Two new Malagasy species of genus *Piper* L. (Piperaceae): *Piper malgassicum* and *Piper tsarasotrae* and their phylogenetic position

Enrico PALCHETTI[1], Stefano BIRICOLTI [1], Massimo GORI[1], Giulia ROTA NODARI[1], Nicola GANDOLFI[2], Alessio PAPINI[3,*]

[1]Department of Agrifood and Environmental Science (DISPAA), University of Florence. Piazzale delle Cascine n. 18 – 50144 - Florence, Italy

[2]Association Tsyriparma: Volounteer association for protection of Malagasy forests. Ambositra, Madagascar

[3]Dept. of Biology, Univ. of Florence. Via Micheli n. 3, Firenze, Italy

*Correspondence: alpapini@unifi.it

Abstract: We describe here two new species of genus *Piper* L. from Madagascar: *Piper malgassicum* and *Piper tsarasotrae*, the species names referring to the currently known distribution areas. These two species contribute, at least in part, to the production the local voatsiperifery pepper, probably mixed together with *P. borbonense* and are, therefore, economically relevant as spices. We used a selected set of characters (those more easily observable on herbarium samples) for a Principal Component Analysis, to assess the relative distance between both species, including in the analysis the autochthonous species of *Piper* known from Africa and Madagascar. In order to check the identity and to assess the phylogenetic position of the two species, we also sequenced the chloroplast gene *ndh*F and the *trn*L intron and the nuclear gene G3PDH. On the basis of these results, we show here the relationships between these two new *Piper* taxa and the most closely related species within the genus (excluding *P. heimii* and *P. pachyphyllum*, for which only morphological characters were available).

Keywords: DNA sequencing, Madagascar, Principal Component Analysis, *Piper*, *Piper malgassicum*, *Piper tsarasotrae,* Piperaceae,

1. Introduction

Most recently, treatments of the pantropical genus *Piper* L. (Piperaceae) included more than 2000 species (Quijano-Abril et al., 2006). The phylogenetic position of *Piper* L. and of family Piperaceae, was inserted within the complex basal group of dicots termed “paleoherbs” (Loconte and Stevenson, 1991). More recently, APG IV (2016) inserted Piperaceae in order Piperales, nested within Magnoliids.

The distribution of *Piper* is pantropical and the genus develops highly variable growth forms and biomechanical organization (Isnard et al., 2012). The highest number of species can be found in America, where earlier 500 species were listed (Burger, 1972; Tebbs, 1993), then increased to at least 1100 (Jaramillo et al., 2008) and most recently up to 1804 (Ulloa Ulloa et al., 2017).

The exact number of *Piper* species and their exact distribution is not easy to ascertain, particularly due to the high number of taxa, some of which are difficult to distinguish one from the other, resulting in many synonyms (Suwanphakdee et al., 2016). Furthermore, some species are widespread, such as *P. umbellatum*, while others, actively cultivated, escaped by accident and may have been naturalized, such as *P. auritum*, *P. nigrum* or *P. methysticum* (Smith et al., 2008). Most species show a restricted distribution area (Marquis, 2004; Quijano-Abril et al., 2006). As a matter of fact, new species were recently described also from old herbarium collections (Görts-Van Rijn and Callejas Posada, 2005).

Only two endemic species are currently known for the African continent, *P. guineense* and *P. capense*. *Piper guineense* is a dioecious vine, relatively similar to the majority of southwestern Asian species, whereas *P. capense* is a shrub with bisexual flowers, hence resembling many species of the American continent (Smith et al., 2008). The knowledge of the genus in Madagascar is far from being complete. Currently *P. heimii* C. DC and *P. pachyphyllum* Baker are indicated for the island, while *P. borbonense* (Miq.) C. DC. was described for the island called at that time Île Bourbon, currently La Reunion (Weil et al., 2017), belonging to Mascarenas Islands, 600 km east of Madagascar. Its presence in Madagascar is a matter of debate, even if De Candolle (De Candolle, 1923; 1869) already assigned some samples from Madagascar and Mauritius to this species (see appendix 1 about herbarium samples by the site http://www.caryologia.unifi.it/tjb/Appendix1.pdf). However, this species is cultivated, which makes it more difficult to assess its natural distribution.

Here, we describe two new species of *Piper* L. from Madagascar on the base of their morphology and supported by molecular data. Both species are mixed with *P. borbonense*, in the so-called high-quality spice voatsiperifery pepper.

