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
esesD4_897          TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
erotP3_922          TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
egod_823            TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
erotD3_1015         TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
ealb_981            TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
egod_819            TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
erotD4_1022         TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
erotD6_1046         TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
erot_926            TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
esesD3_884          TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
erotP4_934          TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
ealt_682            TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
esesP6_871          TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
egod_820            TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
erotP1_1050         TGAAATGGATTTATTTGATTGGTTCATCAATAGTGTTCGGACAGAATCCCCTTTTGACTC
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esesD2_874          TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
efist_1086          TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAATAATTCTATCATAGAGATA
ecan_598            TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTCTATCATAGAGATA
eleu_1125           TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
esesD1_861          TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
esesP2_824          TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
erotP8_972          TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
ecomp_1129          TGGACCATTAATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
erotP9_979          TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
esemi_1030          TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
esesP7_917          TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
esesP3_843          TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
erotP13_1088        TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
esesD8_1096         TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
erotD5_1028         TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
echin_531           TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
emoh_1126           TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
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erotD1_993          TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
esesP1_1075         TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
esesD6_908          TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
esesD7_910          TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
epur_500            TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAATAATTCTATCATAGAGATA
epil_1048           TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
esesP5_855          TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
erotP10_982         TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
erotP6_953          TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
erot_ovat1124       TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
eper_465            TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
esesD5_902          TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
erotP2_1059         TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
erotP11_992         TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
erotD2_999          TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
egod_822            TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
eserot_899          TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
egod_1077           TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
erotP7_963          TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
erotP12_1081        TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
epil_1047           TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
ehys_870            TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAATAATTCTATCATAGAGATA
erotP5_952          TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
egod_821            TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
esesD4_897          TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
erotP3_922          TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
egod_823            TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
erotD3_1015         TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
ealb_981            TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTTTATCATAGAGATA
egod_819            TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
erotD4_1022         TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
erotD6_1046         TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
erot_926            TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
esesD3_884          TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
erotP4_934          TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
ealt_682            TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
esesP6_871          TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
egod_820            TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
erotP1_1050         TGGACCATTCATTCTACTATTATTAGTGAGCAATAATGGAAAAATTATATCATAGAGATA
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esesD2_874          AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATT-TTTTATAGATAGTTCTGC
efist_1086          AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATTGTTTTATAGATCGTTTTGC
ecan_598            AGGGACATAATTCACTAGAAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
eleu_1125           AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
esesD1_861          AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
esesP2_824          AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
erotP8_972          AGGGACATAATTCACTAGAAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
ecomp_1129          AGGGACATAATTCACTAGAAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
erotP9_979          AGGGACATAATTCACTAGAAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
esemi_1030          AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
esesP7_917          AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
esesP3_843          AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
erotP13_1088        AGGGACATAATTCACTAGAAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
esesD8_1096         AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
erotD5_1028         AGGGACATAATTCACTAGAAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
echin_531           AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
emoh_1126           AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
erotD1_993          AGGGACATAATTCACTAGAAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
esesP1_1075         AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
esesD6_908          AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATTTTTTT-ATAGATAGTTCTGC
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esesD7_910          AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATT-TTTTATAGATAGTTCTGC
epur_500            AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATTGTTTTATAGATCGTTTTGC
