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Local plant species delimitation in a highly diverse Amazonian forest: do we all see the same species?

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Abstract

Question: How reliable is the process of delimiting plant species by morphotyping sterile specimens from a highly diverse Amazonian forest plot?

Location: Biological Dynamics of Forest Fragments Project (BDFFP), Central Amazon, Manaus, Brazil.

Methods: A taxonomic exercise was conducted during a Center for Tropical Forest Science (CTFS) Taxonomy Workshop held in Manaus in April 2011, using specimens collected in a 25-ha forest plot. The plant species from this plot had been previously delimited by morphotyping of ca. 80 000 sterile specimens, a process that resulted in the recognition of 115 cases (accounting for 38% of all trees) in which species delimitation was problematic. For the workshop, we selected a subsample of specimens for eight of these difficult cases (taxonomic groups/complexes) and asked 14 participants with different levels of botanical training to independently sort these specimens into morphospecies. We then compared the classifications made by all participants and explored correlations between botanical training and plant classification.

Results: The classification of specimens into morphospecies was highly variable among participants, except for one taxonomic group/complex, for which the median pair-wise similarity was 95%. For the other seven taxonomic groups/complexes, median pair-wise similarity values ranged from 52% to 67%. Training did not increase the similarity in the definition of morphospecies except for two taxonomic groups/complexes, for which there was higher congruence between the classifications made by participants with a high level of botanical training than in comparisons that included less-experienced participants. The total number of morphospecies defined by participants was highly variable for all taxonomic groups/complexes, with the total number varying from 12 to 46 (a 383% difference).

Conclusions: Local plant species delimitation by morphotyping sterile specimens is prone to large uncertainties, and botanical training may not reduce them. We argue that uncertainty in species delimitation should be explicitly considered in plant biodiversity inventories as diversity estimates may be strongly affected by such uncertainties. We recommend that species delimitation and identification be treated as separate processes and that difficulties be explicitly recorded, so as to permit error estimates and the refinement of taxonomic data.

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Introduction

How many species are there? To which species does a particular specimen belong? Which specimens belong to the same species? These are the first questions any biologist needs to ask when working with biodiversity inventories. They are not simple questions, because there is no single way to define species and hence to delimit them (see de Queiroz 2007 for a summary of alternative species concepts; Sites & Marshall 2004). For plant biologists working in highly diverse areas such as the tropics, the species problem is accentuated because there are still many gaps in collecting, and basic research on plants remains scant (Prance 2001). These problems are particularly well known for the Amazon region (Nelson et al. 1990; Hopkins 2007). Indeed, most unknown species are concentrated in areas that are very diverse, poorly sampled and at risk of rapid habitat transformation by humans (Joppa et al. 2011a,b).

In addressing the above questions it is important to distinguish between two distinct but related processes: species delimitation and species identification. Species delimitation is the process by which one recognizes biological units based on some operational criteria (e.g. morphological, ecological or molecular characters). This is a fundamental step towards gaining information on the ecological and taxonomic aspects of the taxa involved, and the most important step for assessing biodiversity in a region. However, delimiting with accuracy is not always easy. In sexually reproducing species, species delimitation may be complicated by convergent evolution, by the difficulty of separating cryptic species or distinguishing intra- from inter-specific variability, and by hybridization. In contrast, species identification is the process of giving a name to a specimen, normally by comparing it with a herbarium specimen assumed to be reliably identified. This second stage is critical for making floristic comparisons with other sites, although purely phylogenetic measures of diversity may turn out to be more cost-effective in the near future, allowing comparisons between areas based on phylogenetic distances and without the need for proper scientific names for species delimited at each local site (see Vamosi et al. 2009; Cadotte et al. 2010; Morlon et al. 2011 for recent reviews).

In the Amazon, there are two main limitations for plant identification in plot-based inventories. First, many species are rare (Hubbell et al. 2008), which means that material for comparisons may be lacking. Second, plants are mostly collected sterile, because of irregular or supra-annual reproductive activity (Haugaasen & Peres 2005; Wright & Calderon 2006; Norden et al. 2007). In addition, there are few taxonomic studies and identification tools for local floras in the Amazon (Prance 2001). As a result, rapid plant inventories in highly diverse forests typically produce a large number of specimens to identify, of which the majority is sterile, and in which juveniles are mixed with adult plants. Without flowers or fruits, fewer characters are available to the taxonomist responsible for both delimiting local species and identifying them. Taxonomic experts frequently do not deal with sterile specimens, and scientists working in plot inventories cannot wait for experts to identify the thousands of sterile specimens collected by plant inventories. Taxonomic monographs offer little insight into local patterns of variation and reference collections in the Amazon region, which are the basis for identification of material for most large-scale inventories, are of poor quality. It is common to find species that co-occur at a site grouped under a single name in herbaria. This may be a source of error in inventory work if, for example, different species at a site are merged under a single name in order to match a herbarium reference that has the wrong circumscription. Thus, despite the fact that species delimitation and identification are different processes, they are frequently mixed up in biodiversity inventory surveys.