2. Materials and methods

2.1. Morphological characters analysis and PCA

Herbarium samples were prepared, among which the type specimens were chosen (see appendix 1 with herbarium samples saved at the permanent link http://www.caryologia.unifi.it/tjb/Appendix1.pdf). A number of characters were observed and measured (where necessary) with a stereomicroscope. The herbarium samples were stored by the Tropical Herbarium of Florence (FT, Centro Studi Erbario Tropicale, Università degli Studi di Firenze).

21 characters (those that showed variation) were coded in a matrix (Table 1) used as input for the Principal Component Analysis with the software PAST 3.16 (Hammer et al., 2001).

2.1. Anatomical characters

Inflorescence stems were cut with a blade, stained with 1% phloroglucinol (w/v) in 12% HCl for 5 min and observed with a bright field light microscope to stain lignin (as in Mosti et al. 2012).

2.3. DNA extraction

For the DNA extraction, leaf samples were collected from the tropical forest of Vohiday (samples PNsv1-10, table 2) and from the Tsarasotra area (samples PNst1-10, table 2). Plant tissue samples were conserved and transported inside 20 mL plastic tubes filled with ethanol 96% (after Murray and Pitas, 1996; Bressan et al., 2014).

For DNA extraction, 40 mg of dry leaf sample were placed into a 2mL tube, together with tungsten carbide beads, frozen in liquid nitrogen and finely ground in a tissue homogenizer (Tissue Lyser ®, Qiagen). DNA was extracted using Invisorb Spin Plant Mini kit (Stratec molecular®). Amplification of the *trn*L intron and the low copy nuclear gene *G3pdh* followed respectively the protocols by Taberlet et al. (1991) and Strand et al. (1997). A set of four primer pairs were designed using the chloroplast genome sequence of *Piper kadsura* (GenBank: KT223569.1) to cover the entire *ndhF* gene.

The InsTAclone PCR Cloning Kit was used to clone *G3pdh* (Thermo Scientific®). Ten samples for each provenience were amplified using the universal primers GPD9R2 and GPD9R4 (Olsen and Schaal, 1999). Up to 15 colonies for single cloned sample were amplified using M13 primers. PCR products were purified using the QIAquick PCR Purification Kit (Quiagen) and sent to the University of Florence internal sequencing service CIBIACI. Manual correction and assembly of the sequences was performed using the software programmes Multaline (Corpet, 1988) and MEGA7 (Kumar et al., 2016).

The new DNA sequences produced during our investigation were deposited in Genbank (Genbank accession numbers are indicated in Table 2).

2.3. Phylogenetic analysis

Together with the here produced new sequences, other sequences used are available in Genbank, more specifically those of genus *Piper* used by Smith et al., (2008). We used one species of *Peperomia* (*Peperomia pellucida*) and *Houttuynia cordata* as outgroups on the basis of the phylogenetic analysis on Piperaceae by Jaramillo and Manos (2001) and Wanke et al. (2007) showing that *Peperomia* is sister group to *Piper s.l.*, while *Houttuynia* is more distantly related to both of these genera (see, for instance, Figure 5 in Wanke et al., 2007). Optimal multiple alignment was obtained with CLUSTALW 1.81 (Thompson et al., 1994). The matrices for each of the three gene sequences employed were combined with the Python (Python version 2.6.4; Biopython 1.57) program combinex2_0.py, written by A. Papini (Lewke Bandara et al., 2013; Simeone et al., 2016), released under GPL licence and available at www.unifi.it/caryologia/PapiniPrograms.html.

A maximum likelihood (Felsenstein, 1981) search was done by preliminarily using MrMODELTEST 2.0 (Nylander, 2004) to evaluate the best likelihood model on the basis of the Akaike information criterion (Akaike, 1974). The model was used as settings for Bayesian Inference with the program MrBayes 3.4b4 (Huelsenbeck and Ronquist, 2001; Ronquist et al., 2012). A maximum likelihood (ML) phylogenetic analysis was done with RaxML (Stamatakis et al., 2012) and the resulting trees were edited with Figtree (Rambaut, 2009). We mapped the support on the tree branches with the results of the Bayesian phylogenetic analysis as follows: after the "burn-in" trees were removed from the data set as in Papini et al. (2007; 2011). The remaining trees were used to produce a 50% majority-rule consensus tree (with PAUP) in which the percentage support was considered equivalent to Bayesian posterior probabilities.