epil_1048           AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
esesP5_855          AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
erotP10_982         AGGGACATAATTCACTAGAAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
erotP6_953          AGGGACATAATTCACTAGAAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
erot_ovat1124       AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
eper_465            AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
esesD5_902          AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATTTTTT-ATAGATAGTTCTGC
erotP2_1059         AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
erotP11_992         AGGGACATAATTCACTAGAAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
erotD2_999          AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
egod_822            AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
eserot_899          AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCCGC
egod_1077           AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
erotP7_963          AGGGACATAATTCACTAGAAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
erotP12_1081        AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
epil_1047           AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCCGC
ehys_870            AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
erotP5_952          AGGGACATAATTCACTAGAAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
egod_821            AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
esesD4_897          AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
erotP3_922          AGGGACATAATTCACTAGAAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
egod_823            AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
erotD3_1015         AGGGACATAATTCACTAGAAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
ealb_981            AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
egod_819            AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
erotD4_1022         AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
erotD6_1046         AGGGACATAATTCACTAGAAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
erot_926            AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCCGC
esesD3_884          AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
erotP4_934          AGGGACATAATTCACTAGAAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
ealt_682            AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
esesP6_871          AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
egod_820            AGGGACATAATTCAC---AAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
erotP1_1050         AGGGACATAATTCACTAGAAACACTCCAAACGCTATCAATTTTTTTATAGATAGTTCTGC
                    ***************   ********************** *** ****** ***  **

esesD2_874          AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATCTCTTCATTT
efist_1086          AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATAAAAATCTCTTCATTT
ecan_598            AACGCATTTTGAATTTGAATTGAAATCATTTTCAAATATCAAATAAAAATCTCTTCATTT
eleu_1125           AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATAAAAATCTCTTCATTT
esesD1_861          AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATCTCTTCATTT
esesP2_824          AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATATCTTCATTT
erotP8_972          AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATCTCTTCATTT
ecomp_1129          AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATAAAAATCTCTTTATTT
erotP9_979          AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATCTCTTCATTT
esemi_1030          AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATCTCTTCATTT
esesP7_917          AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATCTCTTCATTT
esesP3_843          AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATATCTTCATTT
erotP13_1088        AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATCTCTTCATTT
esesD8_1096         AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATATCTTCATTT
erotD5_1028         AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATCTCTTCATTT
echin_531           AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATAAAAATCTCTTCATTT
emoh_1126           AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATAAAAATCTCTTCATTT
erotD1_993          AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATCTCTTCATTT
esesP1_1075         AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATCTCTTCATTT
esesD6_908          AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATCTCTTCATTT
esesD7_910          AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATCTCTTCATTT
epur_500            AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATAAAAATCTCTTCATTT
epil_1048           AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATCTCTTCATTT
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esesP5_855          AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATATCTTCATTT
erotP10_982         AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATCTCTTCATTT
erotP6_953          AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATCTCTTCATTT
erot_ovat1124       AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATAAAAATCTCTTCATTT
eper_465            AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATAAAAATCTCTTCATTT
esesD5_902          AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATCTCTTCATTT
erotP2_1059         AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATCTCTTCATTT
erotP11_992         AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATCTCTTCATTT
erotD2_999          AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATCTCTTCATTT
egod_822            AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATATCTTCATTT
eserot_899          AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATCTCTTCATTT
egod_1077           AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATAAAAATCTCTTCATTT
erotP7_963          AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATCTCTTCATTT
erotP12_1081        AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATCTCTTCATTT
epil_1047           AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATCTCTTCATTT
ehys_870            AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATAAAAATCTCTTCATTT
erotP5_952          AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATCTCTTCATTT
egod_821            AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATATCTTCATTT
esesD4_897          AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATCTCTTCATTT
erotP3_922          AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATCTCTTCATTT
egod_823            AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATATCTTCATTT
erotD3_1015         AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATCTCTTCATTT
ealb_981            AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATAAAAATCTCTTCATTT
egod_819            AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATATCTTCATTT
erotD4_1022         AACGCATTTTTAATTTGAATTGAAATCATTTTCAA-------ATCAAAATCTCTTCATTT
erotD6_1046         AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATCTCTTCATTT
erot_926            AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATAAAAATCTCTTCATTT
esesD3_884          AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATATCTTCATTT
erotP4_934          AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATCTCTTCATTT
ealt_682            AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATCTCTTCATTT
esesP6_871          AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATCTCTTCATTT
egod_820            AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATATCTTCATTT
erotP1_1050         AACGCATTTTGAATTTGAATTGAAATCATTTTCAA-------ATCAAAATCTCTTCATTT
                    ********** ************************       ** ***** **** ****

esesD2_874          CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
efist_1086          CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
ecan_598            CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
eleu_1125           CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAAAAGTAAAACAATTATTTCA
esesD1_861          CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
esesP2_824          CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
erotP8_972          CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
ecomp_1129          CGAAATTTCA------------GGAGAAATGTATTCTCTAACAGTAAAACAATTATTTCA
erotP9_979          CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
esemi_1030          CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
esesP7_917          CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
esesP3_843          CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
erotP13_1088        CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
esesD8_1096         CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
erotD5_1028         CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
echin_531           CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
emoh_1126           CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAAAAGTAAAACAATTATTTCA
erotD1_993          CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
esesP1_1075         CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
esesD6_908          CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
esesD7_910          CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
epur_500            CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
epil_1048           CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
esesP5_855          CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
erotP10_982         CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
erotP6_953          CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
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erot_ovat1124      CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAAAAGTAAAACAATTATTTCA
eper_465           CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAAAAGTAAAACAATTATTTCA
esesD5_902         CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
erotP2_1059        CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
erotP11_992        CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
erotD2_999         CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
egod_822           CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
eserot_899         CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
egod_1077          CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAAAAGTAAAACAATTATTTCA
erotP7_963         CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
erotP12_1081       CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
epil_1047          CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
ehys_870           CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAAAAGTAAAACAATTATTTCA
erotP5_952         CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
egod_821           CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
esesD4_897         CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
erotP3_922         CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
egod_823           CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
erotD3_1015        CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
ealb_981           CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
egod_819           CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
erotD4_1022        CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
erotD6_1046        CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
erot_926           CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAAAAGTAAAACAATTATTTCA
esesD3_884         CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
erotP4_934         CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
ealt_682           CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
esesP6_871         CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
egod_820           CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
erotP1_1050        CGAAATTCCATTGGATTCTAGTGGAGAAATGTATTCTATAACAGTAAAACAATTATTTCA
                   ******* **           *************** *** ******************

esesD2_874         GATTCATCGGAATAAAAATAAAATAAATAGATGTATCAAAGAAATAGAATTGGG
efist_1086         GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
ecan_598           GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
eleu_1125          GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
esesD1_861         GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
esesP2_824         GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
erotP8_972         GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
ecomp_1129         GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
erotP9_979         GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
esemi_1030         GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
esesP7_917         GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
esesP3_843         GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
erotP13_1088       GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
esesD8_1096        GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
erotD5_1028        GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
echin_531          GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
emoh_1126          GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
erotD1_993         GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
esesP1_1075        GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
esesD6_908         GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
esesD7_910         GATTCATCGGAATAAAAATAAAATAAATAGATGTATCAAAGAAATAGAATTGGG
epur_500           GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
epil_1048          GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
esesP5_855         GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
erotP10_982        GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
erotP6_953         GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
erot_ovat1124      GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
eper_465           GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
esesD5_902         GATTCATCGGAATAAAAATAAAATAAATAGATGTATCAAAGAAATAGAATTGGG
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erotP2_1059         GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
erotP11_992         GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
erotD2_999          GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
egod_822            GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
eserot_899          GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
egod_1077           GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