Despite the difficulties in naming sterile specimens, delimiting species locally (e.g. in a single plot) based only on vegetative characters is considered feasible, since at that spatial scale a morphological gap is assumed to indicate a degree of reproductive isolation. In reality, however, morphological gaps may reflect differences between juveniles and adults and/or phenotypic plasticity. By recording appropriate collection data (e.g. height, habitat) some of these issues can be dealt with. However, the cumulative uncertainties in species boundaries can be particularly frequent in highly diverse systems like the *terra firme* forests in Central Amazon, where a large number of closely related, morphologically similar, and hard to distinguish species co-exist (Oliveira & Daly 1999).

Another problem is that the process of delimiting species locally has a component of subjectivity. Morphotyping by overall similarity is a complex cognitive process, and personal judgment plays a part in deciding which differences or similarities among specimens are significant. Different people have different backgrounds and may classify specimens in different ways. This problem is the focus here.

Long-term studies involving large-scale permanent plots have been advocated as cost-effective ways to rapidly accumulate knowledge about tropical biodiversity and to monitor community dynamics and demographics (e.g. Costa & Magnusson 2010). To investigate the effect of some factors that affect species naming, we conducted an exercise of species delimitation using over 1000 sterile specimens collected in a 25-ha plot in a highly diverse forest in Central Amazon. We wanted to determine how much confidence can be placed in species delimitation at that scale, when delimitation relies on sterile specimens

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and vegetative morphological characters alone. We focused on groups in which species delimitation was difficult. These groups represent 38% of all the specimens collected in the plot (ca. 72 800) and roughly 20% of the morphospecies. Our goal was to quantify the uncertainty in plant species delimitation by different people, and highlight the implications of such uncertainties for estimates of diversity. Specifically, our main question was: how reliable is the process of delimiting plant species by morphotyping sterile specimens from a single locality? Since it has been suggested that the number of species recognized by an observer may be influenced by the observer's botanical expertise (Scott & Hallam 2002; Ahrends et al. 2011), we also addressed the question: do experienced botanists show more agreement in the classification of specimens than less experienced botanists?

Methods

Status of data and plant identification before the exercise

The samples used in our study were all collected in a 25-ha permanent plot located ca. 60 km north of the city of Manaus, Amazonas, Brazil (2.4417 S, 59.7858 W). This plot is part of the Center for Tropical Forest Science (CTFS) global network of forest research plots and also part of a local network of permanent plots of the Biological Dynamics of Forest Fragments Project (BDFFP) from the National Institute for Amazonian Research (INPA). The local plot network includes 69 1-ha plots in both fragmented and continuous forests, in which trees $\geq$10 cm at breast height (DBH) have been censused since the early 1980s. The 25-ha plot is a new addition to the network and follows the CTFS protocol, with all trees $\geq$1 cm DBH inventoried. The long-term study at BDFFP has generated thousands of specimens, which have been identified by different people over several years, including prominent experts on the Amazonian flora. In the 69 1-ha plots, 1444 species have been recognized, of which 39.3% are currently morphospecies without a formal name, and species delimitation problems abound. A reference collection for the species in the area has been curated by one of us (A.A.) for many years, and is currently used to help in the identification of new material from the BDFFP plot network.

