

# Identity and delimitation of the American species of *Litsea* Lam. (Lauraceae): a morphological approach

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**Abstract** The genus *Litsea* in America is a small group of species with high variability that has not been evaluated rigorously; authors recognize either three or as many as 11 species because of the unclear delimitation of taxa. Our focus in this study was to resolve the number, names, and distinguishing features of the American species of *Litsea*, based on strict morphological analyses, namely Population aggregation analysis and Multivariate analyses. Population aggregation analysis revealed a large number of polymorphisms in all populations; nevertheless, *L. aestivalis* was distinguished from the other species. Ordination of character variability by principal components analysis and non-metric multidimensional scaling supported the distinction of seven additional species. Finally, eight species of *Litsea* are recognized in America and a key for their identification is provided.

**Keywords** Morphological characters · Multivariate analysis · Non-metric multidimensional scaling · Population aggregation analysis · Principal components analysis

## Introduction

The genus *Litsea* Lam. includes approximately 400 species of dioecious trees and shrubs that mainly inhabit the

tropical and subtropical regions of Asia, the Malayan archipelago, Australia, New Zealand, and other islands in the Pacific basin. A few species are distributed in continental America from the east coast of the United States, most of the Mexican territory (except for the California and Yucatan peninsulas) and in mountainous areas of Central America from Guatemala to Costa Rica (Mez 1889; Allen 1945; Rohwer 1993). Thus, the genus presents the classic pattern of intercontinental disjunction between the eastern areas of North America and Asia (Boufford and Spongberg 1983; Tiffney 1985a, b; Parks and Wendel 1990).

The American species of *Litsea*, except for *L. aestivalis* (L.) Fernald, grow in pine–oak forest, cloud forest, and rarely in the boundaries of tropical dry forest, between 1,000 and 3,000 m of altitude. *Litsea aestivalis* inhabits coastal areas of Alabama, North Carolina, South Carolina, Florida, Georgia, and Virginia, from 10 to 200 m of altitude (Van der Werff 1997).

Some efforts have been made to clarify the taxonomic history and phylogenetic relationships of *Litsea* (Hyland 1989; Li et al. 2004; Fijridiyanto and Murakami 2009), however, these issues are still confusing and controversial. Even in the small American group, taxonomic treatments over the years show strong discrepancies in the recognition of species and subspecies.

Since the revision of the Lauraceae by Meisner (1864), who placed the American species of *Litsea* in *Tetranthera*, and recognized five species, namely *T. californica* Hook. et Arn. (currently *Umbellularia californica* (Hook. et Arn.) Nutt.), *T. geniculata* (Walter) Nees, *T. glaucescens* (Kunth) Spreng. with four varieties (*T. glaucescens* var. *subsolitaria* Meisn. (hereafter cited as var. *glaucescens*, in conformity with nomenclatural rules), *T. glaucescens* var. *subcorymbosa* Meisn., *T. glaucescens* var. *racemosa* Meisn., and *T. glaucescens* var. *major* Meisn.), *T. neesiana*

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Schauer with two varieties (*T. neesiana* var. *corymbifera* Meisn. and *T. neesiana* var. *villosa* (M. Martens et Galeotti) Meisn.), and *T. berteroi* Spreng. (apparently *T. laurifolia* Jacq. (= *Litsea chinensis* Lam.), an introduced species from Asia (Mez 1892)), this group has undergone numerous changes and additions.

Mez (1889) distinguished, now in *Litsea*, six species for America; he retained *L. geniculata* (Walter) G. Nicholson (= *L. aestivalis*), *L. glaucescens* Kunth, and *L. neesiana* (Schauer) Hemsl., and added *L. orizabae* Mez, *L. parvifolia* Mez, and *L. guatemalensis* Mez as new species. Later, Bartlett (1909) recognized 11 species. To the six already recognized by Mez, he added *L. flavescens* Bartlett, *L. novoleontis* Bartlett, *L. pedicellata* Bartlett, *L. pringlei* Bartlett, and *L. schaffneri* Bartlett. The most recent treatment is that by Allen (1945), who accepted four species for Mexico and Central America: *L. muelleri* Rehder, *L. pringlei* (including *L. novoleontis*), *L. parvifolia* (including *L. pedicellata*), and *L. glaucescens* with three varieties—*L. glaucescens* var. *glaucescens*, *L. glaucescens* var. *schaffneri* (Bartlett) C. K. Allen, and *L. glaucescens* var. *flavescens* (Bartlett) C. K. Allen. She subsumed *L. neesiana*, *L. guatemalensis*, and *L. orizabae* as synonyms of *L. glaucescens* var. *glaucescens*.

Regardless of the last revision of the group, there is not yet any consensus on the number of *Litsea* species present in America. Local botanists still apply names to specimens of *Litsea* following Mez or Bartlett instead of Allen, showing they recognize more species than Allen did. In practice, three species complexes can be recognized: the *L. glaucescens* complex (*L. glaucescens* + *L. guatemalensis* + *L. flavescens* + *L. neesiana* + *L. orizabae* + *L. schaffneri*), the *L. parvifolia* complex (*L. pedicellata* + *L. parvifolia*), and the *L. pringlei* complex (*L. pringlei* + *L. novoleontis*). In addition, *Litsea muelleri* seems to be a species clearly different from the others.

Particularly complicated has been the distinction of species within the *L. glaucescens* complex, asserted to be the most widely distributed group of species in the continent (from northern Mexico to Costa Rica). Characters that have been used in several treatments to distinguish species in this group, mainly the form and size of the leaves, and the density of indument on different structures, are very variable. So, while some authors (Meisner 1864; Mez 1889; Bartlett 1909) recognize more than one species in the group, others consider it just as a highly polymorphic species (Allen 1945; van der Werff and Lorea 1997). A similar situation exists in the group of species of northwestern Mexico, where the size of the leaves, rather than the form, has been critical for the recognition of species; such is the case for *L. pringlei*, *L. novoleontis*, *L. parvifolia*, and *L. schaffneri*.

Although high morphological variability has been recognized as the main problem when discerning the number of taxa (Allen 1945, pages 407–409), it has not been

examined rigorously. Recognition of species within the group is currently truly arbitrary. Thus, our focus in this study was to resolve the number, names, and distinguishing features of the American species of *Litsea*, on the basis of strict morphological analyses.

## Materials and methods

A collection of 478 specimens borrowed from several herbaria (see Acknowledgments) was examined in this study. Studied material is listed in Appendix 1. This material covered almost the entire geographic range of the group (no samples from El Salvador and Honduras were available). A set of 36 morphological characters (vegetative and reproductive) was evaluated, including those traditionally used for the recognition of species. Further, a survey of leaf cuticle was carried out in order to consider features previously not assessed in the recognition of species in the group (particular results of this analysis will be published elsewhere). Thus, three characters of leaf epidermis were added to the study, making a total of 39 characters (Table 1).

Values in the counts of secondary veins, flowers, stamens and staminodia are the average of at least three measurements (depending on available material). Percentage of indument density was calculated as the amount of foliar surface covered with indument in a given radial section of the field of the microscope. The continuous variables were measured with callipers or a ruler. Cuticle impressions were made with silicone fluid, following the technique described by Sandoval (2005).

Two different types of analysis were used to resolve the delimitation of American species in *Litsea*. A first approach was achieved applying a Population aggregation analysis (Davis and Nixon 1992). Then, with a different perspective, two multivariate analyses were performed. These had the purpose of assessing the correspondence of taxonomic schemes suggested by Mez (1889), Bartlett (1909), and Allen (1945), with the grouping produced by a phenetic clustering method. In addition, a taxonomic proposal of our own developed during the morphological review of specimens (which considers *L. glaucescens*, *L. guatemalensis*, *L. muelleri*, *L. neesiana*, *L. orizabae*, *L. pringlei*, *L. parvifolia*, and *L. schaffneri* as distinct species) was also evaluated.

