

## A New Species of *Tetramicra* (Orchidaceae: Laeliinae) from Baracoa, Eastern Cuba

Ángel Vale,<sup>1,3</sup> Danny Rojas,<sup>1</sup> Yosvanis Acanda,<sup>1</sup> Natividad L. Sánchez-Abad,<sup>2</sup> and Luis Navarro<sup>1</sup>

<sup>1</sup>Departamento de Biología Vegetal, Universidad de Vigo, Campus Lagoas-Marcosende, CP 36 200, Vigo, España.

<sup>2</sup>Unidad de Servicios Ambientales, Parque Nacional “Alejandro de Humboldt”, CITMA, CP 97 310, Baracoa, Cuba.

<sup>3</sup>Author for correspondence (angel.vale@uvigo.es)

Communicating Editor: Molly Nepokroeff

**Abstract**—A new orchid species, *Tetramicra riparia* is described from eastern Cuba. The new taxon has intermediate traits between the two subgenera of *Tetramicra* currently accepted. In fact, the most useful feature to distinguish *T. riparia* from its congeneric species (the combination of a repent growing habit with a completely entire labellum) is also the main morphological obstacle for classifying it at a subgeneric level. Nevertheless, the classification of the new species in *Tetramicra* subgenus *Tetramicra* is supported by the phylogenetic relationships found for a subset of *Broughtonia* alliance species. In addition, the ecology and conservation status of the new orchid species is discussed and a key for field identification is provided.

**Keywords**—*Broughtonia* alliance, Caribbean, conservation, endangered, riparian, ultramafic rocks.

With more than 10 species, *Tetramicra* Lindley is an orchid genus endemic to the Caribbean islands, among which Hispaniola and Cuba have the majority of the species (Whitner 1996; Nir 2000; Ackerman in press). Recent phylogenetic molecular analyses nested the genus within the *Broughtonia* alliance, which consists of the genera *Broughtonia*, *Psychilis*, *Quisqueya*, and *Tetramicra* (van den Berg et al. 2000; Higgins et al. 2003; van den Berg et al. 2009), and is one of the few groups of plants entirely evolved in the Greater Antilles (Francisco-Ortega et al. 2009). Within this alliance, *Tetramicra* seems to be closer to *Quisqueya* and *Psychilis* than to *Broughtonia* s. l. (Higgins et al. 2003; van den Berg et al. 2009) and differs morphologically from them in the position of the nectar chamber. This structure is at least partially embedded on the pedicel by the fusion of perianth parts and the pedicel in *Broughtonia*, *Psychilis*, and *Quisqueya* but it never reaches the pedicel by being directly formed by the labellum and the column in *Tetramicra* (Dod 1979; Sauleda and Adams 1984; Sauleda 1988). *Tetramicra* is also distinguishable from *Broughtonia* and *Psychilis* in having a terrestrial-rupicolous habit (vs. epiphytic) and smaller flowers. Also, the labellum is completely free from the column in *Quisqueya*, but in *Tetramicra* the labellum and column are frequently fused to form the floral chamber (Dod 1979).

Within *Tetramicra*, the three previously described species endemic to Cuba (*T. eulophiae* Rchb. f. ex Griseb., *T. erosa* Carabia, and *T. simplex* Ames) have an almost entire labellum and short buried rhizomes (Table 1). These species constitute subgenus *Auricula* H. Dietrich section *Auricula* (sensu Dietrich 1985 and Whitner 1996, respectively). In contrast, the species that are not endemic to Cuba have a prominently trilobed labellum and well-exposed stoloniferous rhizomes. These species are included in subgenus *Tetramicra* (Dietrich 1985), which is currently divided into four sections (sensu Whitner 1996): 1) *Tetramicra* (including *T. canaliculata* Urb. and *T. elegans* (Hamilton) Cogn.; 2) *Ekmanii* Whitner (only *T. ekmanii* Mansf.); 3) *Pachycaula* Whitner (only *T. bulbosa* Mansf.), and 4) *Parviflora* Whitner (including *T. parviflora* Lind. ex Rchb. f. and *T. urbaniana* Cogn.) (Table 1). This sectional classification was based on flower size, the degree of fusion between labellum and column, and the presence/absence of pseudobulbs. Subsequent to the publication of Dietrich (1985) and Whitner (1996), two new

species, *Tetramicra malpighiarum* J. A. Hern & M. A. Díaz (Hernández and Díaz 2000) and *T. zanonii* Nir (Nir 2000), were described (from Cuba and Hispaniola, respectively) without reference to subgeneric or sectional classifications.

In this context, our discovery in eastern Cuba of a small orchid with a labellum typical of subgenus *Auricula* and a habit typical of subgenus *Tetramicra*, calls to question the subgeneric classification previously proposed. In this paper we describe this new species and assess its phylogenetic relationships. We also analyzed vegetative and floral characters to detect those traits most useful for distinguishing the new taxon from congeneric species.

### MATERIALS AND METHODS

**Morphological Data**—Morphological data were obtained from natural populations and voucher specimens were deposited at the herbaria indicated in Appendix 1 for almost all species of *Tetramicra* described to date. In the absence of material of the putatively extinct *T. pratensis*, the protologue of this species was used to obtain the data included in this survey. Fresh flowers from 7–15 plants per species were collected in the field in Cuba and Hispaniola and stored in 70% ethanol for measuring floral traits (Appendix 1). *Tetramicra schoenina* (Rchb. f.) Rolfe, *T. schomburgkii* (Rchb. f.) Rolfe, and *T. montecristensis* Dietrich were treated as synonyms of *T. parviflora*, *T. canaliculata*, and *T. ekmanii* respectively, following Ackerman (in press). For the species complexes *T. urbaniana*-*T. parviflora*, *T. elegans*-*T. canaliculata*, and *T. eulophiae*-*T. erosa*, flower samples and sheets from populations of each species s. s. were processed to characterize each taxon s. s. However, taking into account the difficulty of delimiting species in the field as a consequence of intrinsic variability and frequent between-species hybridization episodes, we used conservative criteria (following Ackerman in press) in the field key and, thus, we pooled species in their respective complexes to write the field key.

Measurements of floral characters (Fig. 1) were taken from photographs of flowers well displayed on herbarium sheets (i.e. with all the structures of interest accessible to be measured) and spirit samples of flowers taken from wild populations (see Appendix 1 and Vale et al. 2011 for methodological details). When more than one locality per species was available all the data were pooled to obtain the arithmetic mean and standard deviation.

**DNA Samples and Molecular Procedures**—Five species of *Tetramicra* that represent all sections sensu Whitner (1996) were sampled for molecular phylogenetic analyses. To test if the new taxon is most closely related to *Tetramicra* or to any related genera, one species of each genus of the *Broughtonia* alliance was included. In addition, *Jacquinella teretifolia*, a widespread Neotropical orchid was used as the outgroup (Appendix 2). We used the nuclear ITS region and the plastid *trnL-F* region for the phylogenetic analyses. The former is

TABLE 1. Subgeneric classification, vegetative habit, floral morphology and distribution of the species of the genus *Tetramicra*. Data of the protologus of *T. pratensis* (Reichenbach, 1862) were used to characterize this species. Distribution abbreviated as E. = eastern, W. = western, C. = central, Hisp. = Hispaniola, I. J. = Isla de la Juventud, Jam. = Jamaica, L. A. = Lesser Antilles, P. Rico = Puerto Rico, V. I. = Virgin Islands (for the other abbreviations see Methods).

	<i>T. riparia</i>	<i>T. erosa</i>	<i>T. eulophiae</i>	<i>T. simplex</i>	<i>T. canaliculata</i>	<i>T. elegans</i>
Subgenus	<i>Tetramicra</i>	<i>Auricula</i>	<i>Auricula</i>	<i>Auricula</i>	<i>Tetramicra</i>	<i>Tetramicra</i>
Section	Unknown	<i>Auricula</i>	<i>Auricula</i>	<i>Auricula</i>	<i>Tetramicra</i>	<i>Tetramicra</i>
Root	Spongy and smooth	Thick-fleshy	Thick-fleshy	Thick-fleshy	Spongy and smooth	Spongy and smooth
Rhizome	Caespitose to repent	Erect	Erect	Erect	Repent	Repent
Shoot	A pseudobulb	Acaule	Acaule	Acaule	Thin and straight	Thin and straight
Leaf type	Sub-cylindrical	Cylindrical	Cylindrical	Cylindrical	Sub-cylindrical	Sub-cylindrical
Flower size (mm)	6.5–15.5	16–22	19–25	11–18.5	20–25	14–22
PL/CL ratio	3.73 ± 0.27	1.85 ± 0.35	1.39 ± 0.25	2.22 ± 0.47	2.69 ± 0.37	2.64 ± 0.43
SDC-L	First third	First third	First third	Second third	First third	First third
Dorsal sepal length (mm)	6.78 ± 0.99	7.89 ± 0.49	9.17 ± 0.97	7.03 ± 0.66	8.93 ± 0.99	7.64 ± 1.47
Labellum length (mm)	8.89 ± 2.85	9.85 ± 0.82	11.22 ± 1.38	9.78 ± 1.82	15.36 ± 1.39	10.84 ± 1.85
Column length (mm)	4.34 ± 0.32	5.65 ± 0.37	6.30 ± 0.60	4.66 ± 0.56	5.49 ± 0.62	4.77 ± 0.57
LLL/CLL ratio	0	0.14 ± 0.08	0.26 ± 0.06	0.09 ± 0.10	0.83 ± 0.07	0.83 ± 0.10
Distribution	E Cuba	C to E Cuba	W Cuba & I. J	W Cuba & I. J.	Hisp.	P. R., V. I. & L.A.
Sample size	12	15	15	14	12	7

(continued)

biparentally inherited and is considered the fastest evolving sequence region in the plant genome (Álvarez and Wendel 2003; Hughes et al. 2006). It has been shown to be of great utility for phylogenetic analysis among closely related species of orchids (van den Berg et al. 2005; Gustafsson et al. 2010) and flowering plants in general (Baldwin 1992; Baldwin et al. 1995). The *trnL-F* region has also been shown to be informative among closely related species (Wikström et al. 1999) and is widely used in orchids as well (van den Berg et al. 2000, 2005, 2009; Kennedy and Watson 2010; Monteiro et al. 2010), albeit less variable than ITS.