In the 25-ha plot, which was set up in 2005, specimens from 72 800 trees (50% of the total) were collected for local species delimitation. We followed a protocol for processing these specimens, explicitly separating the processes of delimiting and identifying species. Two of us (A.C.S.G. and J.B. da Silva) initially delimited species in this material taking a rather narrow (splitting) approach, on the premise that it is easier to merge than to split afterwards. During this process, all cases were recorded in which species delimitation was problematic, by defining *species groups* and *species complexes*. The species group category was used for cases in which multiple local species could actually represent a single species due to high morphological similarity. The species complex category was used for cases in which a recognized local species presented high morphological variation, suggesting that it could potentially contain multiple species that could not be clearly separated. Therefore, both categories were used to explicitly record problems in species delimitation. This process allowed us to recognize 1369 putative local species for the 25-ha permanent plot. Within these taxa, we identified 115 problematic cases, including groups containing different local species with very similar sterile morphology (92 species groups), and single local species that we suspected to include multiple, morphologically similar species (23 species complexes). Although relatively few in number, these cases accounted for 38% of the ca. 72 800 specimens collected in the 25-ha CTFS-BDFFP plot. The identities of most of these morphospecies were later obtained by comparison with the BDFFP reference collection and the INPA herbarium, but we did not allow the circumscription of the local species to change when using these reference collections to name them. Only dried herbarium specimens were used for species delimitation.

Selection of samples for the exercise

The data presented in this paper are the result of an exercise conducted during the First CTFS Taxonomy Workshop, held in April 2011 in Manaus, Brazil. For this exercise, we selected seven previously defined species groups and species complexes that represented cases for which species delimitation was considered problematic. For the purpose of comparison, we also included one group composed of two similar morphospecies that we considered easy to separate because of obvious vegetative characters (Table 1). The selected problematic cases represented groups containing a large number of specimens and groups with the largest number of putative species involved. The exercise was thus carried out with eight taxonomic groups/complexes: three species groups ('*Ocotea*', '*Pouteria*' and '*Myrcia*'), four species complexes ('*Cupania*', '*Lacistema*' and two cases including species of *Protium*) and an easy group ('*Eugenia*'; Table 1).

For each taxonomic group/complex we either used all material available for the group or, when the group was too large to be managed during the workshop, used a randomly selected subsample of specimens (Table 1). We then asked 14 participants to sort all the specimens of each taxonomic group/complex into as many morphospecies they thought the set contained. Participants were asked to do the work alone, were not told how the taxonomic groups/complexes had been initially classified, and were not

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Table 1. Taxonomic groups/complexes used to assess the congruence in morphospecies delimitation among 14 participants; putative species and morphospecies of each taxonomic group/complex as recognized by A.C.S.G. and J.B. da Silva and the number of plant specimens (*n*) sorted into morphospecies by all participants.

Taxonomic groups/complexes	Species	Family	*n*
1. *Protium altsonii/laxiflorum*[1]	*Protium altsonii* Sandwith	Burseraceae	156
	Protium laxiflorum Engl.		
2. *Cupania rubiginosa* s.l.[1]	*Cupania rubiginosa* (Poir.) Radlk.	Sapindaceae	151
	Cupania scrobiculata Rich.		
	Cupania sp.1[2]		
3. *Protium carnosum-crassipetalum-rubrum*[1]	*Protium carnosum* A.C.Sm.	Burseraceae	81
	Protium crassipetalum Cuatrec.		
	Protium rubrum Cuatrec.		
4. *Lacistema*[1]	*Lacistema aggregatum* (P.J. Bergius) Rusby	Lacistemataceae	202
	Lacistema polystachyum Schnizl.		
5. *Ocotea cernua* s.l.[3]	*Ocotea cernua* (Nees) Mez	Lauraceae	195
	Ocotea aff. *pauciflora*[2]		
	Ocotea sp.E[2]		
6. *Pouteria cuspidata* s.l.[3]	*Pouteria cuspidata* subsp. *dura* (Eyma) T.D. Penn.	Sapotaceae	131
	Pouteria cuspidata (A.D.C.) Baehni subsp. *cuspidata*		
	Pouteria cuspidata morph. acuminada[2]		
7. *Myrcia*[3]	*Myrcia* falax-deflexa[2]	Myrtaceae	99
	Myrcia magnoliifolia DC.		
8. *Eugenia*[4]	*Eugenia agathopoda* Diels	Myrtaceae	53
	Eugenia illepida McVaugh		

[1]Species complex, [2]morphospecies, [3]species groups, [4]the 'easy group'.

allowed to discuss among themselves how morphospecies should be recognized. They were permitted to use hand lenses or dissecting microscopes, but not external references from the herbarium or the literature. Participants were asked to record the criteria they used to classify the specimens into morphospecies. However, the idea was simply to visually classify specimens collected at a single site into morphospecies. No names were used, only letters or numbers for the morphospecies classes. Each participant carried out this exercise for all taxonomic groups/complexes; the time spent for each taxonomic group/complex was not standardized among the participants.