### Population aggregation analysis (PAA)

This method implies comparisons of the distribution of character states between populations and involves the search for fixed attributes that eventually, based on the differences among them, indicate the different species. To do so, 45 populations were defined across the geographic

**Table 1** Morphological and anatomical characters of *Litsea* considered in the population aggregation analysis

## Characters relative to leaves

1. Persistence of leaves: 0, persistent; 1, deciduous
2. Shape of leaf blade: 0, elliptic; 1, ovate; 2, rounded
3. Shape of leaf base: 0, attenuate-acute; 1, obtuse-cordate
4. Shape of leaf apex: 0, acuminate; 1, acute; 2, obtuse
5. Foliar mucro: 0, absent; 1, present
6. Color of adaxial surface of leaves: 0, green; 1, white
7. Consistency of leaves: 0, membranous; 1, chartaceous; 2, coriaceous
8. Indument on abaxial surface of leaf blade: 0, absent; 1, scattered; 2, dense
9. Type of indument on abaxial surface of leaf blade: 0, pubescent; 1, tomentose
10. Indument on adaxial surface of leaf blade: 0, absent; 1, scattered; 2, dense
11. Type of indument on adaxial surface of leaf blade: 0, pubescent; 1, tomentose
12. Color of indument of leaf blade: 0, translucent; 1, cinereous; 2, ochraceous-ferruginous
13. Distribution of indument on adaxial surface of leaf blade: 0, near the base; 1, on entire blade
14. Indument on abaxial surface of leaf blade: 0, absent; 1, scattered; 2, dense
15. Indument on adaxial surface of midvein: 0, absent; 1, scattered; 2, dense
16. Distribution of indument on adaxial surface of midvein: 0, only within proximal half; 1, along all midvein
17. Indument on petiole: 0, absent; 1, scattered; 2, dense
18. Type of indument on petiole: 0, pubescent; 1, tomentose
19. Color of indument on petiole: 0, translucent; 1, cinereous; 2, ochraceous-ferruginous
20. Distribution of indument on petiole: 0, near blade insertion; 1, on all petiole

## Characters relative to inflorescences and flowers

21. Position of inflorescences: 0, solitary; 1, racemose
22. Indument on peduncle: 0, absent; 1, scattered; 2, dense
23. Type of indument on peduncle: 0, pubescent; 1, tomentose
24. Orientation of indument on peduncle: 0, appressed; 1, ascending to erect
25. Color of indument on peduncle: 0, translucent; 1, cinereous; 2, ochraceous-ferruginous
26. Indument on male inflorescence bracts: 0, absent; 1, scattered; 2, dense
27. Type of indument on male bracts: 0, pubescent; 1, tomentose
28. Indument on female inflorescence bracts: 0, absent; 1, scattered; 2, dense
29. Type of indument on female bracts: 0, pubescent; 1, tomentose
30. Indument on male flower pedicel: 0, absent; 1, scattered; 2, dense
31. Type of indument on male flower pedicel: 0, sericeous; 1, other
32. Shape of male flower pedicel: 0, obconic; 1, cylindrical

**Table 1** continued

33. Indument on abaxial surface of male flower tepals: 0, absent; 1, scattered; 2, dense

34. Indument on adaxial surface of male flower tepals: 0, absent; 1, scattered; 2, dense

## Characters relative to branchlet

35. Indument on branchlets: 0, absent; 1, scattered; 2, dense

36. Type of indument on branchlet: 0, pubescent; 1, tomentose

## Characters relative to foliar epidermis

37. Epidermal cell contour on adaxial surface: 0, polygonal; 1, sinuous

38. Epidermal cell contour on abaxial surface: 0, polygonal; 1, sinuous

39. Relative size between epidermal cells of adaxial and abaxial surface: 0, not similar; 1, similar

distribution of the genus using the boundaries of major river basins, plus other topographic features (like mountain ranges within basins) that may act as natural limits for distribution (Fig. 1). Profiles of character states for each population were constructed and were compared with each other (Table 2). In this case the whole set of 39 characters was used.

## Multivariate analyses

*Principal components analysis (PCA)*

This part of the study is based on a subset of the specimens examined in the previous analysis, which were chosen for being fertile and representative of the whole morphology to be compared (Appendix 1). Three matrices of data were constructed, because exclusive characters for each group were measured; one containing 85 flowering male specimens and 33 variables, a second matrix with 55 flowering female samples and 27 variables, and a third with 87 specimens with fruits and 28 variables (Table 3; data matrices are available from the corresponding author). The specimens examined represent all the entities proposed in the different taxonomic treatments (except for *L. aestivalis*), and covered almost all the geographical distribution registered for the group. The analysis was executed by use of the software Statistica v. 6.0 (StatSoft Inc. 1998) for each of the matrices. Because the variables were of different types, the data were transformed by columns to log<sub>10</sub> (length measures), arc sine (ratio measures), or square root (counts), and the analyses were based on matrices of correlations.

*Non-metric multidimensional scaling (NMDS)*

A basic matrix of 90 specimens and nine qualitative characters (Table 4; the matrix is available from the

corresponding author) was constructed for this analysis. Later a similarity matrix was calculated using percentage disagreement as measurement of distance, because this is recommended for categorical variables. The analysis was executed by use of the software Statistica v. 6.0 (StatSoft Inc. 1998).

The analyses were executed making no assumption about the identity of specimens. Later, on the resulting graphs, putative names according to the different taxonomic schemes were added for each specimen.

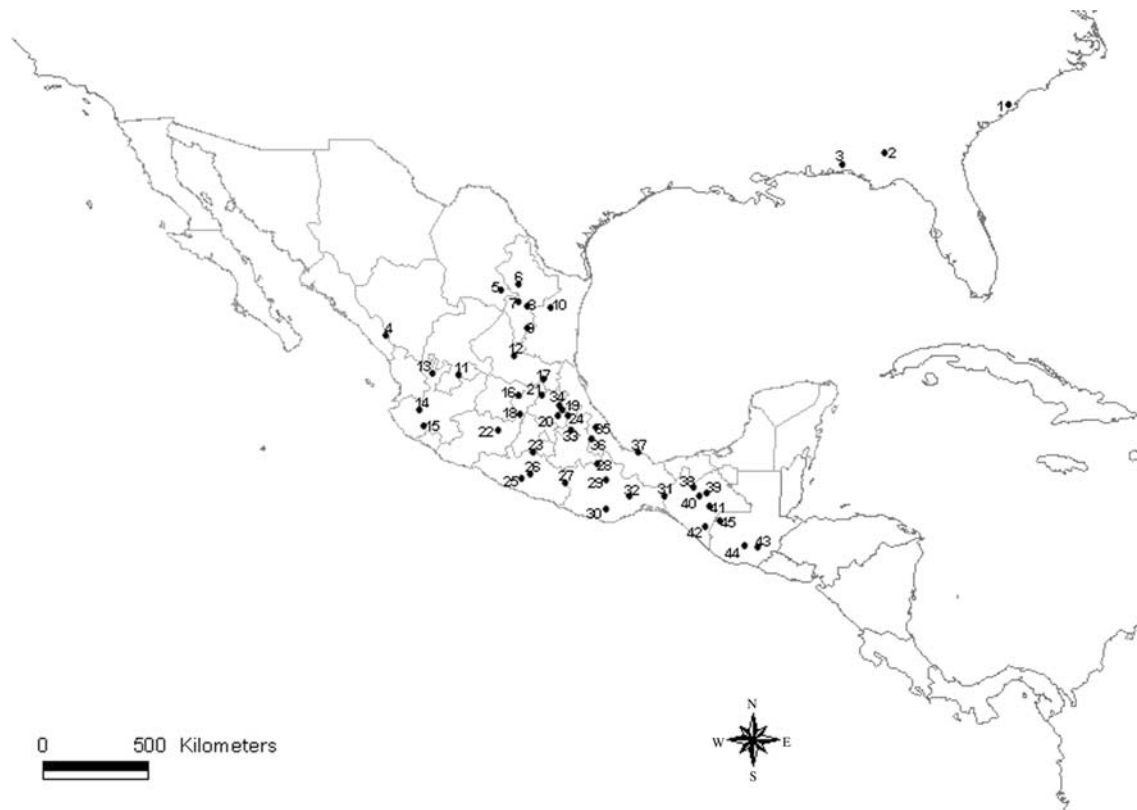
The differences among the resulting groups were subsequently tested for significance with ANOSIM (an analysis of similarity) using Primer v 5. The *R* statistic generated by ANOSIM is a relative measurement of separation of the a priori-defined groups and ranges from  $-1$  to  $+1$ . A value of  $-1$  indicates that all the samples within the groups are less similar to one another than to any other sample from different groups; zero (0) indicates that there are no differences between the groups; and a value of  $+1$  indicates that all the samples within each group are more similar among themselves than to any other sample of a different group (Clarke and Gorley 2001). ANOSIM test of groups derived from PCA was based on the normalized euclidean distance matrix, whereas for groups obtained by NMDS it was based on the original distance matrix.

## Results

### Population aggregation analysis

Population profiles show that six of the characters considered are fixed—the deciduous character and membranous consistency of the leaves, the absence of a mucro, indument on the midvein only towards the base on the lower surface, indument on the proximal section of the petiole, and the difference in size of the epidermal cells between the upper and lower surfaces (Table 2). These attributes clearly separate *L. aestivalis* from the other species.