Dried floral or foliar tissues (60 mg) of each taxon were macerated in liquid nitrogen and total genomic DNA was isolated using the PowerPlant® DNA isolation kit (Mo Bio Laboratories Inc., Carlsbad, California) following the manufacturer's protocol. The *trnL-F* region

was amplified using the primers c and f (Taberlet et al. 1991), and the ITS region with the primers 17SE and 26SE (Sun et al. 1994). Difficult samples (including the new taxon) were reamplified with specific primers designed with Gene Runner v3.01 (Hasting Software Inc., Las Vegas, Nevada) from sequences of the *Broughtonia* alliance species that we previously obtained and others published in GenBank. New specific primers used for the *trnL-F* region were: forward T2F (5' ATTCAGAGA AACCCTGGAATAA 3') and reverse T38R (5' TATCCCGACGATTT CCCC 3'); and for the ITS region: forward I4F (5' AGGAGAAGTCG TAACAAGGT 3') and reverse I4R (5' GGGGTCGCATTCCAA 3'). All PCR reactions were performed in 20 µl volumes containing 40 ng DNA template, 200 µM of each dNTP, 0.5 µM of each primer, 1.0 U Green *Taq* DNA polymerase (GenScript, Aachen, Germany), 1.5 mM MgCl<sub>2</sub>, and 2 µL 10× *Taq* reaction buffer. The PCR reactions were conducted in an

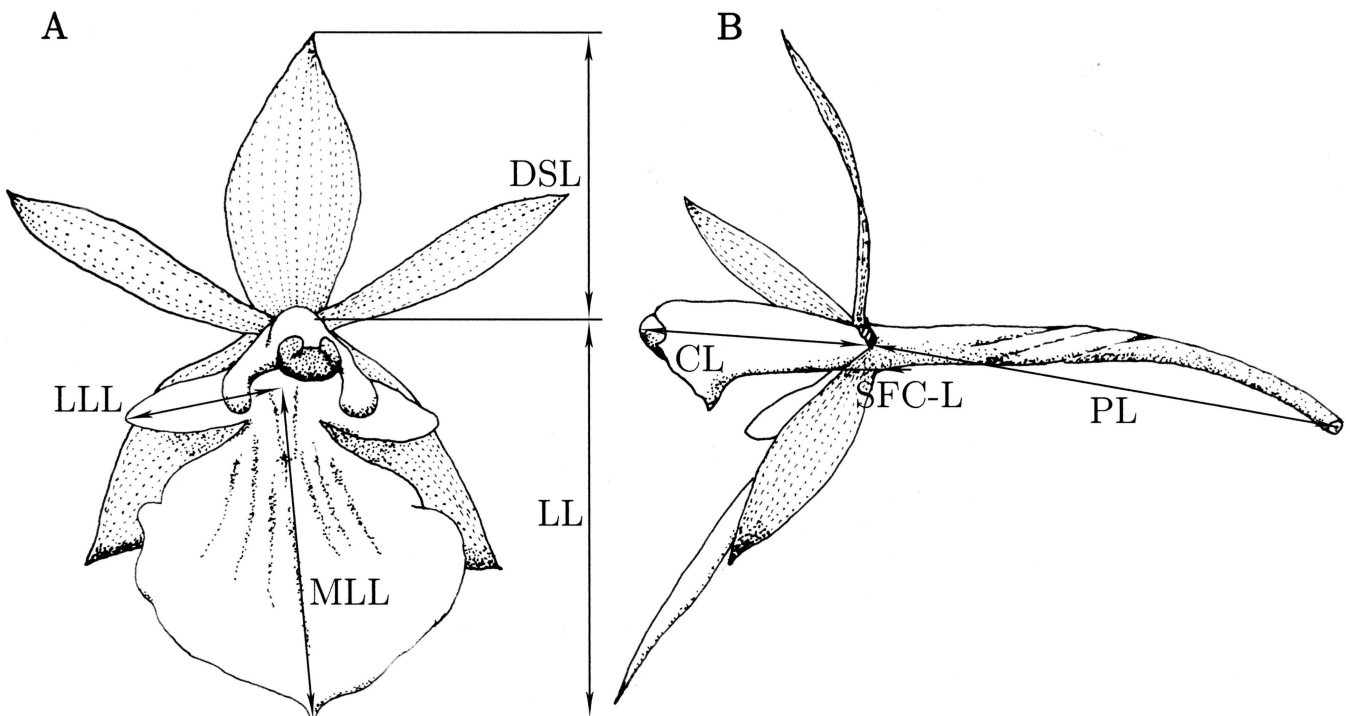


FIG. 1. Morphological measurements of the flowers of *Tetramicra*. A. Front view. B. Lateral view. CL: Column length, DSL: Dorsal sepal length, LL: labellum length, LLL: lateral lobe length, MLL: mid lobe length, PL: pedicel length, and SFC-L: segment of fusion between column and labellum. Drawn by Á. V. after a spirit sample of *T. eulophiae*.

TABLE 1. Continued.

<i>T. ekmanii</i>	<i>T. bulbosa</i>	<i>T. parviflora</i>	<i>T. urbaniana</i>	<i>T. malpighiarum</i>	<i>T. pratensis</i>	<i>T. zanonii</i>
<i>Tetramicra Ekmanii</i>	<i>Tetramicra Pachycaula</i>	<i>Tetramicra Parviflora</i>	<i>Tetramicra Parviflora</i>	Unknown	Unknown	<i>Tetramicra Parviflora</i>
Spongy and smooth	Spongy and smooth	Spongy and sub-fleshy	Spongy and sub-fleshy	Spongy, rugose-pustulate	Spongy and smooth	Spongy and smooth
Repent	Caespitose	Repent	Repent	Erect	Repent	Repent
Thin, short and straight	A pseudobulb	Acaule	Acaule	Acaule	Thin and straight	Acaule
Cylindrical	Flattened to sub-cylindrical	Flattened to sub-cylindrical	Flattened	Cylindrical	Sub-cylindrical	Flattened
18–30	15.5–25.5	9–14	9–12	13.5–16	n. 16	
2.69 ± 0.34	2.91 ± 0.88	2.60 ± 0.63	2.21 ± 0.46	1.93 ± 0.17	3	3.61 ± 0.78
Last third	First third	First third	First third	First third	First third	First third
9.10 ± 1.07	8.63 ± 0.95	5.44 ± 0.32	4.50 ± 0.60	7.29 ± 0.45	6.5	6.24 ± 1.36
15.60 ± 2.93	11.12 ± 2.40	6.71 ± 0.91	6.19 ± 0.88	7.61 ± 0.41	9.5	7.09 ± 2.17
6.35 ± 0.81	6.44 ± 1.00	3.60 ± 0.43	3.52 ± 0.84	4.12 ± 0.33	5	3.45 ± 0.69
0.93 ± 0.07	0.87 ± 0.10	0.59 ± 0.15	0.73 ± 0.06	0	1	0.88 ± 0.14
E. Cuba & Hisp.	Jam. & Hisp.	Jam. & Hisp.	W. Cuba & Bahamas	C. and E. Cuba	Hisp.	E. Hisp.
8	9	13	8	9	-	6

iCycler thermocycler (Bio-Rad, Hercules, California) and consisted of a denaturation step at 94°C for 3 min, 30 cycles of 30 s at 94°C, 30 s at 50°C, and 45 s at 72°C, and one final extension at 72°C for 2 min. The PCR products were checked with agarose gel electrophoresis and purified using EasySpin PCR purification kit (Citomed, Lisbon, Portugal). Samples were sequenced using BigDye® v3.1 cycling sequencing kit and the genetic analyzer ABI Prism 3130 (Applied Biosystems, Foster City, California). Species with illegible sequences or double peaks (*T. eulophiae*, *T. ekmanii* and the new taxon) were cloned into pTZ57R/T vector using InsTAclone PCR cloning kit (Fermentas, St. Leon-Rot, Germany). Recombinant plasmids were purified from 6 ml of *Escherichia coli* JM107 precultures using GeneJET plasmid miniprep kit (Fermentas). Plasmids were double-digested (*Xba*I/*Bam*HI) and digestion products were separated by agarose gel electrophoresis in order to select recombinant clones. Four recombinant clones per transformation were sequenced as previously mentioned, and a consensus sequence was built for each taxon. All sequences were deposited in GenBank (see accession numbers in Appendix 2).

**Phylogenetic Analysis**—Individual alignments of the two loci were conducted in MUSCLE 3.6 (Edgar 2004). We determined the substitution models that best fit the data in jModelTest 0.1.1 (Posada 2008): the general time reversible model (GTR) for *trnL-F*, and GTR with a gamma distribution of rate variation (GTR +  $\Gamma$ ) for ITS. Phylogenetic analyses were conducted on concatenated sequences previously partitioned by locus, and on each locus independently. In each case we conducted four independent Markov chain Monte Carlo (MCMC) analyses in Bayes-Phylogenies (Pagel and Meade 2006). Each analysis was run for 10 million generations, sampling from the chain every 1,000 generations. Convergence was assessed with Tracer 1.5 (Rambaut and Drummond 2007) after a burn-in value of 75% of the samples. Results of the analyses were combined in a single file with a sample size of 10,000 trees. Then the results were summarized with a 50% majority-rule consensus tree.

We also conducted maximum-likelihood (ML) analyses in RAxML 7.0.3 with the rapid hill-climbing algorithm (Stamatakis 2006). Support values were obtained through a rapid bootstrap (BS) algorithm (Stamatakis et al. 2008) with 5,000 iterations.

## RESULTS

**Subgeneric Classification Based on Morphology**—The new *Tetramicra* has characters of both subgenera and at least three sections (Table 1). For example, it has true pseudobulbs like *T. bulbosa* (Figs. 2, 3). The growth habit of the rhizome of seedlings is caespitose (as in *T. bulbosa* plants, section *Pachycaula*) but in mature plants it changes to a repent habit (as occurs in the species of the section *Parviflora*). Its flower size is similar to that of *T. parviflora* and *T. urbaniana*

(Table 1). On the other hand, the floral pedicel of the new species is large in comparison to its flower size (Figs. 2, 3). Consequently, the species possesses the highest values of the PL/CL ratio in the genus, overlapping only with *T. zanonii* (Fig. 4). This remarkable aspect separates the new species from the other Cuban endemic species (and from members of subgenus *Auricula*). The elements mentioned above suggest this new species should be classified into subgenus *Tetramicra*. On the other hand, it has a completely entire labellum, a feature only shared with *T. malpighiarum*, which suggests that these two species may be most closely related to species of subgenus *Auricula* (Fig. 4). The species of subgenus *Auricula* are characterized by LLL/CLL ratios that are less than half the value of those of subgenus *Tetramicra*. Therefore, according to the morphological comparison here, the inclusion of the new species in either subgenus or section is not clear.