Of the 14 participants, 13 had formal training in biological sciences (referred hereafter as botanists), and one was a field technician (with no formal training) who has worked for 6 yr in the 25-ha plot. Among the botanists, three have worked with plant identification in other CTFS permanent plots in Latin America, four had no previous experience in plant identification, and the remaining six had varying levels of experience with plant identification. The person who curated the BDFFP collection over the last 10 yr and the two people who delimited and named the specimens from the 25-ha plot prior to the workshop were among the participants.

Data analysis

The similarity in morphospecies classification among participants was estimated as follows for each taxonomic group/complex. The classification of each person was recorded in a table of specimen vs morphospecies. We then converted this table into a binary (0 or 1) matrix of specimen vs specimen, in which 0 (zero) meant that two specimens were classified as belonging to the same morphospecies, and 1 (one) meant that the pair of specimens was classified as belonging to different morphospecies. The extent of the similarity for all participant vs participant comparisons was calculated as the proportion of values in a participant's binary matrix that matched the values in the other participant's binary matrix.

To test if the observed classification similarities among participants were different than expected by chance, we carried out randomization tests by generating a distribution of classification similarities produced by 100 randomizations of the original classifications per taxonomic group/complex (specimen morphospecies was randomly assigned, maintaining constant the number of morphospecies and the number of specimens per morphospecies per participant). We used the three steps described above to obtain random classification similarity values for all participant vs participant comparisons. The observed classification similarities were then compared to the distribution of the expected similarity for the random classification of specimens in each taxonomic group/complex, to test whether the observed similarities were higher, lower, or the same as expected by chance, at a significance level of 0.05.

To test the effect of experience (training) in plant classification, we performed a correlation (Mantel) test between

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the matrix of classification similarities among participants per taxonomic group/complex and the distance matrix of levels of experience. This distance matrix was calculated as follows: (1) participants were ranked into levels of expertise, from 1 (no expertise) to 4 (high expertise), based on two criteria: years of experience in plant identification and knowledge of the local flora; (2) we calculated the expertise distance between two participants as the sum of the rank values of each one. The correlation between the classification similarities and the level of experience was considered significant if $P < 0.05$.

We restricted ourselves to comparisons of the morphospecies recognized by the participants; there was no 'true' classification to which we could refer. All analyses were performed using R (R Development Core Team 2011; R Foundation for Statistical Computing, Vienna, AT). Supplemental online material includes the data and all scripts used for generating the analyses and figures.

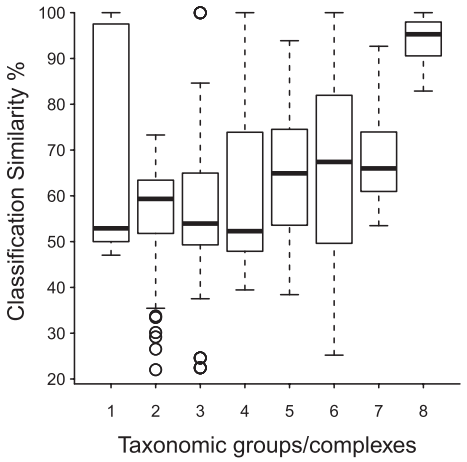

Fig. 1. Pair-wise similarities (%) in morphospecies delimitation among 14 participants, for each taxonomic group/complex (numbered according to Table 1).

Results

Overall, there was high variability in the way the 14 participants sorted the specimens into morphospecies, except for the easy group ('*Eugenia*'). The highest similarity values were found for the '*Eugenia*' group (Fig. 1), with a median pair-wise similarity of 95% and minimum and maximum values of 83% and 100%, respectively. For all other taxonomic groups/complexes, the median values ranged from 52% to 67% (Fig. 1). Botanical experience did not increase classification similarity for most species (Fig. 2). Only for the '*Ocotea cernua* s.l.' and '*Pouteria cuspidata* s.l.' species-groups were there more cases of good congruence for high-experience pairs of participants than for low-experience pairs ($R = 0.69$, $P < 0.01$ for '*Ocotea cernua* s.l.'; and $R = 0.42$, $P < 0.05$ for '*Pouteria cuspidata* s.l'; Fig. 2). For all other groups similarity in the classification was independent of botanical experience.