For the other records, the large number of polymorphisms in all populations and all characters is notorious. Characters such as the form of the lamina (2), the shape of the foliar base (3) and apex (4), indument type (9, 10) and color (12), and type of inflorescence (21), that have traditionally been used to distinguish species of *Litsea* in America are not fixed and were found to be very variable within populations. This situation made it impossible to group populations. Only populations 20 and 23, which correspond to the center of Hidalgo and some localities of Guerrero, State of Mexico and Morelos, were identical. No other group was found with this analysis.



**Fig. 1** Map showing location of 45 populations of *Litsea* studied with PAA. The numbers correspond to the populations indicated in [Appendix 1](#)

**Table 2** Populations character profiles in *Litsea*

Population(no. specimens)	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1,2,3(2,2,2)	1	3	1	0	0	0	0	0	na	1	1	0	0	0	2	0	1	1	0	0	±
4(4)	0	0	±	0	1	1	1	±	0	±	±	±	1	±	±	1 <sup>a</sup>	±	0 <sup>a</sup>	±	1 <sup>a</sup>	1
5(4)	0	±	±	1	±	1	2	±	0 <sup>a</sup>	±	0 <sup>a</sup>	0 <sup>a</sup>	1 <sup>a</sup>	±	±	1 <sup>a</sup>	±	0 <sup>a</sup>	0 <sup>a</sup>	1 <sup>a</sup>	±
6(25)	0	±	±	±	1	±	2	0	na	0	na	na	na	0	0	na	±	0 <sup>a</sup>	0 <sup>a</sup>	1 <sup>a</sup>	±
7(15)	0	±±	±	±	1	±	2	±	±	±	±	0	1	±	±	1	±	±	±	1	±
8(20)	0	±	±	±	1	±	2	0	na	±	±	0 <sup>a</sup>	1 <sup>a</sup>	0	±	1 <sup>a</sup>	±	1 <sup>a</sup>	0 <sup>a</sup>	1 <sup>a</sup>	±
9(23)	0	±	0	±	1	±	±	±	0 <sup>a</sup>	±	1 <sup>a</sup>	0 <sup>a</sup>	1 <sup>a</sup>	±	±	1 <sup>a</sup>	±	1 <sup>a</sup>	0 <sup>a</sup>	1 <sup>a</sup>	±
10(6)	0	0	0	1	1	1	±	0	na	0	na	na	na	0	0	na	0	na	na	na	±
11(5)	0	0	±	0	1	±	1	±	0 <sup>a</sup>	±	0 <sup>a</sup>	0 <sup>a</sup>	1 <sup>a</sup>	±	±	1 <sup>a</sup>	±	0 <sup>a</sup>	0 <sup>a</sup>	1 <sup>a</sup>	0
12(14)	0	±	0	0	1	±	±	0	na	0	na	na	na	0	0	na	0	na	na	na	±
13(4)	0	0	0	0	1	±	1	0	na	±	0 <sup>a</sup>	0 <sup>a</sup>	1 <sup>a</sup>	±	±	1 <sup>a</sup>	±	0 <sup>a</sup>	0 <sup>a</sup>	1 <sup>a</sup>	±
14(16)	0	0	0	0	1	±	1	0	na	±	0 <sup>a</sup>	0 <sup>a</sup>	1 <sup>a</sup>	0	±	1 <sup>a</sup>	±	1 <sup>a</sup>	0 <sup>a</sup>	1 <sup>a</sup>	±
15(8)	0	0	±	0	1	±	1	0	na	0	na	na	na	0	0	na	±	0 <sup>a</sup>	0 <sup>a</sup>	1 <sup>a</sup>	±
16(8)	0	±	0	0	1	±	±	0	na	0	na	na	na	0	0	na	0	na	na	na	±
17(25)	0	±	±	0	1	±	1	0	na	±	0 <sup>a</sup>	±	1 <sup>a</sup>	±	±	1 <sup>a</sup>	±	0 <sup>a</sup>	±	1 <sup>a</sup>	±
18(2)	0	0	0	0	1	±	2	0	na	0	na	na	na	0	0	na	0	na	na	na	1
19(2)	0	0	0	1	1	1	1	0	na	±	0 <sup>a</sup>	0 <sup>a</sup>	1 <sup>a</sup>	0	±	1 <sup>a</sup>	±	0 <sup>a</sup>	0 <sup>a</sup>	1 <sup>a</sup>	1
20,23(7,7)	0	0	0	0	1	±	1	0	na	±	0 <sup>a</sup>	0 <sup>a</sup>	1 <sup>a</sup>	±	±	1 <sup>a</sup>	±	0 <sup>a</sup>	0 <sup>a</sup>	1 <sup>a</sup>	±
21(12)	0	0	0	0	1	±	±	±	±	±	±	±	1 <sup>a</sup>	±	±	1	±	±	2 <sup>a</sup>	1 <sup>a</sup>	±
22(3)	0	0	0	0	1	1	1	0	na	±	0 <sup>a</sup>	0 <sup>a</sup>	1 <sup>a</sup>	0	±	1 <sup>a</sup>	±	0	±	1	0
24(5)	0	0	0	0	1	±	1	0	na	0	na	na	na	0	0	na	0	na	na	na	1
25(8)	0	0	0	0	1	±	1	0	na	0	na	na	na	0	0	na	0	na	na	na	±
26(11)	0	0	0	0	1	1	1	0	na	0	na	na	na	0	0	na	0	na	na	na	±
27(8)	0	0	±	0	1	1	1	±	1 <sup>a</sup>	±	1 <sup>a</sup>	±	1	±	±	1 <sup>a</sup>	±	1 <sup>a</sup>	±	1 <sup>a</sup>	±
28(3)	0	0	0	0	1	1	1	±	1 <sup>a</sup>	±	1 <sup>a</sup>	2 <sup>a</sup>	1 <sup>a</sup>	±	±	1 <sup>a</sup>	±	1 <sup>a</sup>	2 <sup>a</sup>	1 <sup>a</sup>	0
29(28)	0	0	0	0	1	1	1	±	0 <sup>a</sup>	±	±	±	1 <sup>a</sup>	±	±	1 <sup>a</sup>	±	±	±	1	±
30(7)	0	0	0	0	1	1	1	±	0 <sup>a</sup>	±	±	0 <sup>a</sup>	1 <sup>a</sup>	±	±	1 <sup>a</sup>	±	±	±	1	±
31(2)	0	0	±	1	1	1	1	0	na	0	na	na	na	0	0	na	0	na	na	na	1
32(2)	0	0	0	0	1	1	1	0	na	0	na	na	na	0	0	na	0	na	na	na	0
33(41)	0	0	0	0	1	±	1	0		±	±	±	1 <sup>a</sup>	0	±	1 <sup>a</sup>	±	±	±	1 <sup>a</sup>	±
34(7)	0	±	0	0	1	±	1	±	0 <sup>a</sup>	±	0 <sup>a</sup>	0 <sup>a</sup>	1 <sup>a</sup>	±	±	1 <sup>a</sup>	±	0 <sup>a</sup>	0 <sup>a</sup>	1 <sup>a</sup>	1
35(15)	0	0	0	0	1	±	1	±	0	±	1 <sup>a</sup>	2 <sup>a</sup>	1 <sup>a</sup>	±	±	1 <sup>a</sup>	±	1 <sup>a</sup>	2 <sup>a</sup>	1 <sup>a</sup>	±
36(29)	0	0	0	0	1	±	1	±	1 <sup>a</sup>	±	1 <sup>a</sup>	±	1 <sup>a</sup>	±	±	1 <sup>a</sup>	±	±	±	1 <sup>a</sup>	±
37(6)	0	0	0	0	1	1	1	±	0 <sup>a</sup>	0	na	na	na	0	0	na	±	1 <sup>a</sup>	0 <sup>a</sup>	1 <sup>a</sup>	±
38(4)	0	0	0	0	1	±	1	±	1 <sup>a</sup>	±	1 <sup>a</sup>	0 <sup>a</sup>	1 <sup>a</sup>	±	±	1 <sup>a</sup>	±	±	±	1 <sup>a</sup>	±
39(2)	0	0	0	0	1	±	1	0	na	±	1 <sup>a</sup>	0 <sup>a</sup>	1 <sup>a</sup>	0	±	1 <sup>a</sup>	±	1 <sup>a</sup>	2 <sup>a</sup>	1 <sup>a</sup>	1
40(49)	0	0	±	0	1	±	1	±	±	±	±	±	1 <sup>a</sup>	±	±	1 <sup>a</sup>	±	±	±	1 <sup>a</sup>	±
41(14)	0	0	±	1	1	1	1	±	±	±	±	±	1 <sup>a</sup>	±	±	1 <sup>a</sup>	±	±	±	1 <sup>a</sup>	±
42(2)	0	0	0	0	1	±	1	0	na	0	na	na	na	0	0	na	0	na	na	na	1
43(4)	0	0	0	0	1	±	1	0	na	0	na	na	na	0	0	na	0	na	na	na	1
44(18)	0	0	±	0	1	±	1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	1
45(6)	0	0	0	0	1	±	1	0,2	1 <sup>a</sup>	2	2	2	1	2	2	1	2	1	2	1	±
45(488)																					
Population(no. specimens)	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39			
1,2,3(2,2,2)	0	na	na	na	0	na	nd	nd	0	na	1	0	0	0	na	0	0	1			
4(4)	±	1	1	2	1	0	nd	nd	2	0	0	3	nd	±	±	0	0	0			
5(4)	±	0 <sup>a</sup>	1 <sup>a</sup>	±	0	na	nd	nd	±	0 <sup>a</sup>	0	0	0	±	0 <sup>a</sup>	±	±	0			
6(25)	±	0 <sup>a</sup>	1 <sup>a</sup>	0 <sup>a</sup>	0	na	±	0 <sup>a</sup>	0	na	0	0	0	±	na	1	1	0			
7(15)	±	±	1	±	0	na	±	0 <sup>a</sup>	2	±	0	±	±	±	±	±	±	0			
8(20)	±	0.1	1 <sup>a</sup>	±	0	na	0	na	0	na	0	0	0	±	1 <sup>a</sup>	1	1	0			
9(23)	±	±	1 <sup>a</sup>	±	±	0 <sup>a</sup>	0	na	±	±	0	0	±	±	1 <sup>a</sup>	±	±	0			