**Phylogenetic Analysis**—The ITS sequences of clones from difficult species were identical or differed at few (one to three) positions. This was also found among the four clones obtained of the new species differing in only two positions. Thus, the ITS trees obtained with the inclusion of the sequences of those clones was the same at the species level as those obtained using one single consensus sequence for each species. Maximum-likelihood (ML) analyses were consistent with the Bayesian phylogenetic estimation (thus results not showed). The sequenced *trnL-F* comprised 967 bases, and ITS totaled 653. All phylogenetic reconstructions (with the two independent loci and with both of them concatenated) indicated that the new species belongs to the genus *Tetramicra*, subgenus *Tetramicra* (Fig. 5A-C). In all cases, the genus *Tetramicra* was strongly supported (BPP between 0.98 and 1) as a monophyletic clade.

## DISCUSSION

**The Generic and Subgeneric Position of the New Species**—The new orchid species here described possesses a unique combination of characters. In addition, its inclusion in *Tetramicra* is unequivocal according to both morphological and molecular evidence (Fig. 5). Nevertheless, additional species of

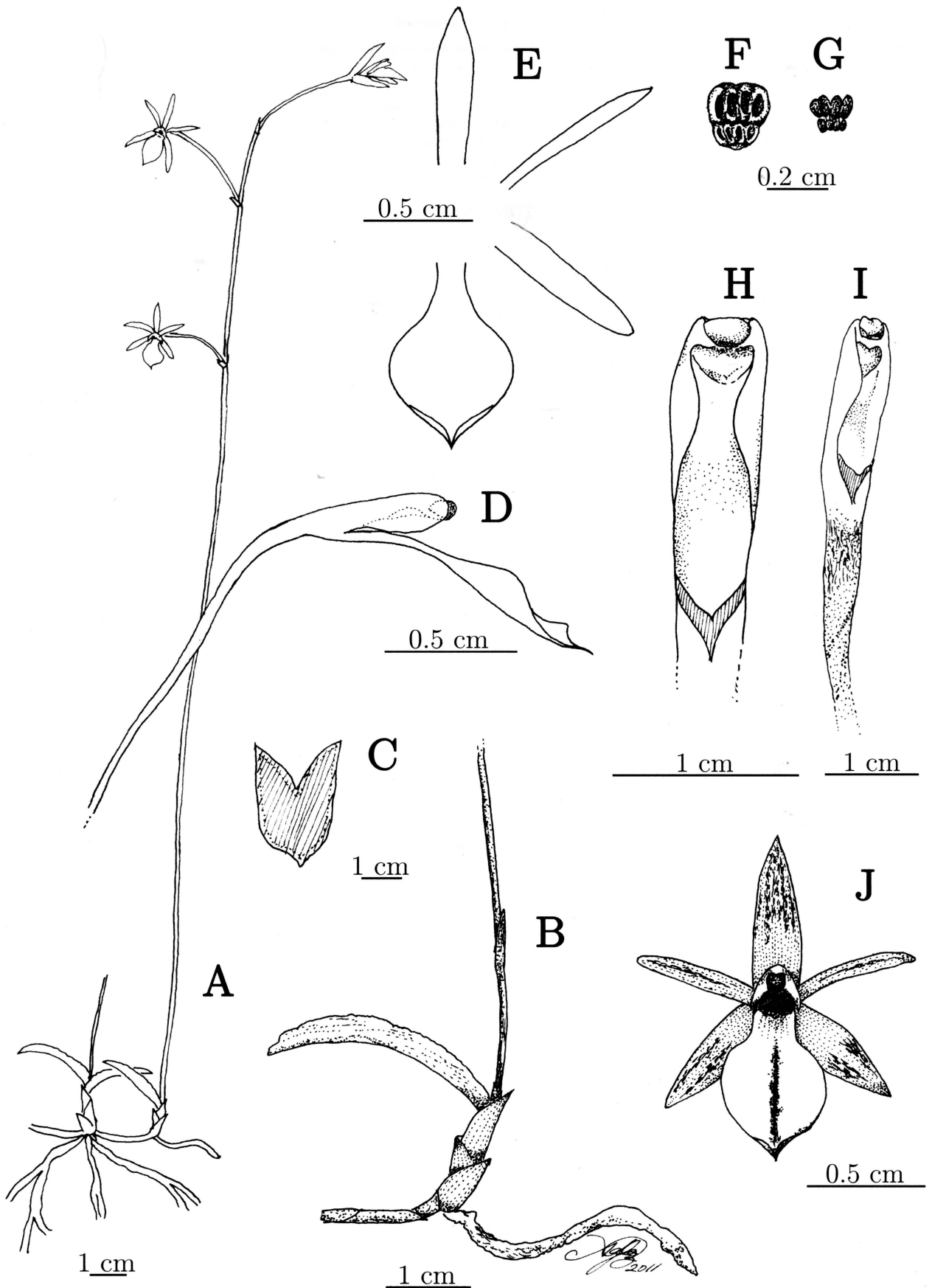


FIG. 2. *Tetramicra riparia*. A. Plant habit. B. Detail of internode, shoot and leaf. C. Transverse section of leaf. D. Lateral view of flower. E. Perianth pieces removed. F. Anthercap in ventral view. G. Pollinarium. H. Column in ventral view. I. Column and part of pedicel in lateral view. J. Flower in frontal view. Drawn by A.V. from holotype.

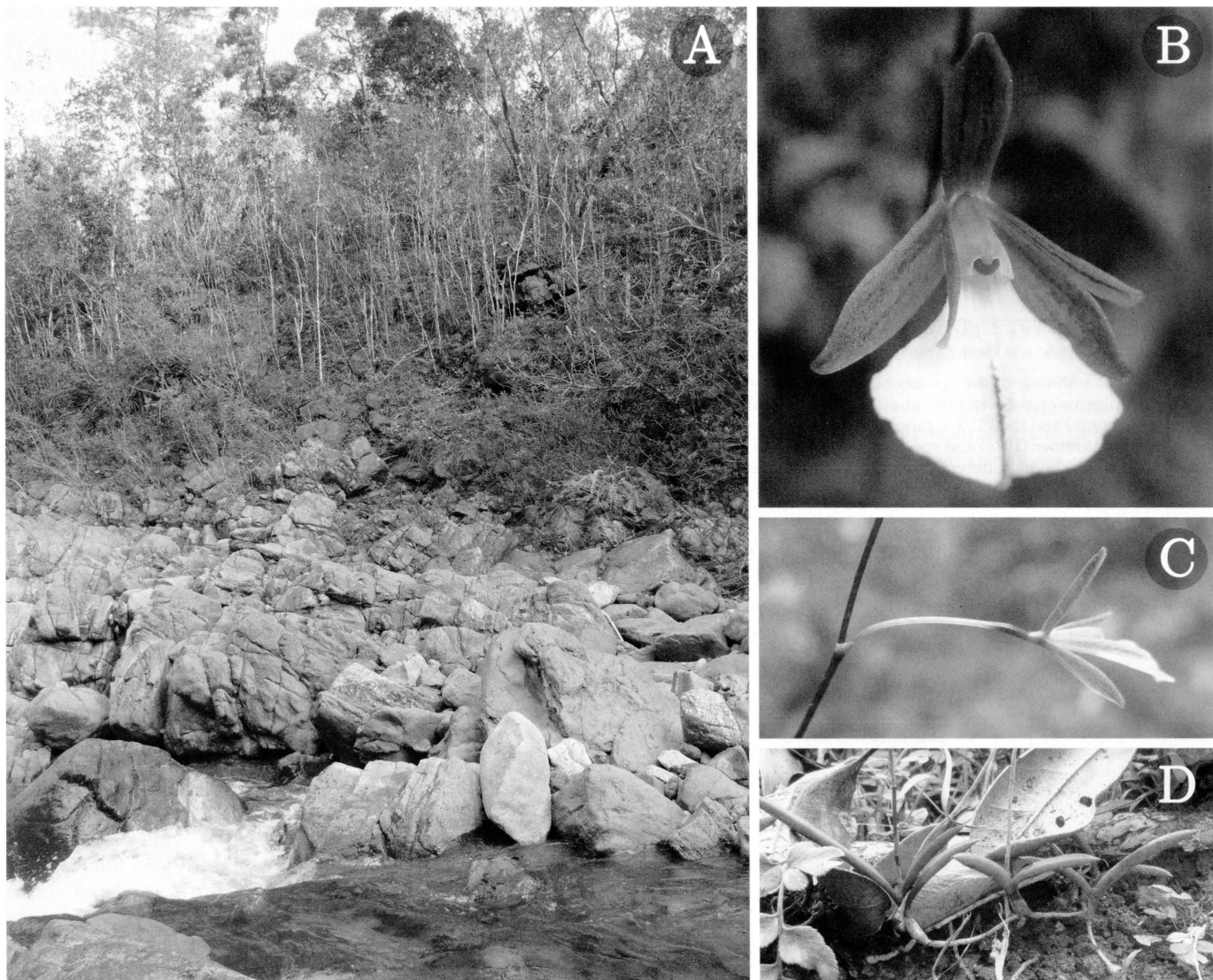


FIG. 3. Habitat and plant details of *Tetramicra riparia*. A. Sunny slopes of the riverbank bordered by a gallery shrub forest inhabited by the species. B. Flower frontal view. C. Flower lateral view, note the large pedicel with regard to the column. D. Plant habit.