There was also considerable variation in the number of morphospecies sorted by the participants for all taxonomic groups/complexes (Fig. 3). This variation was particularly striking for the '*Cupania rubiginosa*' species complex and for the '*Ocotea cernua*' and '*Pouteria cuspidata*' species groups, for which participants defined from one to eight, three to ten, and one to six morphospecies, respectively. As expected, the lowest variation in the number of morphospecies sorted by the participants was for the easy group ('*Eugenia*'), for which almost all participants recognized two morphospecies (Fig. 3). Taking all the taxonomic groups/complexes together, the total number of morphospecies classified varied from 12 to 46 (a difference of 383%).

With the exception of the '*Cupania rubiginosa* s.l.' species complex, more than 50% of pair-wise comparisons for all taxonomic groups/complexes had similarity values higher than those expected by chance (Table 2), and this proportion was especially high (100%) for the easy group ('*Eugenia*'), in which all pair-wise similarities were significantly higher than expected by chance. Because all pair-wise comparisons in which both participants sorted specimens into only one morphospecies were necessarily equal to 100%, the results of simulations for these cases were meaningless, because a pair-wise similarity of 100% is equal to that expected by chance. We reported these cases as 'comparisons equal to chance', along with a few other comparisons in which participants recognized more than one morphospecies and classification similarities were indeed not different than expected by chance (Table 2). In a few cases, pair-wise similarities were lower than expected by chance, ranging from 8% ('*Lacistema'* species complex) to 29% of the pair comparisons ('*Myrcia*' species group; Table 2). In the '*Cupania*' species complex, 58% of pair-wise comparisons had similarity values lower than expected (Table 2).

Considering only the three participants with good knowledge of the local flora, a group that included two botanists and the field technician, classifications varied greatly and were lowest for the '*Cupania rubiginosa'* species complex. The only group in which there was absolute concordance in species delimitation among these three participants was the easy group ('*Eugenia'*; Table 3). There was reasonable concordance among the three participants for the '*Ocotea*', '*Pouteria*' and '*Myrcia*' species groups, with pair-wise similarities varying from 81% to 97% (Table 3). However, there was high variation in morphospecies delimitation for the species complexes (the first four in Table 3), except for '*Protium carnosum*/*crassipetalum*/*rubrum*', with pair-wise similarities

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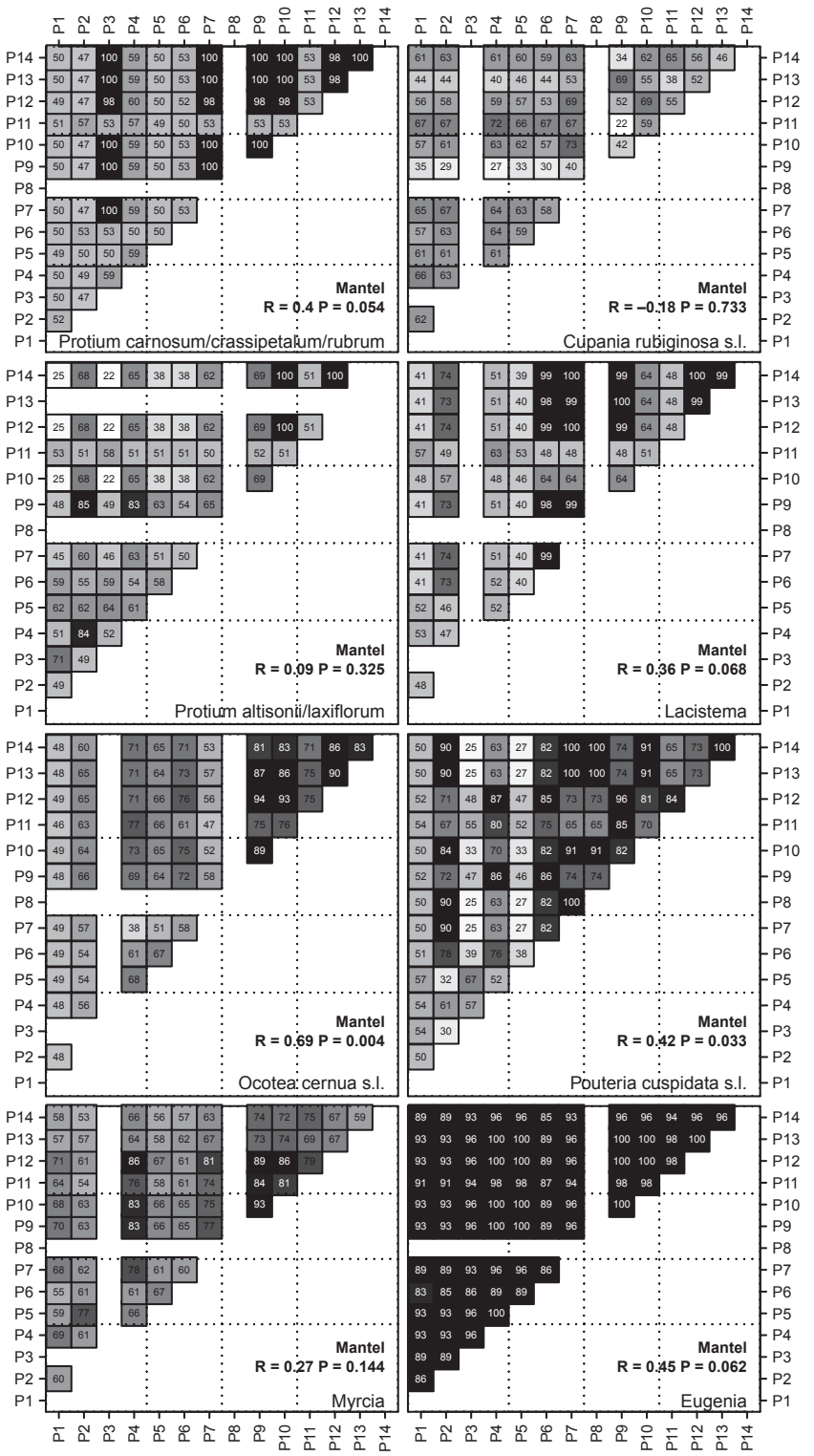