**Table 2** continued

Population(no. specimens)	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39
10(6)	0	na	na	na	nd	nd	nd	nd	nd	nd	nd	nd	nd	0	na	1	1	0
11(5)	±	0 <sup>a</sup>	1 <sup>a</sup>	0 <sup>a</sup>	0	na	nd	nd	2	0	0	0	0	±	±	0	0	0
12(14)	0	na	na	na	0	na	0	na	1	±	0	0	0	0	na	0	0	0
13(4)	1	0	1	0	0	na	nd	nd	2	1	0	0	0	±	0 <sup>a</sup>	0	0	0
14(16)	±	0 <sup>a</sup>	1 <sup>a</sup>	0 <sup>a</sup>	0	na	nd	nd	0	na	0	0	0	±	0 <sup>a</sup>	±	±	0
15(8)	±	0 <sup>a</sup>	1 <sup>a</sup>	0 <sup>a</sup>			0	na	2	1	0	0	1	±	0 <sup>a</sup>	0	0	0
16(8)	0	na	na	na	nd	nd	0	na	±	1 <sup>a</sup>	0	0	1	0	na	±	±	0
17(25)	±	0 <sup>a</sup>	1 <sup>a</sup>	±	±	0 <sup>a</sup>	0	na	2	0	0	0	±	±	0 <sup>a</sup>	±	±	0
18(2)	0	na	na	na	0	na	nd	nd	1	0	0	0	0	0	na	0	0	0
19(2)	±	0 <sup>a</sup>	1 <sup>a</sup>	2 <sup>a</sup>	0	na	nd	nd	2	0	0	0	nd	±	0 <sup>a</sup>	0	0	0
20,23(7,7)	±	±	1 <sup>a</sup>	2 <sup>a</sup>	0	na	0	na	±	0 <sup>a</sup>	±	0	±	±	0 <sup>a</sup>	0	0	0
21(12)	±	±	1 <sup>a</sup>	±	1	0	0	na	±	±	0	0	0	±	±	±	±	0
22(3)	±	1	1	2	±	0 <sup>a</sup>	nd	nd	2	0	0	0	±	±	±	±	0	0
24(5)	0	na	na	na	0	na	nd	nd	0	na	0	0	0	0	na	±	±	0
25(8)	0	na	na	na	0	nd	0	nd	2	1	±	0	0	0	na	0	0	0
26(11)	0	na	na	na	0	na	0	na	±	±	0	0	0	0	na	±	±	0
27(8)	±	±	1 <sup>a</sup>	±	±	1 <sup>a</sup>	0	na	2	0	0	2	nd	±	1 <sup>a</sup>	0	0	0
28(3)	1	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	±	1 <sup>a</sup>	0	0	0
29(28)	±	±	1	2	±	0 <sup>a</sup>	±	0 <sup>a</sup>	±	±	0	±	1	±	±	0	0	0
30(7)	±	±	1 <sup>a</sup>	±	nd	nd	1	±	nd	nd	nd	nd	nd	±	1	0	0	0
31(2)	±	1,na	1,na	2,na	nd	nd	±	0,na	nd	nd	nd	nd	nd	0	na	1	1	0
32(2)	0	na	na	na	0	na	nd	nd	nd	nd	nd	nd	nd	0	na	0	0	0
33(41)	±	±	±	±	0	na	±	0 <sup>a</sup>	±	±	0	0	0	±	±	±	±	0
34(7)	±	0 <sup>a</sup>	1 <sup>a</sup>	0 <sup>a</sup>	nd	nd	1	0	2	0	0	0	±	±	±	±	±	0
35(15)	±	±	±	±	0	na	±	0 <sup>a</sup>	±	±	0	±	±	±	1 <sup>a</sup>	1	1	0
36(29)	±	±	1 <sup>a</sup>	±	±	±	±	1 <sup>a</sup>	±	±	0	0	0	±	1 <sup>a</sup>	±	±	0
37(6)	±	0 <sup>a</sup>	1 <sup>a</sup>	0 <sup>a</sup>	0	na	nd	nd	2	0	0	0	0	±	0 <sup>a</sup>	0	0	0
38(4)	±	±	1 <sup>a</sup>	2 <sup>a</sup>	2	0	nd	nd	2	0	0	0	1	±	±	0	0	0
39(2)	±	1 <sup>a</sup>	1 <sup>a</sup>	2	nd	nd	±	1 <sup>a</sup>	nd	nd	nd	nd	nd	±	1 <sup>a</sup>	0	0	0
40(49)	±	±	1 <sup>a</sup>	±	±	±	±	±	2	±	±	0	±	±	±	0	0	0
41(14)	±	±	±	2 <sup>a</sup>	±	0 <sup>a</sup>	±	1	2	0	±	0	±	±	1 <sup>a</sup>	0	0	0
42(2)	±	1	1	2	nd	nd	nd	nd	2	1	0	0	0	±	1 <sup>a</sup>	0	0	0
43(4)	0	na	na	na	nd	nd	nd	nd	nd	nd	nd	nd	nd	0	na	0	0	0
44(18)	±	1 <sup>a</sup>	1 <sup>a</sup>	0 <sup>a</sup>	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	0	0	0
45(6)	±	±	1 <sup>a</sup>	2 <sup>a</sup>	nd	nd	2	1	nd	nd	nd	nd	nd	2	±	0	0	0
45(488)																		

±, polymorphism; na, not applicable; nd, not determined

<sup>a</sup> Character present but not fixed

Multivariate analyses

*Principal components analysis*

In this case the three matrices analyzed yielded similar results (Table 5), i.e. the first three components represented 50–54% of the variance. The first component explained between 21 and almost 24% in all cases, with a large to moderate contribution from characters concerning the length and density of trichomes. In particular, the length of the trichomes on the midvein on the lower surface of the leaves was the variable with the largest value in the three analyses.

The second component explains 15–19% of the variance and encompasses large values for characters related to the size of leaves and petioles. Finally, the third component explained between 11 and 13% of total variance, where mainly reproductive characters contribute the most (Table 6).