erotP7_963          GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
erotP12_1081        GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
epil_1047           GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
ehys_870            GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
erotP5_952          GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
egod_821            GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
esesD4_897          GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
erotP3_922          GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
egod_823            GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
erotD3_1015         GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
ealb_981            GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
egod_819            GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
erotD4_1022         GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
erotD6_1046         GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
erot_926            GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
esesD3_884          GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
erotP4_934          GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
ealt_682            GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
esesP6_871          GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
egod_820            GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
erotP1_1050         GATTCATCGGAATA------AAATAAATAGATGTATCAAAGAAATAGAATTGGG
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esesD2_874          TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
efist_1086          TCCTACGTCAATTCCATATATGGATTAATAACTACATATATTGAAGATAGAAGCTAATAT
ecan_598            TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
eleu_1125           TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
esesD1_861          TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
esesP2_824          TCCTACGTCAATTCCATATATGGATTCATAACTACATATATGGAATATAGAAGCTAATAT
erotP8_972          TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
ecomp_1129          TCCTA---------------------------------------------------
erotP9_979          TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
esemi_1030          TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
esesP7_917          TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
esesP3_843          TCCTACGTCAATTCCATATATGGATTCATAACTACATATATGGAATATAGAAGCTAATAT
erotP13_1088        TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
esesD8_1096         TCCTACGTCAATTCCATATATGGATTCATAACTACATATATGGAATATAGAAGCTAATAT
erotD5_1028         TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
echin_531           TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
emoh_1126           TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
erotD1_993          TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
esesP1_1075         TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
esesD6_908          TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
esesD7_910          TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
epur_500            TCCTACGTCAATTCCATATATGGATTAATAACTACATATATTGAAGATAGAAGCTAATAT
epil_1048           TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
esesP5_855          TCCTACGTCAATTCCATATATGGATTCATAACTACATATATGGAATATAGAAGCTAATAT
erotP10_982         TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
erotP6_953          TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
erot_ovat1124       TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
eper_465            TCCTACGTCAATTCCATATATGGAGTCATAACTACATATATTGAATATAGAAGCTAATAT
esesD5_902          TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
erotP2_1059         TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
erotP11_992         TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
erotD2_999          TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
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egod_822          TCCTACGTCAATTCCATATATGGATTCATAACTACATATATGGAATATAGAAGCTAATAT
eserot_899        TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
egod_1077         TCCTACGTCAATTCCATATATGGAGTCATAACTACATATATTGAATATAGAAGCTAATAT
erotP7_963        TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
erotP12_1081      TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
epil_1047         TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
ehys_870          TCCTACGTCAATTCCATATATGGAGTCATAACTACATATATTGAATATAGAAGCTAATAT
erotP5_952        TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
egod_821          TCCTACGTCAATTCCATATATGGATTCATAACTACATATATGGAATATAGAAGCTAATAT
esesD4_897        TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
erotP3_922        TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
egod_823          TCCTACGTCAATTCCATATATGGATTCATAACTACATATATGGAATATAGAAGCTAATAT
erotD3_1015       TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
ealb_981          TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
egod_819          TCCTACGTCAATTCCATATATGGATTCATAACTACATATATGGAATATAGAAGCTAATAT
erotD4_1022       TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
erotD6_1046       TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
erot_926          TCCTACGTCAATTCCATATATGGAGTCATAACTACATATATTGAATATAGAAGCTAATAT
esesD3_884        TCCTACGTCAATTCCATATATGGATTCATAACTACATATATGGAATATAGAAGCTAATAT
erotP4_934        TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
ealt_682          TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
esesP6_871        TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
egod_820          TCCTACGTCAATTCCATATATGGATTCATAACTACATATATGGAATATAGAAGCTAATAT
erotP1_1050       TCCTACGTCAATTCCATATATGGATTCATAACTACATATATTGAATATAGAAGCTAATAT
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esesD2_874        AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
efist_1086        AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACTAATAGAGA
ecan_598          AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACTAATAGAGA
eleu_1125         AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
esesD1_861        AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
esesP2_824        AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
erotP8_972        AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
ecomp_1129        ----------------------------------------------AATAGAGA
erotP9_979        AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
esemi_1030        AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
esesP7_917        AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
esesP3_843        AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
erotP13_1088      AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
esesD8_1096       AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
erotD5_1028       AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
echin_531         AGTATACCAACTTTATTA----CAATTTTATACACTACTATAATATAACACTAATAGAGA
emoh_1126         AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
erotD1_993        AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
esesP1_1075       AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
esesD6_908        AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
esesD7_910        AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