*Quisqueya* should be included in future studies to assess if these two genera are reciprocally monophyletic. In addition, a new phylogeny considering most species of *Tetramicra* (including the most recently described species) is also needed to reevaluate the putative boundaries among subgeneric and sectional categories that are currently assumed on the basis of morphological traits. For the moment, regarding the position of the new species, two levels of incongruence between our results and the infrageneric classification of Dietrich (1985) hinder our ability to assign this species to subgenus. Firstly, the new species is vegetatively reminiscent of subgenus *Tetramicra* (see Table 1; Fig. 2A, B; Fig. 3D), but florally it is more like subgenus *Auricula* (see Table 1; Fig. 2; 3B, C). The molecular phylogenetic results are consistent with assigning the new taxon to subgenus *Tetramicra* (see Fig. 5A-C), but this is inconsistent with all other members of the subgenus having a trilobed labellum.

**Exploring a Putative Hybrid Origin**—Alternatively, a hybrid origin hypothesis could explain the intermediate morphological nature of *T. riparia*. Hybridization usually results in intermediate features in orchid species (Cozzolino and Aceto

1994; Caputo et al. 1997; Peakall et al. 1997; Borba and Semir 1998; Barkman and Simpson 2002; Wallace 2003; Pansarin and Amaral 2008). This phenomenon is common in many families of flowering plants (Wolfe et al. 1998; Sang and Zhang 1999; Seehausen 2004; Friar et al. 2008) and is especially frequent among orchids (Klier et al. 1991; Cozzolino et al. 2006; Kennedy and Watson 2010; Pinheiro et al. 2010).

Within the *Broughtonia* alliance, natural hybrids have been reported within *Broughtonia* (*B. × jamaicensis*; Sauleda and Adams 1984), *Psychilis* (*P. × tudiana* and *P. × raganii*; Dod 1983 and Sauleda 1938, respectively), and even between these two genera in the natural hybrid formed by *Broughtonia domingensis* (Lindl.) Rolfe and *Psychilis olivacea* (Cogn.) Sauleda (Sauleda 1988). In light of the hypothesis of a hybrid origin of *T. riparia*, the plastid and nuclear topologies, which differ in the position of *T. parviflora* (see Fig. 5A, B), reflect the history of each locus (Farris et al. 1995; Soltis and Kuzoff 1995; Sang and Zhong 2000; Sang 2002). In fact, particularly for the plastid marker, 99% of the genetic information of the new species and *T. bulbosa* are identical (data in GenBank and not shown here), which

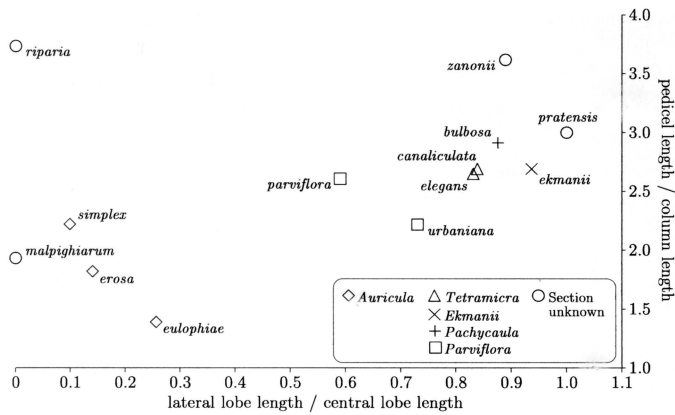


FIG. 4. The ordering of the species of the genus *Tetramicra* plotted on two non-dimensional traits: the LLL/CLL ratio (lateral lobes length/central lobe length) and the PL/CL ratio (pedicel length/column length). Sections sensu Whitner (1996) and species not included in scheme of this author are symbolized by different figures. *T. pratensis* was characterized from data of its protologue. Standard deviations for the two ratios are shown in Table 1.

may explain the sister relationship between them in the *trnL-F* tree (Fig. 5C). If the new species is a hybrid-derived species then it might have inherited its chloroplast genome from *T. bulbosa* or the ancestor of this species. This puta-

tive maternal role of *T. bulbosa* (or an ancestor) for the new taxon is also supported by the occurrence in both species of pseudobulbs and other morphological similarities mentioned above (see Table 1). In addition, there is an entire sequence of 11 bases between the positions 130 and 140 of the *trnL-F* marker that is absent in the new *Tetramicra* and *T. bulbosa* but that is found in the other species.

If a hybrid origin is the cause of the intermediate morphology of the new *Tetramicra* species, the male parent may be among the Cuban species where the presence of an entire or weakly trilobed labellum is a common feature. Among the current Cuban species, those most similar morphologically to *T. riparia* are *T. malpighiarum* and *T. erosa*. The former also has an entire labellum and the latter has auricles that become shorter in populations toward the eastern region (i.e. nearer to Mina Amores). *Tetramicra malpighiarum* is ecologically different from *T. riparia*. It is reported only as epiphytic in calcareous coastal ecosystems in three localities in eastern Cuba (Hernández and Díaz 2000). By contrast, *T. erosa* and *T. riparia* are terrestrial-rupicolous and occur in ultramafic ecosystems (Nir 2000; Llamacho and Larramendi 2005; Barreto et al. 2008). At this point, additional studies of the karyotypes of the species of *Tetramicra* (including *T. riparia* and the species suspected as its possible parents) could help to confirm the probability of a hybrid origin of this new taxon (Wallace 2003).

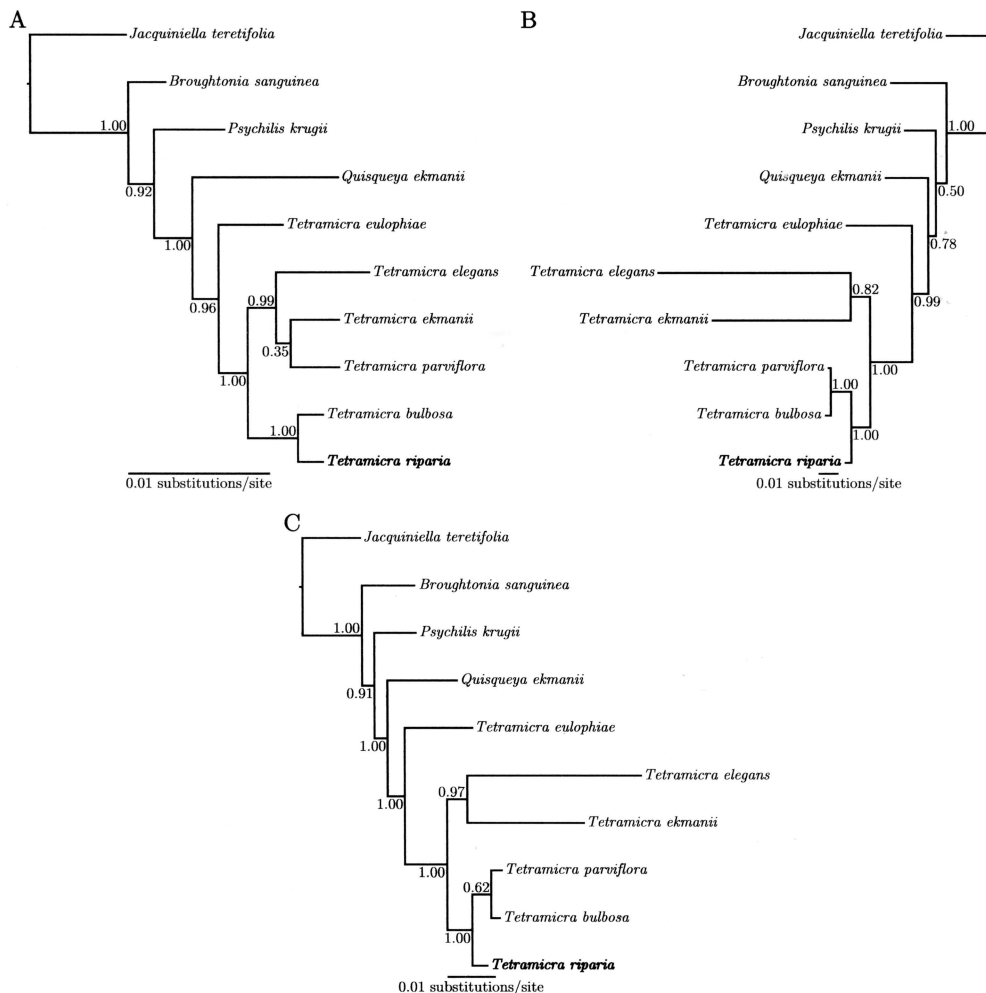


FIG. 5. Majority-rule consensus trees of 10,000 trees resulting from Bayesian inference analyses of: A. Sequences of the *trnL-F* region. B. ITS region. C. concatenated sequences of ITS and *trnL-F* regions together. Values correspond to Bayesian posterior probabilities. The new taxon described in this study is shown in bold italics.

## TAXONOMIC TREATMENT

***Tetramicra riparia*** Vale, Sánchez-Abad & L. Navarro. sp. nov.—TYPE: CUBA. Provincia Guantánamo, Baracoa, Orilla del Río Báez, Mina Amores (20° 25'57"N & 74° 36'31"W), cerca de la mina de cromo (56 m. s. n. m.), 26 February 2010. Á. Vale, N. L. Sánchez-Abad & J. A. Rodríguez AVG2010-04 (holotype: HAC; isotypes: BSC, M, SANT).