Specimens identified with the same species name are hardly visualized as different groups in the corresponding PCA graphs for all the taxonomical schemes (Figs. 2, 3). However, the ANOSIM permutation test shows that when the groups obtained are evaluated for distinctiveness, those corresponding to the classification hypothesis advanced in this study are supported with the highest value of *R* for the



**Table 3** Morphological characters of *Litsea* evaluated in principal components analysis

Male flowers	Abbreviation
1. Secondary nerve pairs	SNP
2. Flowers per inflorescence	FIN
3. Stamens number	SN
4. Trichomes on abaxial surface of midvein (%)	TAbM
5. Trichomes on abaxial surface of leaf blade (%)	TAbB
6. Trichomes on adaxial surface of midvein (%)	TAdM
7. Trichomes on adaxial surface of leaf blade (%)	TAdB
8. Length of trichomes on abaxial surface of leaf blade (mm)	TLabB
9. Length of trichomes on abaxial surface of midvein (mm)	TLabM
10. Length of trichomes on adaxial surface of leaf blade (mm)	TLabB
11. Length of trichomes on adaxial surface of midvein (mm)	TAdM
12. Length of leaves maximum (cm)	LLMax
13. Length of leaves minimum (cm)	LLmin
14. Width of leaf maximum (cm)	LWMax
15. Width of leaf minimum (cm)	LWmin
16. Foliar ratio: length/width maximum	FRLWMax
17. Foliar ratio: length/width minimum	FRLWmin
18. Maximum length of petiole (cm)	PLMax
19. Minimum length of petiole (cm)	PLmin
20. Ratio: length of petiole/length of leaves maximum	RPFMax
21. Ratio: length of petiole/length of leaves minimum	RPFmin
22. Length of peduncles in inflorescence (cm)	PLI
23. Maximum length of pedicel (mm)	PdLMax
24. Minimum length of pedicel (mm)	PdLmin
25. Length of filaments in external verticils (mm)	FLVext
26. Length of anther in external verticils (mm)	ALVext
27. Ratio: length of filament/length of anther in external verticils	RFAVext
28. Length of filaments in internal verticils (mm)	FLVint
29. Length of anther in internal verticils (mm)	ALVint
30. Ratio: length of filament/length of anther in internal verticils	RFAVint
31. Length of tepals (mm)	TL
32. Width of tepals (mm)	TA
33. Ratio: length/width of tepals	RTLW

Female flowers	Abbreviation
1. Secondary nerve pairs	SNP
2. Flowers per inflorescence	FIN
3. Staminodia number	StN
4. Trichomes on abaxial surface of midvein (%)	TAbM
5. Trichomes on abaxial surface of leaf blade (%)	TAbB
6. Trichomes on adaxial surface of midvein (%)	TAdM
7. Trichomes on adaxial surface of leaf blade (%)	TAdB
8. Length of trichomes on abaxial surface of leaf blade (mm)	TLabB
9. Length of trichomes on abaxial surface of midvein (mm)	TLabM
10. Length of trichomes on adaxial surface of leaf blade (mm)	TLabB
11. Length of trichomes on adaxial surface of midvein (mm)	TAdM
12. Maximum length of leaves (cm)	LLMax
13. Minimum length of leaves (cm)	LLmin
14. Maximum width of leaf (cm)	LWMax
15. Minimum width of leaf (cm)	LWmin

**Table 3** continued

Female flowers	Abbreviation
16. Foliar ratio: length/width maximum	FRLWMax
17. Foliar ratio: length/width minimum	FRLWmin
18. Maximum length of petiole (cm)	PLMax
19. Minimum length of petiole (cm)	PLmin
20. Ratio: length of petiole/length of leaves maximum	RPFMax
21. Ratio: length of petiole/length of leaves minimum	RPFmin
22. Length of peduncles in inflorescence (cm)	PLI
23. Maximum length of pedicel (mm)	PdLMax
24. Minimum length of pedicel (mm)	PdLmin
25. Length of tepals (mm)	TL
26. Width of tepals (mm)	TA
27. Ratio: length/width of tepals	RTLW

Fruits	Abbreviation
1. Secondary nerve pairs	NPVS
2. Trichomes on abaxial surface of midvein (%)	TAbM
3. Trichomes on abaxial surface of leaf blade (%)	TAbB
4. Trichomes on adaxial surface of midvein (%)	TAdM
5. Trichomes on adaxial surface of leaf blade (%)	TAdB
6. Length of trichomes on abaxial surface of leaf blade (mm)	TLabB
7. Length of trichomes on abaxial surface of midvein (mm)	TLabM
8. Length of trichomes on adaxial surface of leaf blade (mm)	TLabB
9. Length of trichomes on adaxial surface of midvein (mm)	TAdM
10. Maximum length of leaves (cm)	LLMax
11. Minimum length of leaves (cm)	LLmin
12. Maximum width of leaf (cm)	LWMax
13. Minimum width of leaf (cm)	LWmin
14. Foliar ratio: length/width maximum	FRLWMax
15. Foliar ratio: length/width minimum	FRLWmin
16. Maximum length of petiole (cm)	PLMax
17. Minimum length of petiole (cm)	PLmin
18. Ratio: length of petiole/length of leaves maximum	RPFMax
19. Ratio: length of petiole/length of leaves minimum	RPFmin
20. Length of peduncle in infrutescence (cm)	PLIfr
21. Maximum length of pedicel (mm)	PdLMax
22. Minimum length of pedicel (mm)	PdLmin
23. Maximum thickness of pedicel (mm)	PdTMax
24. Minimum thickness of pedicel (mm)	PdTmin
25. Maximum thickness of pedicel beneath the cupule (mm)	PdLCMax
26. Minimum thickness of pedicel beneath the cupule (mm)	PdLCmin
27. Maximum size of fruit (mm)	FSmax
28. Minimum size of fruit (mm)	FSFmin

three matrices analyzed. In contrast, Allen’s scheme is qualified with the lowest *R* figures (Table 7).

*Non-metric multidimensional scaling*

Global values of *R* and *p* derived from ANOSIM for the NMDS analyses again indicated that the classification hypothesis advanced here is the one with the greatest support (Table 8). The resulting diagram of the arrangement of

**Table 4** Morphological characters of *Litsea* used for the non-metric multidimensional scaling

1. Shape of leaf blade: 0, elliptic; 1, ovate; 2, round
2. Shape of leaf apex: 0, acuminate; 1, acute; 2, obtuse
3. Shape of leaf base: 0, attenuate-acute; 1, obtuse-cordate
4. Color of adaxial foliar surface: 0, green; 1, white
5. Consistency of leaf blade: 0, membranous; 1, chartaceous; 2, coriaceous
6. Epidermal cell contour: 0, polygonal; 1, sinuous; 2, lobed; 3, cleft
7. Density of trichomes on adaxial surface of midvein: 0, absent; 1,  $\leq 25\%$ ; 2,  $\leq 50\%$ ; 3,  $\leq 75\%$ ; 4,  $\leq 100\%$
8. Length of trichomes on adaxial surface in midvein: 0, absent; 1,  $< 0.1$  mm; 2,  $> 0.5$  mm
9. Type of trichomes: 0, absent; 1, straight; 2, curly

**Table 5** Eigenvalues of the first three components from principal components analyses for the three matrices analyzed in American *Litsea*

	Component	Eigenvalues	Percentage of total variance
Females	1	5.945989	22.02218
	2	5.236695	19.39517
	3	3.034947	11.24054
Accumulated		14.21763	52.6579
Males	1	7.075618	21.44127
	2	4.952597	15.00787
	3	4.504548	13.65014
Accumulated		16.53276	50.0993
Fruits	1	6.718414	23.99434
	2	4.977201	17.77572
	3	3.533252	12.61876
Accumulated		15.22887	54.3888

the NMDS analysis for this hypothesis is given in Fig. 4, which with a stress value of 0.122, represents a good adjustment. In this case the ANOSIM permutation test significantly separated every pair of groups at the level  $p < 0.01$  (Table 9), except for *L. neesiana* and *L. orizabae*, for which no significant differences were observed ( $R = -0.009$ ,  $p < 0.426$ ). The principal coordinates derived from this analysis are shown in Appendix 2.