epur_500          AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACTAATAGAGA
epil_1048         AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
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erotP6_953        AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
erot_ovat1124     AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
eper_465          AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
esesD5_902        AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
erotP2_1059       AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
erotP11_992       AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
erotD2_999        AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
egod_822          AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
eserot_899        AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
egod_1077         AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
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erotP7_963          AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
erotP12_1081        AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
epil_1047           AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
ehys_870            AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
erotP5_952          AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
egod_821            AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
esesD4_897          AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
erotP3_922          AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
egod_823            AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
erotD3_1015         AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
ealb_981            AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
egod_819            AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
erotD4_1022         AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
erotD6_1046         AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
erot_926            AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
esesD3_884          AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
erotP4_934          AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
ealt_682            AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
esesP6_871          AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
egod_820            AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
erotP1_1050         AGTATACCAACTTTATTAGAAACAATTTTATACACTACTATAATATAACACGAATAGAGA
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efist_1086          GTATGGTAAGTAAGAAATAAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
ecan_598            GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
eleu_1125           GTATGGTAAGTAAGAAATTGATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
esesD1_861          GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
esesP2_824          GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
erotP8_972          GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
ecomp_1129          GTATGGGAAGTAAGAAATTGATTTCTTACTTCCCATACTCTCGGATCTCATAGAATACCG
erotP9_979          GTATGGTAAGTAAGAAATTGATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
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esesP7_917          GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
esesP3_843          GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
erotP13_1088        GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
esesD8_1096         GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
erotD5_1028         GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
echin_531           GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
emoh_1126           GTATGGTAAGTAAGAAATTGATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
erotD1_993          GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
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esesD7_910          GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
epur_500            GTATGGTAAGTAAGAAATAAATTTCTTACTTACCATACTCTCGGATCTCGTAGAATACCG
epil_1048           GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
esesP5_855          GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
erotP10_982         GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
erotP6_953          GTATGGTAAGTAAGAAATTGATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
erot_ovat1124       GTATGGTAAGTAAGAAATTGATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
eper_465            GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
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erotP11_992         GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
erotD2_999          GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
egod_822            GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
eserot_899          GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
egod_1077           GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
erotP7_963          GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
erotP12_1081        GTATGGTAAGTAAGAAATTGATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
epil_1047           GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
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ehys_870          GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
erotP5_952        GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
egod_821          GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
esesD4_897        GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
erotP3_922        GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
egod_823          GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
erotD3_1015       GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
ealb_981          GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
egod_819          GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
erotD4_1022       GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
erotD6_1046       GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
erot_926          GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
esesD3_884        GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
erotP4_934        GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
ealt_682          GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
esesP6_871        GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
egod_820          GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
erotP1_1050       GTATGGTAAGTAAGAAATCAATTTCTTACTTACCATACTCTCGGATCTCATAGAATACCG
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esesD2_874        CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
efist_1086        CCTATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTGATTT
ecan_598          CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
eleu_1125         CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
esesD1_861        CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
esesP2_824        CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
erotP8_972        CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
ecomp_1129        CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTGTATTT
erotP9_979        CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
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esesP7_917        CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