Herb, caespitose to repent, rupicolous to terrestrial, 18 (8–26) cm tall including the inflorescence, and 2.5 (1–4) cm when sterile. Roots from the nodes, glabrous, white, 2 (1–4) mm thick. Rhizome stoloniferous. Internodes mainly showy, rarely absent, 15 (0.1–40) mm long and 2–4 mm wide, covered by adpressed papyraceous sheaths when young. Rhizome sheaths lanceolate ca. 0.6 (0.3–1.2) mm long × 0.3 (0.1–0.4) mm wide. Vertical pseudobulbs of 13–30 mm including the leaves. Sheath of the shoots (2–4) amplexicaulous, papyraceous, oblong to lanceolate, 1–5 mm long × ca. 0.4–1.5 mm wide. Leaves dark green, sometimes purplish-green when new and purple in plants growing on sunny surfaces. Leaves 2 (1–4) per shoot, distichous, articulate, succulent, rugose, conduplicate, erose-denticulate, ligulate to narrowly elliptical, and acute to acuminate; blades 21 (14–30) mm long, 3 (2–4) mm wide and 2.2 (2–3) mm high. Inflorescence terminal, erect when young and erect to slender at anthesis, 15 (9–23) cm long and up 1.5 mm across, bearing 3 (1–5) spirally arranged flowers that open successively. Bracts of the raceme more distant toward the apex, tightly sheathing, 2–3 × 0.5–1 mm, lanceolate and acuminate, rarely cup-like; the fertile ones and near ones cup-like and less adpressed than the sterile ones. Flowers tiny, resupinate with the perianth spreading to 10–17 mm long (from the dorsal sepal apex to the labellum apex). Tepals brownish green and brown-spotted, labellum white. Petals larger than sepals but frequently thinner (by up to a half), open to the same extent as the sepals (or sometimes less) and projected toward the labellum. Pedicel of 16.15 (15.3–18.6) mm twisted, including an ovary 4.1 (4–4.5) mm long. Sepals similarly wide, the laterals somewhat oblique and sometimes shorter than the dorsal. Dorsal sepal of 6.8 (5.4–7.8) × 1.34 (1.2–1.9) mm, lanceolate. Petals 7.1 (5.7–8.4) mm × 0.63 (0.32–0.94) mm, linear-lanceolate. Labellum 9.5 (8.6–11) × 5.4 (4.7–6) mm completely white with 1–3 purple veins along the distal half of the central line. Labellum basally fused to the column in the basal third. Column of 4.34 (3.79–4.83) × 1.9 (1.3–2.2) mm. Anther cap white to purple, of ca. 2 mm. Stigma of 0.52 (0.36–0.71) × 0.78 (0.65–1.12) mm. Fruit unknown. Figures. 2, 3.

**Diagnostic Features**—*Tetramicra riparia* can be distinguished from the other *Tetramicra* species mainly by three features: 1) the combination of a repent habit and an entire labellum, 2) the presence of a rhizome with massive internodes and pseudobulbs less than 1.5 cm, and 3) the presence of a pedicel almost four times larger than the column (Figs. 2D, 3C, 4). The new taxon also differs from other small species (i.e. *T. parviflora*, *T. urbaniana*, and *T. zanonii*) because these species have filiform internodes and a trilobed labellum (Table 1; Fig. 4). *Tetramicra riparia* differs from *T. malpighiarum* in growth habit (rupicolous in the former and epiphytic in the latter), as well as in the color of the labellum (pinkish in *T. malpighiarum* and white in *T. riparia*) (Fig. 3B). The other species (namely *T. bulbosa*, *T. canaliculata*, *T. ekmanii*, *T. elegans*, *T. erosa*, *T. euphoniae*, *T. pratense*, and *T. simplex*) are much larger plants and have larger flowers than *T. riparia* (Table 1) (i.e. flower diameter is larger than 16 mm). In particular, the species of subgenus *Tetramicra* (i.e. *T. bulbosa*, *T. canaliculata*, *T. ekmanii*, *T. elegans*, and *T. pratensis*) can also be easily distinguished from *T. riparia* because of the prominently trilobed labella they possess.

**Distribution and Ecology**—*Tetramicra riparia* is only known from eastern Cuba. It grows along a short segment of the southern riverbank of Río Báez on slopes with a mix of gabbros and ultramafic rocks, sometimes growing in interstitial mud (Fig. 3A, D). The site is part of the UN Biosphere Reserve “Cuchillas del Toa,” near the border with the “Alejandro de Humboldt” National Park. The richness of these rocks in chrome, aluminum, and other heavy metals, suggests this orchid could be a specialized serpentinophyte, which is a common phenomenon among the endemic flora of this site (Sánchez-Abad 2008). The population occurs in the ecotone between the riparian bushy forest (Sánchez-Abad 2008) and a charrascal (a sub-spiny shrubby forest) with elements of mountain rain forest (Capote and Berazaín 1984). In this area, *T. riparia* grows associated with *Anemia coriacea*, *Rhinchospora* sp., *Dorstenia* sp., *Encyclia moebusii*, *Selaginella* sp., *Gundlachia* sp., *Pitcairnia cubensis*, and *Blettia antillana*.

**Conservation Assessment**—Between 2007 and 2011 the number of reproductive plants in the population of *Tetramicra riparia* changed from 35–42 individuals. The only known population of these plants is distributed along the southern riverbank of Río Báez, covering an area of 200 m<sup>2</sup>. Therefore, we propose classifying *T. riparia* under the IUCN (2001, 2006) category CR B1a; D.

**Etymology**—The specific epithet refers to its habit on rocky riverbanks (Fig. 3A).

KEY TO SPECIES AND SPECIES COMPLEXES OF THE GENUS *TETRAMICRA*

1. Labellum with the lateral lobes equal or larger than the mid lobe ..... 2
2. Shoots with pseudobulbs ..... *T. bulbosa*
2. Shoot without pseudobulbs ..... 3
3. Leaves more or less flattened (diameter > 12 mm) ..... 4
4. Flower diameter < 14 mm; pedicel length/column length ratio < 3 ..... *T. parviflora*-*T. urbaniana* complex
4. Flower diameter > 23 mm; pedicel length /column lengthratio > 3 ..... *T. zanonii*
3. Leaves terete (diameter < 7 mm wide) ..... 5
5. Dorsal sepals and lateral petals forming an obtuse angle; distal portions of lateral petals hidden behind the labellum ..... *T. ekmanii*
5. Dorsal sepals and lateral petals forming an acute angle; lateral petals almost completely visible ..... 6
6. Labellum disk basally bimamillate, with a narrow crest from the mammilla toward the middle apex ..... *T. pratensis*
6. Disk without mammilla ..... *T. canaliculata* – *T. elegans* complex

1. Labellum entire or lateral lobes rudimentary or much shorter than the mid lobe ..... 7
7. Internodes and pseudobulbs conspicuous ..... *T. riparia*
7. Internodes inconspicuous without pseudobulbs ..... 8
8. Plant growing on trunks, with visible pustulate roots; leaves < 5 cm ..... *T. malpighiarum*
8. Plant growing on the ground, with the roots completely buried; leaves > 5 cm ..... 9
9. Labellum as long as wide or wider than long; labellum touching the column along the first and second third of the column length; growing exclusively in white sands savannas of Pinar del Río and Isla de la Juventud ..... *T. simplex*
9. Labellum longer than wide; labellum touching the column only in the first third of the column length; in sandy and rocky habitats (especially in serpentine and schistose soils) of Cuba and Isla de la Juventud ..... *T. eulophiae*-*T. erosa* complex

ACKNOWLEDGMENTS. The study was supported by Agencia Española de Cooperación Internacional para el Desarrollo (AECID) PhD grants to AVG and YAA, a "María Barbeito" PhD grant from the Xunta de Galicia to DRM, the Project A/023710/09 from AECID, the grant CGL2009-10466 of the Spanish Dirección General de Investigación, Ciencia y Tecnología; FEDER funds from the European Union; the project CYTED 409AC0369 and the grant INCITE09-3103009PR of the Xunta de Galicia. We are also grateful to the cited herbaria by physical/digital access to specimens, especially to HAC, SANT, BSC, JBSD, HAJB, and M for receiving types and vouchers. We thank C. Hamilton, J. Cordero, M. Islam, R. Oviedo, Y. Domínguez, M. Faife, S. Martín-Rodríguez, K. Hennessey, S. Pagliuca, J. Llamacho, and F. Jiménez for samples and assistance in Jamaica, Hispaniola, and Cuba. Thanks to J. D. Ackerman by providing Puerto Rican and Lesser Antillean samples and data in press. Thanks to M. Rey and V. Ferrero for supporting lab and office work. We also thank to the National Park Alejandro de Humboldt's staff and the St. Augustine-Baracoa Friendship Association for field assistance. Special thanks to C. Hamilton for spelling and grammar support.