Fig. 2. Matrix of participant vs participant classification similarity values (%) in plant species delimitation for all taxonomic groups/complexes. Participants are ordered by experience ranks: (**a**) P1–P4, no previous experience; (**b**) P5–P7, some experience in morphotyping and no training in taxonomy; (**c**), P8–P10, good experience in morphotyping specimens and good knowledge of the local flora; and (**d**), P11–P14, more than 10 yr of experience and plot taxonomy as a major research focus. The darker the cell colour the higher the similarity. Gaps in a matrix indicate the participant did not process the material for the taxonomic group/complex. Statistics of Mantel tests are shown in the lower right corner of each matrix.

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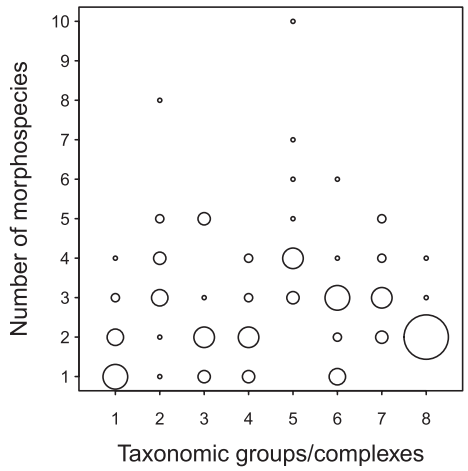

Fig. 3. Number of morphospecies recognized by all participants for the eight taxonomic groups/complexes. The size of the circles is proportional to the number of participants; the smallest circle represents just one participant. Taxonomic groups/complexes are numbered according to Table 1.

varying from 42% to 100% (Table 3). The classification similarities between the field technician and the two botanists were not lower than those between the two botanists. Furthermore, classification similarity values between the field technician and the three most experienced participants, and those between these experienced participants and the two botanists with better knowledge of the local flora were alike.

Discussion

The results indicate that the morphotyping process has a large component of subjectivity. There was high variation in the number of morphospecies recognized by the participants and a fairly low concordance in the classification of the same species groups/complexes by different people. While our focus was on difficult groups, there was even some divergence in the number of morphospecies recognized for the easy group (Fig. 3), in which the lowest concordance between participants was 83%. However, the median pair-wise similarity of 95% for the easy group ('*Eugenia*') does indicate that, in some cases, vegetative morphological characters alone may well allow accurate delimitation of local species.

Clearly, working with sterile material has a number of limitations. Delimiting species using vegetative morphological characters is more difficult when morphological variation appears to be continuous and overlapping, as in the species complexes in particular. This variation may be the result of morphological plasticity due to the environment or to ontogenetic differences between adults and juveniles. This variation is usually not considered by taxonomists, because taxonomic work is usually done by describing a few fertile specimens from adult plants. Sterile specimens are the norm in plot-based inventories, as specimens are collected in a short period of time when most trees are sterile. Therefore, most forest plot inventories are based in practice on sterile material. Even when flowers or fruits are available, these are mostly used for superficial comparisons, rather than dissected for the detailed study required to compare floral characters.