**Discussion**

The results of the PAA revealed unequivocally that *L. aestivalis* is the most distinct species among the American group of *Litsea*. This result was expected because this species is ecologically and geographically isolated, growing in coastal environments at an altitude of 10–200 m

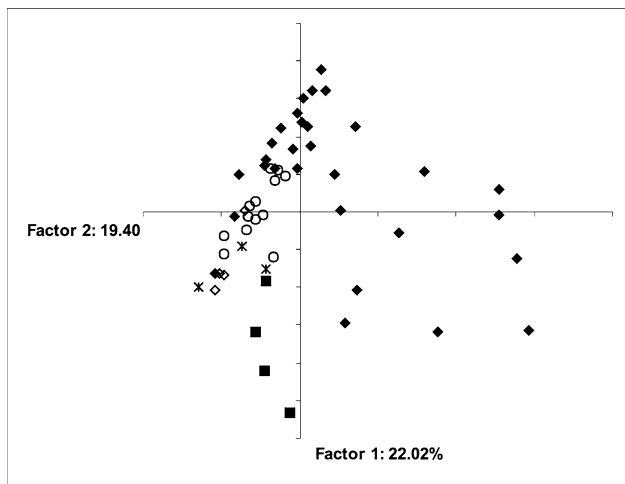
**Table 6** Results from principal components analysis

	Factor 1	Factor 2	Factor 3
<b>Males</b>			
TlAbM	-0.695509		
TAbM	-0.688174	0.536716	
TlAbB	-0.685619		
TlAdM	-0.670938		
TlAdB	-0.669953		
PLMax		-0.766901	
PLmin		-0.728887	
LLMax		-0.716661	
LLmin		-0.689224	
ALVext			-0.747299
ALVint			-0.733579
FRLWmin			0.670700
FRLWMax			0.669322
FLVint			-0.617675
<b>Females</b>			
TlAbM	0.854222		
TlAbB	0.798480		
TlAdM	0.744012		
TAbM	0.738549		
TAbB	0.732199		
LLMax		0.794134	
LLmin		0.785288	
PLMax		0.734067	
PLmin		0.716525	
SNP		0.635491	
LWMax			0.771859
LWmin			0.679737
FRLWmin			-0.677250
FRLWMax			-0.639578
PLI			0.534593
<b>Fruits</b>			
TlAbM	-0.892782		
TlAbB	-0.887620		
TAbM	-0.825616		
TlAdM	-0.820975		
TlAdB	-0.808450		
PLMax		0.885308	
PLmin		0.869477	
LLMax		0.850838	
LLmin		0.823149	
FRLWmin		0.508637	
FSmax			0.675380
FSFmin			0.669719
PdLMax			0.658549
PdTMax			0.649495
PdTmin			0.625930

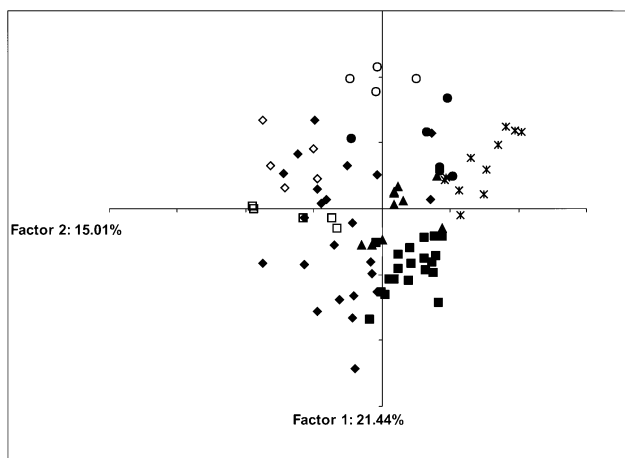
The variables are arranged in descending order according to their contribution to the first three components

Only the five variables with the largest values for each factor are indicated





**Fig. 2** First two principal components for 27 quantitative variables of 55 female specimens of *Litsea* with mature flowers grouped under the Allen (1945) classification scheme. Open circles, *L. pringlei*; filled squares, *L. parvifolia*; open diamonds, *L. muelleri*; closed diamonds, *L. glaucescens* var. *glaucescens*; asterisks, *L. glaucescens* var. *schaffneri*



**Fig. 3** First two principal components for 33 quantitative variables of 85 male specimens of *Litsea* with mature flowers grouped under the classification proposal advanced in this study. Open circles, *L. muelleri*; filled triangles, *L. pringlei*; filled squares, *L. glaucescens*; filled diamonds, *L. guatemalensis*; asterisks, *L. schaffneri*; open diamonds, *L. neesiana*; open squares, *L. orizabae*; filled circles, *L. parvifolia*

(Van der Werff 1997), unlike the other species, which are distributed mainly in pine and oak forest at an altitude of 900–3,000 m. The populations of the remaining species are mostly sympatric and this condition diminishes the power of the PAA to distinguish, in this case, the other taxa.

As the ANOSIM test results show, the classification scheme advanced here is the best for describing the number of species recognized as *Litsea* in Mesoamerica. We

**Table 7** Results of the ANOSIM test for each classification scheme evaluated by PCA, showing global *R* values for each of the three matrices and the global significance level

	Mez (1889)	Bartlett (1909)	Allen (1945)	This study
Female	$R = 0.532,$ $p < 0.001$	$R = 0.408,$ $p < 0.001$	$R = 0.12,$ $p < 0.07$	$R = 0.569,$ $p < 0.001$
Male	$R = 0.475,$ $p < 0.001$	$R = 0.538,$ $p < 0.001$	$R = 0.246,$ $p < 0.002$	$R = 0.599,$ $p < 0.001$
Fruit	$R = 0.491,$ $p < 0.001$	$R = 0.526,$ $p < 0.001$	$R = 0.245,$ $p < 0.001$	$R = 0.546,$ $p < 0.001$

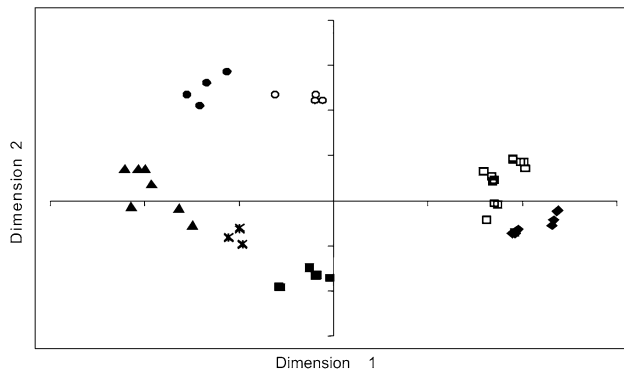
**Table 8** Results of the ANOSIM test for each classification scheme evaluated by NMDS, showing global *R* and *p* values

Mez (1889)	Bartlett (1909)	Allen (1945)	This study
$R = 0.603,$ $p < 0.001$	$R = 0.624,$ $p < 0.001$	$R = 0.444,$ $p < 0.07$	$R = 0.924,$ $p < 0.001$

consider that lower values of *R* obtained in the ANOSIM test for the other classification schemes evaluated mean that combinations of characters used for circumscription of the corresponding species do not categorize adequately the morphological variation observed in the group. So, Bartlett judged that some expression of character variation was of major importance in the identification of species and recognized twice as many as Mez did. Allen, on the other side, was right in pointing out, on the basis of the geography of character variation, that some of the species described by Bartlett were spurious. She, however, failed to distinguish other species and subsumed them in *L. glaucescens*, creating a very loose concept for this taxon.

The classification scheme advanced in this study suggested the recognition of eight species. Ordination of character variability through PCA and NMDS analyses, however, result in distinction of only seven additional species. There was no support to maintain *L. neesiana* distinct from *L. orizabae*. The accepted species for Mesoamerica are *L. glaucescens*, *L. guatemalensis*, *L. muelleri*, *L. neesiana*, *L. parvifolia*, *L. pringlei*, and *L. schaffneri*, which are discussed next.

*Litsea muelleri*, is a homogeneous species of restricted distribution, mainly from the area of the Cerro Potosí in Galeana, Nuevo León, in northeastern Mexico. In this study, nine specimens were considered, the majority from the locality mentioned, two from Hidalgo, and one from a small population within the Biosphere Reserve El Cielo in Tamaulipas. *Litsea muelleri* occurs close to *L. guatemalensis* and *L. neesiana*, because of its dense indumentum of



**Fig. 4** Graph from NMDS analysis for nine qualitative variables of 90 specimens of *Litsea* grouped under the scheme advanced in this study. Open circles, *L. muelleri*; filled triangles, *L. pringlei*; filled squares, *L. glaucescens*; filled diamonds, *L. guatemalensis*; asterisks, *L. schaffneri*; open squares, *L. neesiana* and *L. orizabae*, filled circles *L. parvifolia*

**Table 9** Results of the ANOSIM test showing all pair differences between the eight *Litsea* species, for the classification scheme advanced here evaluated by NMDS