esesP3_843        CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
erotP13_1088      CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
esesD8_1096       CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
erotD5_1028       CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
echin_531         CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
emoh_1126         CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
erotD1_993        CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
esesP1_1075       CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
esesD6_908        CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
esesD7_910        CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
epur_500          CCTATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTGATTT
epil_1048         CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
esesP5_855        CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
erotP10_982       CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
erotP6_953        CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
erot_ovat1124     CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
eper_465          CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
esesD5_902        CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
erotP2_1059       CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
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erotD2_999        CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
egod_822          CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
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epil_1047         CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
ehys_870          CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
erotP5_952        CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
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esesD4_897          CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
erotP3_922          CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
egod_823            CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
erotD3_1015         CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
ealb_981            CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
egod_819            CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
erotD4_1022         CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
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erot_926            CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
esesD3_884          CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
erotP4_934          CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
ealt_682            CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
esesP6_871          CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
egod_820            CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
erotP1_1050         CCGATTATAGCCCGTTGATTTCATTTAAGACGCAAAAATAGAATACTTTTCATTTTATTT
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esesD2_874          ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
efist_1086          CTTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAAGTAA
ecan_598            ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
eleu_1125           ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
esesD1_861          ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
esesP2_824          ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
erotP8_972          ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
ecomp_1129          ATTCAACTATTGATAAGAATTCCGAAGCCAAGTTTCATTTCAACTAA
erotP9_979          ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
esemi_1030          ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
esesP7_917          ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
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erotP13_1088        ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
esesD8_1096         ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
erotD5_1028         ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
echin_531           ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
emoh_1126           ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
erotD1_993          ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
esesP1_1075         ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
esesD6_908          ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
esesD7_910          ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
epur_500            CTTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAAGTAA
epil_1048           ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
esesP5_855          ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
erotP10_982         ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
erotP6_953          ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
erot_ovat1124       ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
eper_465            ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
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erotD2_999          ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
egod_822            ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
eserot_899          ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
egod_1077           ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
erotP7_963          ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
erotP12_1081        ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
epil_1047           ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
ehys_870            ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
erotP5_952          ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
egod_821            ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
esesD4_897          ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
erotP3_922          ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
egod_823            ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
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ealb_981            ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
egod_819            ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
erotD4_1022         ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
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erot_926            ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
esesD3_884          ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
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esesP6_871          ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
egod_820            ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
erotP1_1050         ATTCAACTATTGATAAGAATTCAGAAGTCAAGTTTCATTTCAACTAA
                     ********************* **** ************** ***
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APPENDIX B-12: *Eupatorium rotundifolium* and *E. sessilifolium* ISSR NEXUS files for PAUP*

Eupatorium rotundifolium

```
#NEXUS

begin taxa;
 dimensions ntax=128;
 taxlabels

RD1993 RD1994 RD1995 RD2997 RD2998 RD2999 RD21000 RD21001 RD21002 RD21003
RD21004 RD21005 RD21006 RD31007 RD31008 RD31009 RD31010 RD31011 RD31012
RD31013 RD31014 RD31015 RD31016 RD41019 RD41020 RD41021 RD41022 RD41023
RD41024 RD41025 RD41026 RD41027 RD51028 RD51029 RD51030 RD51031 RD51032
RD51034 RD51035 RD51036 RD61037 RD61038 RD61039 RD61040 RD61041 RD61042