## LITERATURE CITED

- Ackerman, J. D. Flora of the Greater Antilles. *Memoirs of the New York Botanical Garden* (in press).
- Álvarez, I. and J. F. Wendel. 2003. Ribosomal ITS sequences and plant phylogenetic inference. *Molecular Phylogenetics and Evolution* 29: 417–434.
- Baldwin, B. G. 1992. Phylogenetic utility of the internal transcribed spacers of nuclear ribosomal DNA in plants: an example from the Compositae. *Molecular Phylogenetics and Evolution* 1: 3–16.
- Baldwin, B. G., M. J. Sanderson, M. J. Porter, M. F. Wojciechowski, C. S. Campbell, and M. J. Donoghue. 1995. The ITS region of nuclear ribosomal DNA: a valuable source of evidence on angiosperm phylogeny. *Annals of the Missouri Botanical Garden* 82: 247–277.
- Barkman, T. J. and B. B. Simpson. 2002. Hybrid origin and parentage of *Dendrochilum acuíferum* (Orchidaceae), inferred in a phylogenetic context using nuclear and plastid DNA sequence data. *Systematic Botany* 27: 209–220.
- Barreto, A., J. Ávila, N. Enríquez, R. Oviedo, B. L. Toscano, and G. Reyes. 2008. Flora y vegetación de la propuesta de reserva florística manejada "Meseta de San Felipe," Camagüey, Cuba. *Foresta Veracruzana* 10: 9–24.
- Borba, E. L. and J. Semir. 1998. *Bulbophyllum* × *cipoense* (Orchidaceae), a new natural hybrid from the Brazilian campos rupestres: description and biology. *Lindleyana* 13: 113–120.
- Capote, R. P. and R. Berazain. 1984. Clasificación de las formaciones vegetales de Cuba. *Revista del Jardín Botánico Nacional* 5: 1–49.
- Caputo, P., S. Aceto, S. Cozzolino, and R. Nazzaro. 1997. Morphological and molecular characterization of a natural hybrid between *Orchis laxiflora* and *O. morio* (Orchidaceae). *Plant Systematics and Evolution* 205: 147–155.
- Cozzolino, S. and S. Aceto. 1994. Morphological and molecular characterization of ×*Orchiaceras bergonii* (Nanteuil) E. G. Cam. *Giornale Botanico Italiano (Florence, Italy)* 128: 861–867.
- Cozzolino, S., A. M. Nardella, S. Impagliazzo, A. Widmer, and C. Lexer. 2006. Hybridization and conservation of Mediterranean orchids: should we protect the orchid hybrids or the orchid hybrid zones? *Biological Conservation* 129: 14–23.
- Dietrich, H. 1985. Orchidaceae cubanae novae V. Taxa nova et critica ex generibus *Tetramicra*, *Encyclia*, *Stelis* et *Malaxis*. *Feddes Repertorium* 96: 559–573.
- Dod, D. D. 1979. *Quisqueya* - a new and endemic genus from the island of Hispaniola. *American Orchid Society Bulletin* 48: 140–150.
- Dod, D. D. 1983. Orquídeas (Orchidaceae) nuevas para La Española y otras notas. IV. *Moscosa* 2: 2–18.
- Edgar, R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Research* 32: 1792–1797.
- Farris, J. S., M. Källersjö, A. G. Kluge, and C. Bult. 1995. Testing significance of incongruence. *Cladistics* 10: 315–319.
- Francisco-Ortega, J., E. Santiago-Valentín, P. Acevedo-Rodríguez, C. Lewis, J. Pipoly III, A. W. Meerow, and M. Maunder. 2009. Seed plant genera endemic to the Caribbean island biodiversity hotspot: A review and a molecular phylogenetic perspective. *Botanical Review* 73: 183–234.
- Friar, E. A., L. M. Prince, J. M. Cruse-Sanders, M. E. McGlaughlin, C. A. Butterworth, and B. G. Baldwin. 2008. Hybrid origin and genomic mosaicism of *Dubautia scabra* (Hawaiian silversword Alliance; Asteraceae, Madiinae). *Systematic Botany* 33: 589–597.
- Gustafsson, A. L. S., C. F. Verola, and A. Antonelli. 2010. Reassessing the temporal evolution of orchids with new fossils and a Bayesian relaxed clock, with implications for the diversification of the rare South American genus *Hoffmannseggella* (Orchidaceae: Epidendroideae). *BMC Evolutionary Biology* 10: 177–189.
- Hernández, J. A. and M. A. Díaz. 2000. A new species of *Tetramicra* (Orchidaceae) from eastern Cuba. *Harvard Papers in Botany* 5: 189–192.
- Higgins, W. E., C. van den Berg, and W. M. Whitten. 2003. A combined molecular phylogeny of *Encyclia* (Orchidaceae) and relationships within Laeliinae. *Selbyana* 24: 165–179.
- Holmgren, P. K. and N. H. Holmgren. 1998 [continuously updated]. Index Herbariorum: A global directory of public herbaria and associated staff. New York Botanical Garden's Virtual Herbarium. <http://sweetgum.nybg.org/ih/>.
- Hughes, C. E., R. J. Eastwood, and C. D. Bailey. 2006. From famine to feast? Selecting nuclear DNA sequence loci for plant species-level phylogeny reconstruction. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences* 361: 211–225.
- IUCN. 2001. 2001 IUCN red list categories and criteria: Version 3.1.: 32 p. Gland, Switzerland and Cambridge, UK: IUCN Species Survival Commission. [[http://www.iucnredlist.org/info/categories\\_criteria2001](http://www.iucnredlist.org/info/categories_criteria2001), accessed on 1 November 2007].
- IUCN. 2006. Guidelines for using the IUCN red list categories and criteria. Version 6.2. Gland and Cambridge: IUCN. [<http://app.iucn.org/webfiles/doc/SSC/RedList/RedListGuidelines.pdf>, accessed on 1 November 2007].
- Kennedy, A. H. and L. E. Watson. 2010. Species delimitations and phylogenetic relationships within the fully myco-heterotrophic *Hexalectris* (Orchidaceae). *Systematic Botany* 35: 64–76.
- Klier, K., M. J. Leoschke, and J. F. Wendel. 1991. Hybridization and introgression in white and yellow lady's slipper Orchids (*Cypripedium candidum* and *C. pubescens*). *The Journal of Heredity* 82: 305–318.
- Llamacho, J. A. and J. A. Larramendi. 2005. *The orchids of Cuba / Las orquídeas de Cuba*. Lleida: Greta Editores.
- Monteiro, S. H., A. Selbach-Schnadellbach, R. P. de Oliveira, and C. van den Berg. 2010. Molecular phylogenetics of *Galeandra* (Orchidaceae: Catasetinae) based on plastid and nuclear DNA sequences. *Systematic Botany* 35: 476–486.
- Nir, M. 2000. *Orchidaceae Antillanae*. New York: DAG Media Publishing.
- Pagel, M. and A. Meade. 2006. Bayesian analysis of correlated evolution of discrete characters by reversible-jump Markov Chain Monte Carlo. *American Naturalist* 167: 808–825.
- Pansarin, E. R. and M. C. E. Amaral. 2008. Reproductive biology and pollination mechanisms of *Epidendrum secundum* (Orchidaceae)—floral variation: a consequence of natural hybridization? *Plant Biology* 10: 211–219.