Groups	R statistic	Significance level ( <i>p</i> )
<i>L. muelleri</i> , <i>L. pringlei</i>	0.94	0.003
<i>L. muelleri</i> , <i>L. parvifolia</i>	0.734	0.001
<i>L. muelleri</i> , <i>L. glaucescens</i>	1.0	0.002
<i>L. muelleri</i> , <i>L. schaffneri</i>	1.0	0.001
<i>L. muelleri</i> , <i>L. guatemalensis</i>	1.0	0.001
<i>L. muelleri</i> , <i>L. neesiana</i>	1.0	0.002
<i>L. muelleri</i> , <i>L. orizabae</i>	1.0	0.003
<i>L. pringlei</i> , <i>L. parvifolia</i>	0.334	0.003
<i>L. pringlei</i> , <i>L. glaucescens</i>	0.988	0.001
<i>L. pringlei</i> , <i>L. schaffneri</i>	0.893	0.001
<i>L. pringlei</i> , <i>L. guatemalensis</i>	1.0	0.001
<i>L. pringlei</i> , <i>L. neesiana</i>	1.0	0.001
<i>L. pringlei</i> , <i>L. orizabae</i>	1.0	0.001
<i>L. parvifolia</i> , <i>L. glaucescens</i>	0.99	0.001
<i>L. parvifolia</i> , <i>L. schaffneri</i>	0.907	0.001
<i>L. parvifolia</i> , <i>L. guatemalensis</i>	0.993	0.001
<i>L. parvifolia</i> , <i>L. neesiana</i>	1.0	0.001
<i>L. parvifolia</i> , <i>L. orizabae</i>	1.0	0.002
<i>L. glaucescens</i> , <i>L. schaffneri</i>	0.971	0.001
<i>L. glaucescens</i> , <i>L. guatemalensis</i>	0.999	0.001
<i>L. glaucescens</i> , <i>L. neesiana</i>	1.0	0.001
<i>L. glaucescens</i> , <i>L. orizabae</i>	1.0	0.002
<i>L. schaffneri</i> , <i>L. guatemalensis</i>	1.0	0.001
<i>L. schaffneri</i> , <i>L. neesiana</i>	1.0	0.001
<i>L. schaffneri</i> , <i>L. orizabae</i>	1.0	0.001
<i>L. guatemalensis</i> , <i>L. neesiana</i>	0.907	0.001
<i>L. guatemalensis</i> , <i>L. orizabae</i>	0.849	0.001
<i>L. neesiana</i> , <i>L. orizabae</i>	0.003	0.354

long trichomes (Fig. 5c, d) but, unlike them, it always presents leaves almost round with cordate bases that do not exceed 5 cm in length (Fig. 6a).

*Litsea pringlei* and *L. parvifolia* are clearly differentiated entities (Fig. 4, Table 9). They share coriaceous leaves with cordate, subcordate, obtuse, or rounded leaf bases (Fig. 6b, c), and their epidermal cells have cleft anticlinal walls (Fig. 7b). However, leaf blades in *L. pringlei* are always glabrous whereas in *L. parvifolia* they consistently have short erect, dispersed trichomes underneath (Fig. 5e, f). These two species are restricted to the Mexican states of Coahuila, Nuevo León, and Tamaulipas. Bartlett (1909) recognized *L. pringlei* and *L. parvifolia* as different species, in addition to *L. novoleontis* and *L. pedicellata*, all sharing the distinctive character of subcordate to rounded leaf base. He considered that *L. novoleontis* differed from *L. pringlei*, claiming the former presented a solitary, fasciculate inflorescence whereas the latter had terminal and axillary corymbs. He drew a similar conclusion for *L. pedicellata* and *L. parvifolia*; whereas *L. pedicellata* was distinguished for having paniculate inflorescences, *L. parvifolia* was characterized by bearing solitary or rarely fasciculate inflorescences. Although terminal inflorescences do not exist within the group, inflorescence structure is a very variable character both in single plants and among individuals of the same species.

Allen (1945) gave taxonomic importance to the form of the base and size of the leaves, and considered *L. schaffneri* a variety within *L. glaucescens*. The results of our study differ from Allen's appreciation, and agree with the judgement of Bartlett in that these two taxa represent different species. *L. schaffneri* has narrow and coriaceous leaves (never rounded), glabrous, with acute base and epidermal cells with sinuous anticlinal walls (Figs. 6d, 7b). The species is found mainly in Hidalgo, Guanajuato, Querétaro, San Luis Potosí, and a few places in Tamaulipas.

*Litsea glaucescens* has been considered the most common and widely distributed *Litsea* species; it is also attributed with a considerable variation in vegetative characters, for example the type and density of pubescence on different parts of the plant and the form and size of leaves. Indeed, Allen (1945) considers it a too highly polymorphic species in which three varieties could be distinguished. An outcome of our study, perhaps the most unexpected, is that in contrast with the persistent concept of *L. glaucescens*, it was found that it forms a coherent entity differentiated from the rest of the species by the glabrous condition of its almost membranous leaves with petioles longer than 1 cm (Fig. 6e). The species exhibits a unique epidermal cell pattern with irregular lobed contours (Fig. 7c). Thus, circumscribed, the species is reduced in distribution to the environs of

the Trans-Mexican volcanic belt, with some isolated locations in Querétaro and Jalisco. This is the most popularly used *Litsea* species (locally called “laurel”).

*Litsea guatemalensis* and *L. neesiana* are characterized by having the densest indument and the longest trichomes within the American species of *Litsea*. In the Mez (1889) and Bartlett (1909) classifications these taxa appear as three different species (because they considered *L. orizabae* different from *L. neesiana*), but Allen (1945) regarded them as synonyms of *L. glaucescens* var. *glaucescens*. Inspection of the individuals assembled in these species reveals they share epidermal cells with (mostly) polygonal contour (Fig. 7a). This type of epidermal cell pattern is similar to that found in *L. aestivalis*, and clearly distinguishes these from the other species. On the other hand, they differ in the morphological types of trichomes found in their indument—long and straight in *L. guatemalensis* and long and curly in *L. neesiana*.

Therefore, *Litsea guatemalensis* is here characterized by the presence of long, straight trichomes, of variable density (pubescence can be lost with age), leaves with acute to attenuate base, and long acuminate apex (Figs. 5a, b, 6f.). This species is actually, according to this study, the most widely distributed in America, found

from northwest of Mexico through Central America. *Litsea neesiana* is distinguished by having a dense indument of long and curly trichomes (Fig. 5c, d), and mostly oblong, never long acuminate leaves (Fig. 6g). Thus circumscribed, the species would mainly be restricted to the surroundings of the volcano Pico de Orizaba, in Veracruz, Mexico, and to some isolated populations in the center of Chiapas and mountain areas of Oaxaca, Mexico. Analysis of the characters considered in this study does not support the separation of *L. neesiana* from *L. orizabae* (Table 9).

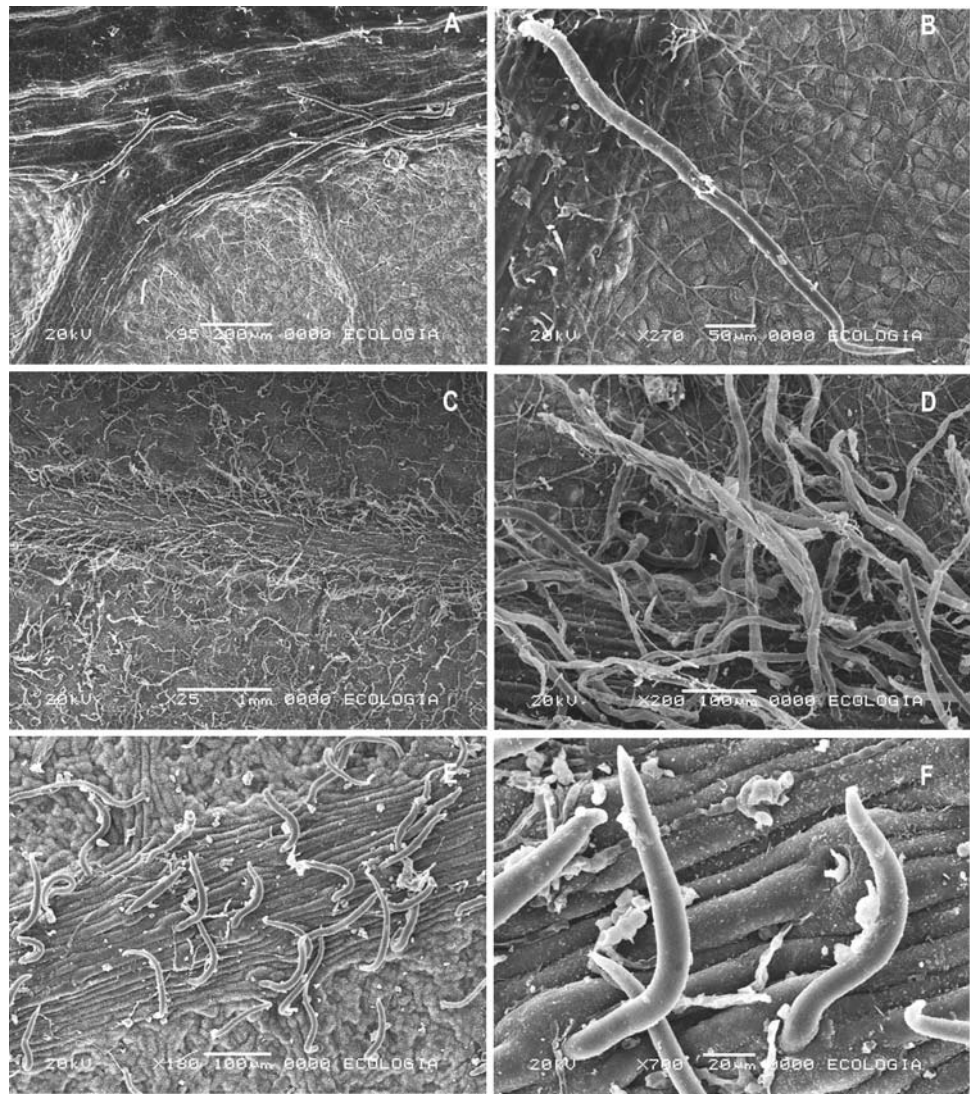
In short, the recognition of eight species of *Litsea* in America is supported by this work on the basis of analysis of qualitative and quantitative characters. Sympatric populations were found throughout the area of distribution, but principally in the environs of the Trans-Mexican volcanic belt and the Sierra Madre Oriental. In this respect, it is important to mention that local people use *Litsea* species, preferring, maintaining, and even propagating the glabrous forms, thus changing the original distribution of populations.