RD61043 RD61044 RD61045 RD61046 RP11050 RP11051 RP11052 RP11053 RP11054
RP11055 RP11056 RP21059 RP21060 RP21061 RP21062 RP21063 RP21064 RP3922 RP3923
RP3924 RP3925 RP3926 RP3927 RP3928 RP3929 RP3930 RP3931 RP4932 RP4933 RP4934
RP4935 RP4936 RP4937 RP4938 RP4939 RP4940 RP4941 RP5948 RP5949 RP5950 RP5951
RP5952 RP6953 RP6954 RP6955 RP6956 RP6958 RP6959 RP6961 RP6962 RP7963 RP7964
RP7965 RP7966 RP7967 RP7970 RP8971 RP8972 RP8973 RP8974 RP9978 RP9979 RP9980
RP10982 RP10983 RP10984 RP11988 RP11989 RP11990 RP11991 RP11992 RP121078
RP121079 RP121080 RP121081 RP121082 RP121083 RP121084 RP131088 RP131089
RP131090 RP131093
   ;
 end;

begin characters;
 dimensions nchar=218;
 format symbols= "01";
 ;

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RD2998
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RP121082
111000110001000010101001010001000010000000001000100000000000000001010001000100010000001010000100010000000000110000110000000000110000100000000100001000010100010100001000010000010100000000000000001
RP121083
111000110001000010101001010001000010000000001000100000000000000001010001000100010000001010000100010000000000110000110000000000110000100000000100001000010100010100001000010000010100000000000000001
RP121084
111000110001000010101001010001000010000000001000100000000000000001010001000100010000001010000100010000000000110000110000000000110000100000000100001000010100010100001000010000010100000000000000001
RP131088
000000010001000010001000000000100000000000000010000001000000000000001010000100000010000100100000100001000000000110000110001000100000100001000010100010100001000010000010100000000000000001
RP131089
000000010001000010001000000000100000000000000010000001000000000000001010000100000010000100100000100001000000000000000100010000100000100001000010100010100001000010000010100000000000000001

RP131090
00000000000100001000100000000100000000000000100000010000000000000101000010000001000001
00100001010000000000000000000001010010010000000001001000010000010000000001100001100010
00100100000100110000000100010000001000100000001
RP131093
00000001000110001000100000000100000001000000100000010000000000000100000010000001000001
00100010010000000010000000000000000010000010000000001000010000010000000000100000100010
00100100000100100001000100010000001000100000001
;
end;

Eupatorium sessilifolium

#NEXUS

begin taxa;
 dimensions ntax=71;
 taxlabels

SD1861 SD1862
SD1863 SD2874 SD2875 SD2876 SD2877 SD2878 SD2879 SD2880 SD2882 SD2883
SD3884 SD3885 SD3886 SD3887 SD3888 SD3889 SD3892 SD3893 SD4895 SD4896
SD4897 SD4898 SD5902 SD5903 SD6904 SD6905 SD6906 SD6907 SD6908 SD6909
SD7910 SD7911 SD7912 SD7913 SD7914 SD81094 SD81096 SD81097 SD81098
SD81099 SD81100 SP11075 SP2824 SP2825 SP2826 SP2827 SP3830 SP3843 SP3844
SP3845 SP3846 SP4847 SP4849 SP4850 SP4851 SP4852 SP4853 SP5854 SP5855
SP5856 SP6865 SP6866 SP6867 SP6871 SP6872 SP6873 SP7917 SP7918 SP7919
;
 end;

begin characters;
 dimensions nchar=157;
 format symbols= "01";
 ;

matrix

SD1861
00001001101001000101000101001010100000000000001100100001000100001000000001000000100100
00100111010100001001000001000000000100000110011000100100001010000000000

SD1862
00001001101001000101000101001010100000000000001100100001000100001000000001000000100100
00100111010100001001000001000000000100000110011000100100001010000000000

SD1863
00001001101001000101000101001010100000000000001100100001000100001000000001000000100100
00100111010100001001000001000000000100000110011000100100001010000000000

SD2874
00000100001010010000001001000100000000000000101100100000010001000100001010010000100100
10000100000000010000100000000000000011000010001100010010010001100001000

SD2875
0000010101101001000100100100010000000000000010110010010001000100010000001001000000010010000101000000100001000000000000000011010010001100010010010000101001000

SD2876
0000010000101001000100100100010000000000000000010010000000001000100000010010000000100100000101000001000010000000000000000010000100011000100100100011010010000

SD2877
0000010101101001000000000100010000000000010011001001000000100010001000100100100001000001000001010000000000010000000000100001101001000110001001001000010000010000

SD2878
0000010001101000000000000100110000000000000001001001001000100010001000000100100000001000010000010000010000000000000010000110100100011000100100100011000000000

SD2879
0000010101101000000100100100010000000000000011011001000000100010001000000100100001001000010000010000010000000000010000000000100001100001000110001001001000010100000000

SD2880
0000010001101000000000100100010000000000000000011101000000100010001000000100100001001000010000010000010000000000010000000000100001100001000110001001001000010100000000

SD2882
0000010001101001000100100100110000000000100110111001001000100011001000100100100101001000100100000100000100000000010000000000100001101001000110001001001000011010000000

SD2883
0000010001101001000100100100010000000000000110110010000001000100010001001001000000010010000100000100000100000000010000011010010001100010010010000110000010000

SD3884
0000010011000010000001000100000000000000001000110010100000100010001000000000010001000010000100000100000100000000010000110100010000001000000000000100110100100001010001000000010000100000

SD3885
1000010011000010010001000100000000000000000011100100001000010000010001000000100000010000001000000100100000100000000010000110100010000001010000000000000001101001000010100010000001000000100100

SD3886
0000010011000010010000010100000000000000000000011010000100000010001000000001000101000000100000100000100000000010000110100010000001010000000000000001101001000010101001000000001000000000

SD3887
0000010001000010000001000100010000000100100001100100001001010000100000000100000100000100000100011001001000010100010000010010010000000

SD3888
0000010000000001000000100010001000000010010000110010000100101000001000000001000000000000100001101010100000001000000000000010111010010000101000100000000000000001000

SD3889

1000010011000010010001000100010000000000000011100100001001010000010000000010100000000010001100010100000001000000000000101110100100001010001000000000101001000

SD3892
0000010011000010010001000100010000000000000011100100001001010000010000000010100000000010001100010000000001000000000000101110100100001010001100000000001001000

SD3893
0000010011000010010001000100010000000000000011100000001001010000010000000000000000000010001100000000000101000000000000100100100100001010001100001001101000000

SD4895
0001000001010000010100000000100000000000000010101000001001000010000000000000000001000000100000010000010000000100000000000000010000000001100000010000000100010000

SD4896
0000000001010000010100000000100000000000000001010000000010000100000000000000000000000100100000001001000010010000000000000000010000000001100000010000000100010000

SD4897
1001001001010000001001000100100000000000000010101000001001000010000010000010010000001001000001001000010010000000000000010010010001100010010000000100010000

SD4898
0011001001010000010101000100101000000000000010110000000001000010000001000000001000001000100001001000001001000000000000010010010001100010010000000100010000

SD5902
0000010001100000100000010100001000000000000000110010000001000100000100000001000100010000100101000000101010000000000000100010001100010010000010000010100000

SD5903
0000010001100000100000010100001000000000000000110010000001000000000100000000000000010000100101000000101010000000000000100010001100010010000010000010100000

SD6904
0000010000100000110010000100000000000011000001110100010010010000010000001010000010000010000010000010000110010000100000000000000100001000011000100100010000110000000

SD6905