For complex groups, we show that there is a large amount of subjectivity and low concordance even among experts. However, despite the high variability in species delimitation, the concordance was higher than chance for seven out of eight taxonomic groups/complexes, which means that the congruence between pairs of participants was not random and, therefore, that vegetative morphological characters do carry taxonomic information. The only exception to this trend was in the *Cupania* species complex, for which there were few cases of concordance higher than chance. This species complex was the most problematic, and yielded high variation in the number of morphospecies and low concordance even among experienced botanists and among the three participants with good knowledge of the local flora. This species complex

Table 2. Simulation results: number of pair-wise comparisons among participants for which the congruencies in classification were significantly greater than, lower than, or not different from those expected by chance; *P*-value of 0.05.

Taxonomic groups/complexes	Comparisons > chance (%)	Comparisons < chance (%)	Comparisons = chance (%)	Total number of comparisons
Cupania rubiginosa s.l.	25 (37.9)	38 (57.6)	3 (4.5)	66
Protium altisonii/laxiflorum	39 (59.1)	16 (24.2)	11 (16.7)	66
Protium carnosum/crassipetalum/rubrum	49 (62.8)	12 (15.4)	17 (21.8)	78
Lacistema	56 (84.8)	5 (7.6)	5 (7.6)	66
Ocotea cernua s.l.	42 (63.6)	13 (19.7)	11 (16.7)	66
Pouteria cuspidata s.l.	80 (87.9)	3 (3.3)	8 (8.8)	91
Myrcia	34 (51.5)	19 (28.8)	13 (19.7)	66
Eugenia	78 (100.0)	0	0	78

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Table 3. Pair-wise classification similarities (%) for the three participants with better knowledge of the local flora.

Taxonomic groups/complexes	Participants 9 vs 12	Participants 9 vs 10	Participants 10 vs 12
Cupania rubiginosa s.l.	51.8	42.3	69.2
Protium altisonii/laxiflorum	68.7	68.7	100.0[a]
Protium carnosum/ crassipetalum/ rubrum	97.5	100.0[a]	97.5
Lacistema	99.0	64.1	63.6
Ocotea cernua s.l.	93.9	88.6	93.2
Pouteria cuspidata s.l.	95.6	82.0	80.7
Myrcia	88.7	92.7	85.6
Eugenia	100.0	100.0	100.0

All values are significantly higher than those expected by chance ($P < 0.001$), with the exception of those marked with the superscript 'a', for which pair-wise similarities were not different than those expected by chance because both participants sorted the specimens into a single morphospecies.

included fuzzy morphological variation and probably various juveniles in the set of specimens sorted. The large number of cases in the *Cupania* complex for which classification similarity was lower than expected by chance is probably related to the ample morphological variation in this group. The fact that classification was less similar than expected may reflect the bias of participants in weighing some characters more than others, as suggested by the notes made during the experiment by each participant.

Unexpectedly, botanical experience did not improve agreement in species delimitation for most taxonomic groups/complexes. We had hypothesized that agreement in species delimitation would be higher between highly experienced botanists because they would have prior experience with the types of vegetative characters thought to be useful for differentiating species. In plant censuses, botanical expertise may be an important factor affecting the quality of vegetation surveys (Scott & Hallam 2002). These authors showed that the misidentification rate among experts (2.7%) was lower than among less experienced observers (14.1%). In another study involving plant censuses, Archaux et al. (2009) found that the risk of overlooking identification errors was reduced with the researchers' familiarity with the local flora. Our study shows that experience has a minor effect in improving the delimitation of species, and that formal training is also not so important. We also tried to extract patterns from the characters the participants said they used for the classifications, but found none. This suggests that participants used characters other than those they said they had used or, alternatively, that they were not able to translate the complex multivariate pattern they used into a few objective characters. The procedure of extracting patterns and creating categories from more or less continuous morphometric data has a large subjective component (Gift & Stevens 1997), and this seems to be true when morphotyping sterile specimens by visual inspection. For the easy group, the relatively high agreement in morphotyping, independently of botanical experience, suggests that if the morphological pattern is obvious, people will process morphological information in a similar way, even if they are not able to explain how they process the information.