Finally, as a synthesis of the diagnostic characters of the species, a key for the identification of the American species of *Litsea* is provided.

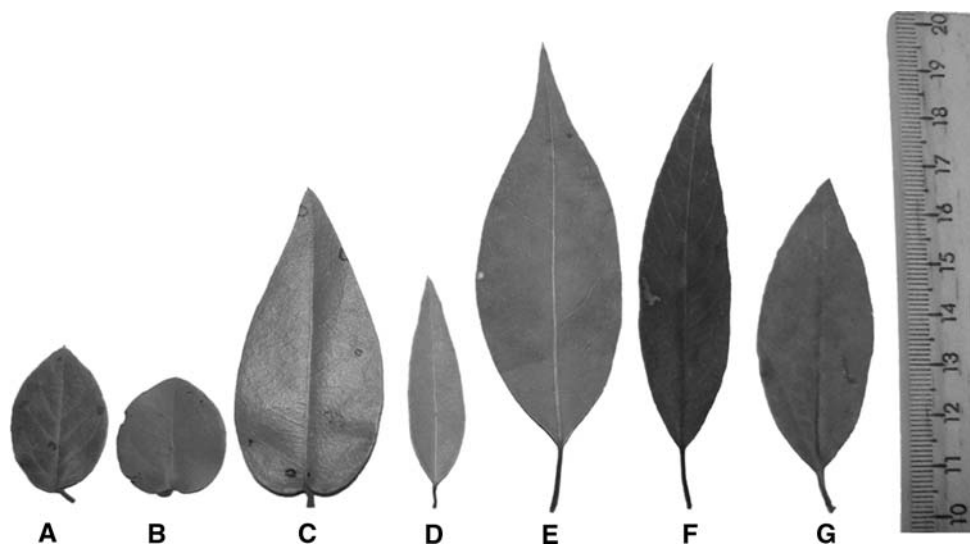
#### IDENTIFICATION KEY FOR AMERICAN SPECIES OF *Litsea*

- |  |                         |
|--|-------------------------|
| 1. Leaves deciduous, blade with agglomerate trichomes only near the base of the midvein on abaxial surface                                   | <i>L. aestivalis</i>    |
| 1. Leaves persistent, blade either glabrous or if pubescent then with trichomes (sometimes scattered) on the entire blade on abaxial surface |                         |
| 2. Leaf blade rounded or ovate, with cordate or obtuse base  |                         |
| 3. Leaf blade glabrous   | <i>L. pringlei</i>      |
| 3. Leaf blade pubescent  |                         |
| 4. Trichomes erect and short, not exceeding 0.1 mm   | <i>L. parvifolia</i>    |
| 4. Trichomes curly-ferruginous, longer than 0.2 mm   | <i>L. muelleri</i>      |
| 2. Leaf blade elliptic with attenuate or acute base, never rounded or cordate  |                         |
| 5. Leaf blade glabrous   |                         |
| 6. Leaf blade elliptic, petioles longer than 1 cm. Anticlinal walls of epidermal cells lobed   | <i>L. glaucescens</i>   |
| 6. Leaf blade linear-lanceolate, petioles of variable length but not reaching 1 cm. Anticlinal walls of epidermal cells sinous               | <i>L. schaffneri</i>    |
| 5. Leaf blade pubescent at least below   |                         |
| 7. Leaf apex long acuminate, lower surface with indument of variable density made of straight and long trichomes                             | <i>L. guatemalensis</i> |
| 7. Leaf apex acute, lower surface with dense indument made of long and curly trichomes   | <i>L. neesiana</i>      |

**Fig. 5** Lower leaf indument. **a** and **b** Straight and long trichomes, *L. guatemalensis* (N. Jiménez P. et al. 1496); **c** and **d** long and curly trichomes, *L. neesiana* (N. Jiménez P. et al. 1504), and **e** and **f** erect and short trichomes, *L. parvifolia* (N. Jiménez P. et al. 1512)

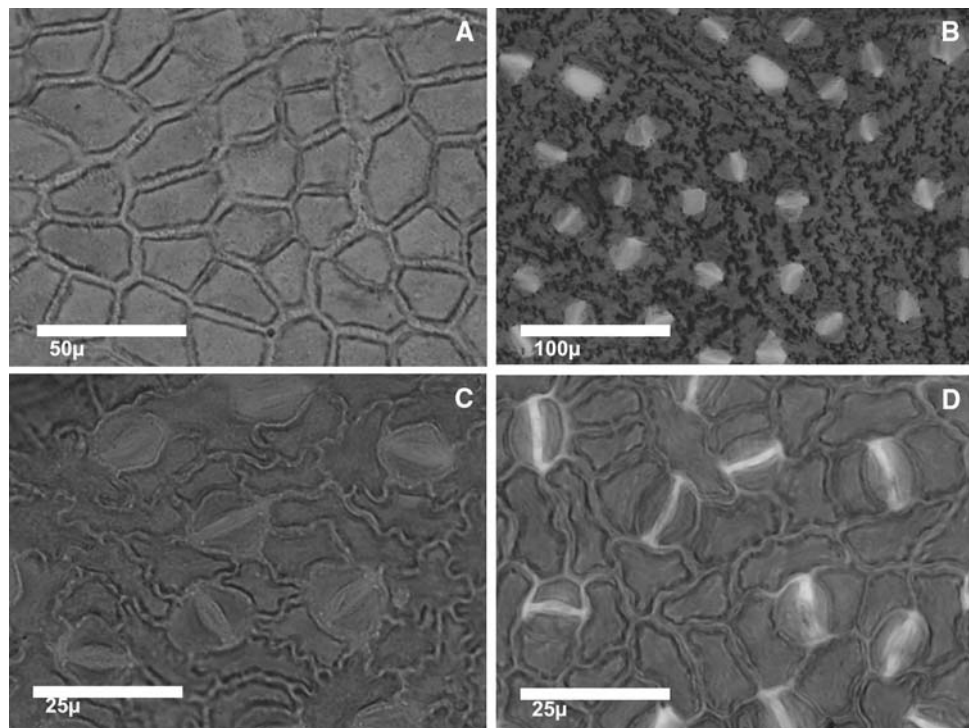


**Fig. 6** Leaf shape of **a** *L. muelleri*, **b** *L. parvifolia*, **c** *L. pringlei*, **d** *L. schaffneri*, **e** *L. glaucescens*, **f** *L. guatemalensis*, and **g** *L. neesiana*





**Fig. 7** Epidermal cell contour. **a** Polygonal cells with straight walls and sharp or rounded points, *L. neesiana* (N. Jiménez P. et al. 1506); **b** cleft cells with numerous deep, round teeth along walls, *L. pringlei* (N. Jiménez P. et al. 1527); **c** lobed cells with few shallow, round teeth along walls, *L. glaucescens* (N. Jiménez P. et al. 1546); **d** sinuous cells with wavy walls, *L. schaffneri* (Hinton 17495)



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## Appendices

Data placed in the Supplementary Information in the online version include a table with the record of the populations and specimens analyzed in each analysis, and the principal coordinates for 90 specimens studied in NMDS.

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