- Peakall, R., C. C. Bower, A. E. Logan, and H. I. Nicol. 1997. Confirmation of the hybrid origin of *Chiloglottis* × *pscottiana* (Orchidaceae: Diurideae). I. genetic and morphometric evidence. *Australian Journal of Botany* 45: 839–855.
- Pinheiro, F., F. Barros, C. Palma-Silva, D. Meyer, M. F. Fay, R. M. Suzuki, C. Lexer, and S. Cozzolino. 2010. Hybridization and introgression across different ploidy levels in the Neotropical orchids *Epidendrum fulgens* and *E. puniceoluteum* (Orchidaceae). *Molecular Ecology* 19: 3981–3994.
- Posada, D. 2008. jModelTest: phylogenetic model averaging. *Molecular Biology and Evolution* 25: 1253–1256.
- Rambaut, A. and A. J. Drummond. 2007. TRACER, v. 1.4. [beast.bio.ed.ac.uk/Tracer](http://beast.bio.ed.ac.uk/Tracer).
- Reichenbach, H. G. 1862. P. 439 *Annales Botanices Systematicae* Vol. 6. Leipzig: Ambrosii Abel.
- Sánchez-Abad, N. L. 2008. *Flora y vegetación de la Cuba, Baracoa, Guantánamo*. M. S. Thesis. Havana: Universidad de La Habana.
- Sang, T. 2002. Utility of low-copy nuclear gene sequences in plant phylogenetics. *Critical Reviews in Biochemistry and Molecular Biology* 37: 121–147.
- Sang, T. and D. Zhang. 1999. Reconstructing hybrid speciation using sequences of low copy nuclear genes: hybrid origins of five *Paeonia* species based on *Adh* gene phylogenies. *Systematic Botany* 24: 148–163.
- Sang, T. and Y. Zhong. 2000. Testing hybridization hypotheses based on incongruent gene trees. *Systematic Biology* 49: 422–434.
- Sauleda, R. P. 1988. A revision of the genus *Psychilis* Rafinesque (Orchidaceae). *Phytologia* 65: 1–34.
- Sauleda, R. P. and R. M. Adams. 1984. A reappraisal of the orchid genera *Broughtonia* R. Br., *Cattleyopsis* Lem. and *Laeliopsis* Lindl. *Rhodora* 86: 445–467.
- Seehausen, O. 2004. Hybridization and adaptative radiation. *Trends in Ecology & Evolution* 19: 198–207.
- Soltis, D. E. and R. K. Kuzoff. 1995. Discordance between nuclear and chloroplast phylogenies in the *Heuchera* group (Saxifragaceae). *Evolution* 49: 727–742.
- Stamatakis, A. 2006. RAxML-VI-HPC: Maximum likelihood based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics* 22: 2688–2690.
- Stamatakis, A., P. Hoover, and J. Rougemont. 2008. A fast bootstrapping algorithm for the RAxML web-servers. *Systematic Biology* 57: 758–771.
- Sun, Y., D. Z. Skinner, G. H. Liang, and S. H. Hulbert. 1994. Phylogenetic analysis of *Sorghum* and related taxa using internal transcribed spacers of nuclear ribosomal DNA. *Theoretical and Applied Genetics* 86: 26–32.
- Taberlet, P., L. Gielly, G. Pautou, and J. Bouvet. 1991. Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molecular Biology* 17: 1105–1109.
- Vale, A., L. Navarro, D. Rojas, and J. C. Álvarez. 2011. Breeding system and pollination by mimicry of the orchid *Tolumnia guibertiana* in Western Cuba. *Plant Species Biology* 26: 163–173.
- van den Berg, C., D. H. Goldman, J. V. Freudenstein, A. M. Pridgeon, K. M. Cameron, and M. W. Chase. 2005. An overview of the phylogenetic relationships within Epidendroideae inferred from multiple DNA regions and recircumscription of Epidendreae and Arethuseae (Orchidaceae). *American Journal of Botany* 92: 613–624.
- van den Berg, C., W. E. Wesley, R. L. Dressler, W. M. Whitten, M. A. Soto Arenas, A. Culham, and M. W. Chase. 2000. A phylogenetic analysis of Laeliinae (Orchidaceae) based on sequence data from internal transcribed spacers (ITS) of nuclear ribosomal DNA. *Lindleyana* 15: 96–114.
- van den Berg, C., W. E. Wesley, R. L. Dressler, W. M. Whitten, M. A. Soto Arenas, and M. W. Chase. 2009. A phylogenetic study of Laeliinae (Orchidaceae) based on combined nuclear and plastid DNA sequences. *Annals of Botany* 104: 417–430.
- Wallace, L. 2003. Molecular evidence for allopolyploid speciation and recurrent origins in *Platananthera huronensis* (Orchidaceae). *International Journal of Plant Sciences* 164: 907–916.
- Wikström, N., P. Kenrick, and M. W. Chase. 1999. Epiphytism and terrestrialization in tropical *Huperzia* (Lycopodiaceae). *Plant Systematics and Evolution* 218: 221–243.
- Whitton, C. L. 1996. *The Cattleyas and their relatives: volume IV. The Bahamian and Caribbean species*. Portland: Timber Press.
- Wolfe, A. D., X. Qiu-Yun, and S. R. Kephart. 1998. Assessing hybridization in natural populations of *Penstemon* (Scrophulariaceae) using hypervariable intersimple sequence repeats (ISSR) bands. *Molecular Ecology* 7: 1107–1125.
- APPENDIX 1. List of taxa used in morphological characterization. Species are listed alphabetically. The information provided corresponds to the consulted sheets and the flower spirit samples (noted by a previous “S:”) in this order. When more than one specimen identified with the same number was consulted, the number of consulted sheets is included as [×n]. Herbarium codes follow HOLMGREN AND HOLMGREN (1998). For *Tetramicra pratensis* (Reich f.) Rolfe, data were obtained from the protologue (Reichenbach 1862).
- Tetramicra* species –1. *Tetramicra bulbosa* Mansf.; HAITI. Dept. Ouest-Sud: Croix des Bouqueds, Camp Franc; 26 Jan 1925; *Ekman* 3075 (IJ). Massif de la Selle, Morne de la Selle, Camp Franc, in steep limestone hillsides; 26 Jan 1925; *Ekman* 3079 (K). Obleon, 3 km desde Furcy en la carretera a Seguin, Massif de la Selle; 17 Nov 1982; *Mejía & Pimentel* 24399 (JBSD). Massif de la Selle, Morne de la Selle, Camp-Franc, on the path to the “Source”; 26 Jan 1925; *Ekman* 3079 (NY).
- REP. DOMINICANA. Pedernales: Canote, Cabo Rojo; Nov 1976; *Dod* 860 (JBSD). Sierra de Bahoruco, 35 km N de Cabo Rojo; 17 Feb 1982; *Zanoni et al.* 19124 (JBSD). 46 km al N de Cabo Rojo, camino minero a Aceitillar, Las Abejas; 7 April 2000; *Jiménez et al.* 3128 (JBSD). Bosque de las Abejas, Sierra de Bahoruco, May 2009, *Vale et al.* AVG2009-01 (JBSD). S: Bosque de las Abejas, Srta. Bahoruco; May 2009; *Vale et al.* AVG2009-01 to AVG2009-09 (SANT). Independencia: 38.6 km al S de Puerto Escondido, camino a caseta No. 2; 19 Mar 1985; *Zanoni et al.* 33953-A (JBSD).
2. *Tetramicra canaliculata* (Aubl.) Urb.; REP. DOMINICANA. Santiago: Hillside La Pradera near 40 km W of Santiago, near Mao road; 10 Jun 1968; *Liogier* 11609 (IJ). Puerto Plata: Maimón, Loma Quemada; 24 May 2000; *Peguero et al.* 1316 (JBSD). Alto de Cofresi, al W de Puerto Plata; 15 Jun 2006; *Greuter & Rankin* 26637 (JBSD). Paraje Palo Indio, propiedad del Sr. Nicolás Domínguez; 23 May 1999; *Clase et al.* 1154 (JBSD). Cofresi; 6 Jun 1973; *Liogier* 19336 (JBSD). Altos de Cofresi, Puerto Plata; 25 Jun 2011; *Vale & Cordero* AVG2011-42 (JBSD); S: Altos de Cofresi; 25 Jun 2011; *Vale & Cordero* AVG2011-30 to AVG2011-41 (SANT). Montecristi: 2 km antes de ciudad Monte Cristi, carr. que va a Villa Vázquez; 16 Aug 1984; *García & Alba* 160 (JBSD). Pozo Bonito 2 km al N de Villa Vázquez; 29 May 1992; *González & McDowell* 208 (JBSD). Tramo entre Palo Verde y Villa García; 9 Jul 2005; *Veloz & Salazar* 3907 (JBSD); Dajabón: Cordillera Central, 2.5 km al NW de Partido, Arroyo Corral, Los Indios; 30 April 1992; *Jiménez & García* 279 (JBSD). Santiago: Platanal afuera, 21 km al W de Santiago; 27 May 1993; *González & McDowell* 199 (JBSD). Santiago Rodríguez: Los Cacaos, salida a Monción, 4 km al S de Monción, carr. a río Mao; 25 May 1988; *Zanoni et al.* 41178 (JBSD).
3. *Tetramicra ekmanii* Mansf.; HAITI. Dept. Nort: Morne La Cidre, M Michel; 20 Nov 1927; *Ekman* 9429 (IJ).
- REP. DOMINICANA. Pedernales, Aceitillar, Sierra de Bahoruco; 11 Feb 1969; *Liogier* 13808 (IJ). Sierra de Bahoruco, Alcoa Exploration Co. Aceitillar; 14 April 1987; *Zanoni et al.* 38958 (JBSD). Isla, W of Hoyo de Pelempito; 11 Feb 1981; *Zanoni et al.* 10925 (JBSD). km 27 de Cabo Rojo, Carr. a Aceitillar; 8 Feb 1986; *Zanoni et al.* 36084 (JBSD). Aceitillar, puesto de guardaparques nacionales; 5 Jun 1999; *Ackerman et al.* 3318 (JBSD). Cayo, Aceitillar; 24–25 Jul 1973; *Liogier & Liogier* 19653a (JBSD). Sierra de Bahoruco, near Altagracia; 28 Mar 1998; *Beurton & Mory* 926 (JBSD). Aceitillar próximo a la carr. abandonada; 4 Dec 1997; *Veloz et al.* 916 (JBSD). S: Hoyo de Pelempito; May 2009; *Vale et al.* AVG2009-10 to AVG2009-16 (SANT). La Vega: Constanza, ridge between Rio Pantuflas Dam and Río Grande de Constanza; 22 Jan 1997; *Ackerman et al.* 2980 (JBSD). NW of Constanza; 10 April 1980; *Mejía & Zanoni* 5012 (JBSD). La Vega: Salto de Constanza; 19–20 Jan 1974; *Liogier* 21114 (JBSD).
- CUBA. Guantánamo: Monte Cristi, altiplano a 700 m; May 1968; *Dietrich* 8903 (HAJB), Felicidad de Yateras, pinar de Monte Cristi, calizas; 13 Mar 1983; *Arias et al.* 49367 (HAJB, B [×2]).
4. *Tetramicra elegans* (Hamilton) Cogn.; PUERTO RICO. Anegada West End area; 30 May 1987; *Proctor* 43593 (IJ). Susua State Forest; 26 Jun 1963; *Liogier* 9740 (IJ). Serpentine barrens, Susua; 28 Dec 1963; *Liogier* 10497 (IJ). Anegada on the sandy plain near West End; 30 Jul 1970; *D’Arcy* 4831 (IJ). Cabo Rojo, llanos costa, Sierra Bermeja, S-facing slope above Rancho Cabassa; 27 Feb 2001; *Breckon* 6364 (JBSD). Susua; 26 Jun 1985; *Dod s. n.* (JBSD). Isla Tórtola; 1 Aug 1980; *Dod* 740 (JBSD).
- US VIRGIN ISLANDS. St. John Cruz Bay Quarter, Maria Bluff Point; 27 Jan 1991; *Acevedo & Angell* 4060 (JBSD).
- FRANCE-GUADALOUPE. Saint Barthélemy, Case Marronne L’Orient; 14 May 1953; *Le Gallo* 2077 (IJ). Saint Barthélemy, Anse Chauvelte, rochers calcaires; 3 Jun 1953; *Le Gallo* 2084 (IJ).
- ANTIGUA AND BARBUDA. St Mary: Johnson’s Point; 28 Jul 1965; *Webster* 13580 (IJ). Lagoon beach ridge station 2; 28 Aug 1970; *Stoddart* 3087 (IJ). Barbuda: Round Hill area of Barbuda; 28 Jul 1977; *Howard* 18514 (JBSD).

NETHERLANDS. Sant Eustatius; 8 Jun 2008; *Ackerman 4226* (UPRRP)—

5. *Tetramicra erosa* Carabia; CUBA. Matanzas: Entre Corral Nuevo y Chirino en la zona de Margot; 19 Jul 1990; *Gutiérrez & Panfét 69291* (HAJB). Cienfuegos: Aguada de Pasajeros, 3 km al N del Km 203 de la autopista nacional; 28 Mar 1980; *Bisse et al. 41729* (HAJB [ $\times 4$ ]). Villa Clara: El Arcoiris, Santa Clara; 15 April 2010; *Faife & Vale AVG2010-03* (SANT). Minas de Motembo; 2 Jan 1919; *Fortín & León 6996* (HAC). Ciego de Ávila: Florencia, entre San Felipe y Guadalupe; 28 April 1987; *Arias et al. 61870* (HAJB). Prov Camagüey: Minas, cuabal S de carr. Minas-Altigracia; 27 April 1984; *Bisse et al. 54103* (HAJB). km 27 carr. Camagüey-Minas; 8 May 1976; *Bisse 31296* (HAJB). Guáimaro, N de Monte Grande; 12 May 1976; *Bisse et al. 31421* (HAJB [ $\times 2$ ]). Cuabales NW de Loma de Maraguán; 23 May 1984; *Bisse et al. 53627* (HAJB [ $\times 4$ ]). Esmeralda, S de Cananova; 21 Jun 1949; *Liogier et al. 1040* (HAC). Llanura de Maraguán; 15 Mar 1989; *Barreto & Pérez 4529* (HAC [ $\times 2$ ]). Sabanas Velasco; April 1966; *Acuña 1159* (HAC). S: Sabana serpentinícola en Los Orientales, afueras de la ciudad de Camagüey; 15 Jun 2009; *Vale et al. AVG2009-31 to AVG2009-45* (SANT). Gramma: Bueycito, Río Yao; Mar 1943; *Victorin & Clemente 21892* (HAC). Santiago de Cuba: Mayarí Arriba, Sierra Cristal; 24 Feb 1976; *Areces et al. 30609* (HAJB). Charrascos de Sacala Lengua; 25 April 1985; *Álvarez et al. 56601* (HAJB). Guantánamo: Baracoa, Pinares W de Yamanigüey; 13 April 1985; *Álvarez et al. 55820* (HAJB). Camino a Cubitas; 1 Aug 1950; *Acuña & Rodríguez 16250* (HAC). Holguín: E de Yamanigüey entronque Mina Potosí y Río Jaguaní; 16 April 1981; *Bisse et al. 44267* (HAJB). Sierra de Nipe, Mayarí abajo, Loma La Mensura; April 1970; *Bisse 15766* (HAJB). Camino de La Breña, después del río; 7 May 1973; *Álvarez & Berazaín 24392* (HAJB). Moa, W de Yamanigüey; 7 May 1980; *Bisse et al. 42950* (HAJB [ $\times 3$ ]). Aeropuerto de Moa; 9 April 1945; *Acuña 12390* (HAC). Moa; 24-28 Feb 1948; *Ferrás 15026* (HAC). Ca. de Loma de Winch, S de Nipe, Mayarí; 21 April 1960; *Liogier et al. 8044* (HAC). Cabezadas de Arroyo Pinalito, Sierra de Nipe; 24 Feb 1956; *López Figueiras 2482* (HAC). Loma El Serrucho camino a Sierra Cristal; 2-7 April 1956; *Liogier et al. 5393* (HAC). El Prado; 2-7 April 1956; *Liogier et al. 5393* (HAC). Bajada del Río Miguel, Mayarí; 2-7 April 1956; *Liogier et al. 5895* (HAC [ $\times 2$ ]). Lomas de la Entrada de Sao Arriba; 24 Feb 1937; *Hno. León 16870* (HAC). Moa, Baracoa; 1939; *Busher 11039* (HAC). Lomas que rodea la ciudad de Holguín; 3 April 1953; *López Figueiras 922* (HAC).