Our focus in this study was on difficult groups, which represent an important component of diversity in the Central Amazonian forests, accounting for ca. 40% of trees in the 25-ha plot, and a probably similar number in other plot inventories in the Amazon region. The variability we observed in the taxonomic groups/complexes can be used to generate an estimate of the error in quantifying diversity. In the Manaus plot we recognized 115 groups (species groups + species complexes) with problems in species delimitation; initial estimates before the April 2011 experiment were that they included 259 morphospecies. When we applied the mean coefficient of variation in the number of morphospecies recognized by the participants to all taxonomic groups/complexes except the easy group (48%), the result suggested that these 115 groups may contain anywhere from 135 to 383 species. Similarly, applying the coefficient of variation for the easy group (27%) to all the 1110 'easily' recognized morphospecies in the Manaus plot (1369 morphospecies in the plot minus 259 belonging to species groups or species complexes), yielded an estimate of species number in this category ranging from 810 to 1410 species. In other words, even for 'easily' defined groups there are still uncertainties in estimates of diversity in highly diverse forests due to variation in morphotyping. Such variation may further increase as one proceeds to the species identification stage (Ahrends et al. 2011).

Emerging technologies and new types of data, such as molecular data (e.g. DNA barcoding, Kress & Erickson 2008; Gonzalez et al. 2009; Kress et al. 2009; Valentini et al. 2009) and near-infrared spectra of leaves or wood (NIRs, Kim et al. 2004; Durgante 2011) may help to solve problems concerning species delimitation and identification, by providing data and analytical methods that are more objective than vegetative morphology for delimiting/identifying species. DNA barcoding may be especially useful in resolving particular problems, such as linking juveniles to adult plants. Gonzalez et al. (2009) found that the correct identification of juveniles increased from 70% to 95% when molecular data were considered, but they also showed that many species and genera in Amazonian forests are not monophyletic, according to several chloroplast markers and internal transcribed spacers (ITS; <70% for both species and genera). The number of markers and, consequently, the necessary funds needed for species iden-

tification may be too high to make this technique a feasible solution. However, the generation of phylogenies may be a by-product (at least for plants) of the barcode initiative (http://www.barcodeoflife.org). Other data, which are promising for plant identification and species delimitation, include absorbance values of NIR light by plant tissues, such as leaves or wood (Kim et al. 2004; Krajsek et al. 2008; Durgante 2011), which may be more cost-effective than DNA barcoding but lack the potential for producing phylogenies. The use of such data in plot work should lower uncertainty in the delimitation of local species. Such data will also permit more accurate comparisons between areas and thus lead to a better understanding of biodiversity (Vamosi et al. 2009; Cadotte et al. 2010; Morlon et al. 2011). However, local species delimitation remains crucial, and it is unlikely that in plot-based inventories the morphotyping process we are discussing here will be changed, since barcoding the numerous plants from inventories is currently not feasible. Our results point to an important source of error in species delimitation that should be explicitly considered. In southwestern Amazonia, Dexter et al. (2010) found that common identification errors for 55 species of the genus *Inga* (Fabaceae) were related to incorrect lumping or splitting of species, and that the total error rates substantially affected the conclusions on species-level analysis of ecological neutral theory. These results emphasize the need to quantify the uncertainty in both species delimitation and species identification in order to reduce error cascades (*sensu* Bortolus 2008) in ecological research, particularly in highly diverse systems such as Amazonian forests. We suggest that species delimitation and species identification should be treated as distinct processes and explicitly considered when dealing with plant inventory data for diversity assessment in the Amazon. Recording problems concerning species delimitation, and perhaps delimiting taxa narrowly, will allow the refinement of plot taxonomy and lead to more precise measurements of diversity.

Conclusion

This study measured the uncertainty in plant species delimitation in a local permanent plot in a highly diverse *terra firme* forest in Central Amazonia. We showed that local species delimitation by morphotyping sterile specimens is prone to large uncertainties, and that botanical experience may not reduce these uncertainties. We argue that uncertainty in species delimitation should be explicitly considered in plant biodiversity inventories, particularly in highly diverse forests. We recommend that species delimitation and identification be treated as separate processes and difficulties explicitly recorded, as we did with the species complexes and species groups, so as to minimize errors and permit the refinement of plot taxonomy. Because species are the working units for most plant ecologists, the uncertainties in the quantification of diversity that have been demonstrated here may have impacts on the interpretation of ecological patterns at a variety of levels.

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Supporting Information

Additional supporting information may be found in the online version of this article:

Data S1. Data and R scripts for analyses and figures.

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