0000000000100000110000010100010000000011000001110100000010010000010000000000000010000010000010000010000110010000100000000000000100001000011000100100010000110000000

SD6906
0000000000100000110010000100010000000000000011001000100000100000100000000000000010000010000010000010000110010000100000000000000100001000011000100100010000110000000

SD6907
0000000000100000110000010100010000000000000011001000000000100000100000000000000010000010000010000010000110010000100000000000000100001000011000100100010000110000000

SD6908
0000010000100000110010010100010000000001000101110100000001001000011000000100000001000001000010000010000110010000100000000000000100110001100000010001000011000000

SD6909
0000010000100000110010010100010000000011000101110100000010010000110000001010000010000010001001000010001001000000000000000000100110001100000010001000011000000

SD7910
0000010100100000100001000010010000001001001001101000001000010101000000000000000010000100100001010010001000000100000001001000000110001001100010010011000000

SD7911
0000010000100000100000010010010000001000000000101000001000010001000000000000000000000000000000001000001010010001000000100000000000000000000100010001000100100100000

SD7912
0000010100100000100000010010010100011000000011101000001000010001000000000000000000000000000000010000010100100010000001000000010010010001100010011000100100110000

SD7913
0000010100000000100001010010010000001000000000110100000100001010100000000000000000000000000000000000001000001001000100000001000000000000000000010001001100010010010000000

SD81094
0000011101110100010001001000010000000001000011100010000001001000001000010000000000101001000011100000100010010000000000100001000001100010100001000100010001000101000

SD81096
0000011101110100010111001000010000000001101011101010100001001000101000010100100001010001000011100110000010010000000001001010000011000101000010001000100010100

SD81097
0100011101110100010100001000010100000001101011101010000001001000101000010100000101010001000011100110000000100000000010010100000100001000000100010001000101000

SD81098
0000010101110100010000001000010000000101000000101010000001001000101000010000100101000001000011100110000000100000000010000100000100001010000100010001000101000

SD81099
0000010101110100000011001001010100000001000001100010000000100100001000001000000000010100100011100000100000010000000100000000000000010000001000101000010001000101000

SD81100
0110010101110100000011001001000100000101101010101010000000100100001000010100100101010010001110000010000001000000010000000001001010000111000101000010001000101000

SP11075
0000010001010000100100101000010000100000000010111001000000100100000100000000000001000010011000101000010001000000100000000000000000100001001100010100001000000100

SP2824

0001000100010000100100010100101000101000000010100001000100010000001000000000000100001000100011000100010000100010100000000100000101000001100101000001010000110

SP2825
0001000100010000100100010100101000101000000010100001000100010000001000000000000100001000100011000100010000100010100000000100000101000001100101000001010000110

SP2826
0001000100010000100100010100101000101000000010100001000100010000001000000000000100001000100011000100010000100010100000000100000101000001100101000001010000110

SP2827
0001000100010000100100010100101000101000000010100001000100010000001000000000000100001000100011000100010000100010100000000100000101000001100101000001010000110

SP3830
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SP3843
0000010000101000100000010101010000111000000010100001000100000100001000000000000100001000101000100100001011001000000001010101010001000011010010010100000100

SP3844
0000010000101000100100010101010000111000000000100001000100000100001000000000000100000000101000100100001011101000000000010001010001000011010010010100000100

SP3845
0000010000101000100100010101010000111000000010100001000100000100001000000000000100001000101000100100001011101000000001010001010001000011010000010100000100

SP3846
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SP4847
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SP4849
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SP4850
0000010001011000010000010101010000001000000000110001000000101000010000000000010000001000010001001000000000

SP4851
0000010001011000010000010101010000001000000000110001000000101000010000000000010000001000010001001000000000

SP4852
0000010001011000010000010101010000001000000000110001000000101000010000000000010000001000010001001000000000

SP4853
0000010001011000010000010101010000001000000000011000100000001010000100000000000010000001
0010000100000100101000111000100000000000001000011001000100010010000000000

SP5854
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0010010100000100000010000001000000010101010100100010010000000100100000000

SP5855
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0001010010010000000010000001000010010101010100100010010100000100100000000

SP5856
0000010000110000100101011101000100110000000000100001000100100010000000000000000000101000
0001010010010000000010000001000010010101010100100010010100000100100000000

SP6865
0000010001111000000010101000001001011000000000010001000100001000010000000000000000100000
0010011010010000100100000000000001101000101010010001001000000010100000011

SP6866
0000010001111000000010101000001001011000000000010001000100001000010000000000000000100000
0010011010010000100100000000000001101000101010010001001000000010100000011

SP6867
0000010001111000000010101000001001011000000000010001000100001000010000000000000000100000
0010011010010000100100000000000001101000101010010001001000000010100000011

SP6871
0000010001111000000010101000001001011000000000011000100010000100001000000000000000100000
0010011010010000100100000000000001101000101010010001001000000010100000011

SP6872
0000010001111000000010101000001001011000000000011000100010000100001000000000000000100000
0010011010010000100100000000000001101000101010010001001000000010100000011

SP6873
0000010001111000000010101000001001011000000000011000100010000100001000000000000000100000
0010011010010000100100000000000001101000101010010001001000000010100000011

SP7917
0000000000100000100010100100010010011000000000100001000000010010001000000000000000000000
1010001100000100001001101001100000010001010100100100010000011010010000000

SP7918
0000000000100000100010100100010010011000000000100001000000010010001000000000000000000000
1010001100000100001001101001100000010001010100100100010000011010010000000

SP7919
0000000000100000100010100100010010011000000000100001000000010010001000000000000000000000
1010001100000100001001101001100000010001010100100100010000011010010000000

;
end;

VITA

Kunsiri Chaw Siripun was born in Bangkok, Thailand. She finished her preliminary studies in Bangkok. She received the Bachelor of Science degree in General Science from Kasetsart University in 1992 and later completed her Master of Science degree in Botany from the same university in 1997. During her master's program she had an opportunity to be an exchange student and studied at Tsukuba University in Japan for one year. In 1998 she started working at Kasetsart University, Kamphangsean Campus with the Faculty of Liberal Arts and Science as a Botany Lecturer.

She received a scholarship from Royal Thai Government to pursue doctoral studies and came to University of Tennessee, Knoxville in Fall 1999. She joined the Botany Department and received her PhD degree in Spring 2004. While at the University of Tennessee, she worked as a graduate assistant and graduate teaching assistant. She is a member of Botanical Society of America.