6. *Tetramicra eulophiae* Rchb. f.; CUBA. Pinar del Río: Guane, Guillén, Lomas Cantadoras; 15 May 1984; *Bisse et al. 54322* (HAJB). La Palma, Loma Preluda de Cajálbana; 4 April 1976; *Bisse et al. 31007* (HAJB). Matahambre, pinares al W del pueblo, en el camino viejo a Macurije; 30 Mar 1982; *Bisse et al. 46646* (HAJB [ $\times 2$ ]). Viñales, pinares cerca del Hotel Los Jazmines; 15 April 1972; *Lippold 22040* (HAJB [ $\times 2$ ]). Matahambre, pinar destruido 4km al S del campamento Malas Aguas; 28 Mar 1982; *Bisse et al. 46534, 46535* (HAJB [ $\times 2$ ]). Mantua, playa La Cana, manigua al sur de los manglares; 29 Mar 1982; *Bisse et al. 46587* (HAJB [ $\times 2$ ]). Near km 10.5 S de Pinar del Río, road to La Coloma; 16 Mar 1957; *Proctor 16328* (IJ). Loma de los Cabrerros. Caracoles, Mantua; 18 May 1983; *Urquiola et al. 524* (HPPR). Ladera N y NW de Cerro de Cabras; 17 April 1987; *Vega et al. 3445* (HPPR). Arroyo de las Microcycas, 5 km al N de Consolación; 14 Mar 1953; *Liogier 2800* (HAC). Pinares de San Vicente y San Cayetano; 17 May 1941; *León et al. 20062* (HAC). San Diego de los Baños; April 1915; *León 4991* (HAC). Cuabal de San Miguel de los Baños; May 1942; *León & Victorin 21003* (HAC). Las Martinas; no date; *Acuña 10973* (HAC). San Cayetano, carr. a Viñales; 8 April 1987; *Moncada 0038795* (HAC). Sierra de La Güira, inmediaciones de un pinar; Mar 2007; *Vale et al. s. n.* (HAC). Isla de La Juventud: Sierra de la Siguanea; 14 April 1974; *Berazaín & Areces 24902* (HAJB). Nueva Gerona, maniguas cerca del Hotel Rancho Tesoro; 1 Jun 1975; *Bisse et al. 26231* (HAJB [ $\times 2$ ]). Loma la Cañada, falda W y cima; 15 Mar 1981; *Álvarez 43980* (HAJB [ $\times 3$ ]). Sierra de la Cañada, manigua abierta y pinares; 1 ene 1975; *Lippold et al. 26274* (HAJB). Pinar en La Demajagua, camino entre Geología y La Esperanza; 5 Jun 2009; *Domínguez & Panfét PFC 85550* (HAJB, SANT). White-sand sabana, Cerro de Cunagua; 18 Mar 1954; *Killip 43663* (HAC). San Francisco de la Piedras; 2 Mar 1954; *Killip 43551* (HAC). Between Mina de Oro and Playa del Soldado; 6 April 1954; *Killip 43920* (HAC). unknown: Locality

unknown; 1860-1864; Wright 3336 (K). S: Inmediaciones de Hotel Colony; 16 Mar 2011; *Vale & Faife AVG2011-15 to AVG2011-29* (SANT).

7. *Tetramicra malpighiarum* J. A. Hernández & Díaz; CUBA. Granma: Cabo Cruz, Guafe; Jun 1996; *Hernández 41199* (HAC). S: Cabo Cruz, Sendero El Guafe; 12 Jun 2009; *Vale et al. AVG2009-46 to AVG2009-54* (SANT). Santiago de Cuba: Sardineras; 25 Jun 1949; *Casas 15557* (HAC).

8. *T. parviflora* Lind. ex Rchb. f.; JAMAICA. Parish St. Andrew: Gorge of Cane River below the Falls; 8 April 1956; *Stern 693* (IJ). Stony Hill; Feb 1955; *Morris & Syme 2033* (IJ). Near Newcastle; April 1955; *Hart s. n.* (IJ).

HAITI. District Ouest: Port-au-Prince, Montfleury; 4 Feb 1925; *Ekman 3027* (IJ)—

REP. DOMINICANA. Pedernales: Sierra de Bahoruco, km 27 del puerto de Cabo Rojo, carr. a Aceitillar; 8 Feb 1986; *Zanoni et al. 36083* (JBSD). In pine forest, Aceitillar, Sierra de Bahoruco; 11 Feb 1969; *Liogier 13806* (JBSD). S: Hoyo de Pelempito; 23 April 2009; *Vale et al. AVG2009-18 to AVG2009-26* (SANT). Santiago Rodríguez: Monción; May 1930; *Valeur 419* (JBSD). Elías Piña: Hondo Valle; Jun 1986; *Dod 127* (JBSD [ $\times 2$ ]). Dajabón: Cerro de Chacuey, Partido; 29 Dec 1975; *Liogier & Liogier 24542* (JBSD). Barahona: Paraíso, a nivel del Mar; dic 1974; *Liogier 22072* (JBSD). Independencia: S: Puerto Escondido; 25 April 2009; *Vale et al. AVG2009-27 to AVG2009-30* (SANT).

9. *Tetramicra riparia* Vale, Sánchez-Abad & L. Navarro; CUBA. Guantánamo: Baracoa, orilla del Río Báez, Mina Amores cerca de la mina de cromo; 26 Feb 2010; *Vale et al. AVG2010-04* (HAC, BSC, M, SANT). S: Baracoa, orilla del Río Báez, Mina Amores cerca de la mina de cromo; 26 Feb 2010; *Vale et al. AVG2010-04 to AVG2010-15* (SANT).

10. *Tetramicra simplex* Ames; CUBA. Pinar del Río: Arenas blancas al S de Ovas; 26 Feb 1972; *Bisse 21612* (HAJB [ $\times 2$ ]). Terreno arenoso y lagunas al SW de Las Ovas; 5 April 1976; *Bisse et al. 39564* (HAC). Isla de la Juventud: Pinares cerca de Las Delicias; 1 May 1975; *Bisse 26198* (HAJB). Between San Francisco de las Piedras and Cerro La Cañada; 27 Jan 1955; *Killip 44636* (HAC). Santa Bárbara; 9 Feb 1953; *Killip 42654* (HAC). Sabana NW del Hotel Colony; 4 Jun 2009; *Domínguez & Panfét PFC 85510* (SANT, HAJB). Near Los Indios; 19 May 1910; *Jennings 644* (AMES [ $\times 2$ ]). S: Isla de la Juventud: Inmediaciones de Hotel Colony; 16 Mar 2011; *Vale & Faife AVG2011-01 to AVG2011-14* (SANT).

11. *Tetramicra urbaniana* Cogn.; BAHAMAS. New Providence; S of Nassau.; 3 April 1903; *Curtiss 145* (K, NY, AMES [ $\times 2$ ]).

CUBA. Matanzas: Canasí, Lomas de Galindo; 15 Mar 1979; *Berazaín et al. 40215* (HAJB [ $\times 4$ ]). Cuabales al SE de Galindo; 28 Jan 1977; *Berazaín 34433* (HAJB). Canasí, lomas de Galindo; Feb 1972; *Bisse 21562* (HAJB [ $\times 3$ ]). Canasí, Lomas de Galindo, Cuabal; 15 Mar 1979; *Berazaín et al. 40215* (JBSD). El Campanario, Santo Tomás, Ciénaga de Zapata; 2 April 2010; *Oviedo et al. s. n.* (HAC [ $\times 4$ ]).

12. *T. zanonii* Nir; REP. DOMINICANA. El Seibo: Parque Nacional Los Haitises entre la caseta No. 1 de (Cueva de Arena) y Boca de Infierno; 9 Jan 1986; *Zanoni et al. 35967* (JBSD, NY). Parque Nacional los Haitises; 25 April 1985; *Zanoni et al. 34369* (JBSD). Manigua sobre rocas calcáreas, Los Haitises, Bahía de Samaná; 19 ene 1977; *Liogier & P. Loggier 26330* (JBSD).

APPENDIX 2. GenBank voucher information for the molecular data. Plant name, collector and voucher number, herbarium, GenBank accession number for ITS region and *TrnL-F* region, in this order. Voucher numbers for ITS clones are separated by commas. Sequence accession numbers in bold typeface correspond to those obtained in this research. Locality information for *Tetramicra* species is in APPENDIX 1.

***Tetramicra* species:** *T. bulbosa* AVG2009-01 (JBSD) (**JQ963170**; **JQ963179**). *T. ekmanii* AVG2009-10 (JBSD) (**JQ963171**; **JQ963180**). *T. elegans*, *Ackerman 4226* (UPRRP) (**JQ963172**; **JQ963181**). *T. eulophiae*; *Vale et al. AVG2007-01* (HAC) (**JQ963173**; **JQ963182**). *T. parviflora* AVG2009-18 (SANT) (**JQ963174**; **JQ963183**). *T. riparia* AVG2010-04 (HAC) (**JQ963175**, **JQ963176**, **JQ963177**; **JQ963184**). **Other genera:** *Broughtonia sanguinea* (Sw.) R. Br. Brieger Coll. 14440 (ESA) (AF260186, AF267015). *Jacquinella teretifolia* Britton & P. Wilson Whitten 97026 (FLAS) (AY008519, AY422390). *Psychilis krugii* (Bello) Saulea Chase O-1062 (K) (AF260157, AF266891). *Quisqueya ekmanii* Dod Higgins 53 (FLAS) (AY008567, AY422447).