3. Results

3.1 Morphological characters analysis and PCA

The characters used for the species description and for comparison were observed and measured with a stereomicroscope on the herbarium samples of the two new species and of the most closely related species of *Piper* (images of the samples and original protologues in appendix 1: herbarium samples). In appendix 1 (http://www.caryologia.unifi.it/tjb/Appendix1.pdf) also a list with the investigated samples (scanned samples, in the majority of cases) of other species is reported. The characters were coded as numeric states (table 1) and analysed with PAST. The Principal Component Analysis (PCA) was based on a set of characters, those most variable and easily observable on herbarium samples. The results of the PCA analysis are shown in Figure 1. The samples from Tsarasotra (from now on *P. tsarasotrae)* were quite isolated, even if quite close to *P. guineense* and to the samples from Vohiday (from now on *P. malgassicum*) and *P. heimii* (Figure 1). Figure 1 shows also that *P. pachyphyllum* and *P. borbonense* are relatively close.

3.2 Phylogenetic analysis

The phylogenetic analysis (Figure 2) showed that *P. malgassicum* and *P. tsarasotrae* are strictly related to each other and to *P. borbonense*, this last clustering together with *P. malgassicum* with 100% Bayesian Support (BS). These two species formed a monophyletic group with *P. tsarasotrae* with 70% BS. The sister group of this cluster was the group of 5 sequences of the African species *P. guineense* (85% BS), while the Asian species *P. caninum* formed the sister group to the former species, but with BS less than 50% (Figure 2).

3.3 Microscopy observations

The observation with a stereomicroscope was useful for the observation of micromorphological character of the flowers, necessary for the following description. In *P. tsarasotrae* male flowers, usually three (very rarely four) stamens are present (Figure 3A) with two anthers with lateral apertures (Figure 3A1). In the female flowers, the number of stigmas may vary from 3 to 4. In Figure 3B a case with three lobed stigmas is shown. In *P. malgassicum* male flowers, stamens are sometimes solitary (Figure 3C) and show two anthers with lateral apertures (Figure 3C1). In female flowers, stigmas are most frequently three, sometimes four, still visible on the enlarged fertilized ovary (Figure 3D). The stigmas are sessile (Figure 3D1).

The observation with the light microscope of cross sections of the stem showed that in *P. tsarasotrae* two circles of bundles are present (Figure 4A): a group of larger more internal bundles and an external group of smaller bundles (Figure 4B).

Also in *P. malgassicum* the inflorescence stem in cross section showed two circles of bundles (Figure 4C): a group of larger more internal bundles and an external group of smaller bundles (Figure 4D). In this species a continuous layer of sclerenchyma enclosed the smaller bundles. (Figure 4C).

3.4 Description of the two new species

The morphological nomenclature here employed followed Simpson (2010).

Piper tsarasotrae Papini, Palchetti, Gori, Rota Nodari spec. nov.

Typus: Collectors Enrico Palchetti and Nicola Gandolfi for samples 1.1 A (female samples, holotype) and 1.1.D (male sample, paratype, as defined in 9.6, ex. 5 of the International Code of Nomenclature for algae, fungi, and plants: Mcneill, 2012);

locality Tsarasotra, Ambositra region (Madagascar); localization: S20° 27' E47° 10'. Deposited by Centro Studi Erbario Tropicale, Università degli Studi di Firenze (FT).

Similar to *Piper guineense* Schumach. & Thonn., but differing since the foliar basis is uneven and acuminate instead of cordate. Dioecious. Shrub, sometimes epilithic and sometimes creeping on the ground, swollen stem nodes. Leaves alternate. Shape oblong ovate, 4.5-8 cm long and only 0.5-2 cm wide. Leaf apex acuminate, while the leaf base is uneven and acuminate. Inflorescence leaf opposite, cylindrical and erect. Female spikes 4-6 cm long, with a peduncle 1-2 cm long, with small sessile spirally arranged flowers. Single ovary, 4 (rarely 3) lobed white stigma, covered by short appendages. Male spikes 3-5 cm long, with a peduncle 1-2 cm long and stamens organized in groups of three. Ripe fruit reddish and rounded, 0.4-0.7 mm long, fruit pedicel 0.8-1.2 cm. Each fruit gives off a single rounded-shaped seed. Inflorescence stem in cross section with two circles of bundles: a group of larger more internal bundles and an external group of smaller bundles.

Living in arid forest. The environment of the species is shown in Figures 5A and 5B, while the female cones are shown in Figures 5C and 5D. Fruits in Figure 5E. In Figure 5F, both the inflorescence and the fruits can be observed on the same individual. Usually three (very rarely four) stamens present with two anthers with lateral apertures. Number of stigmas from 3 to 4.

Piper malgassicum Papini, Palchetti, Gori, Rota Nodari spec. nov.

Typus: Collectors Enrico Palchetti and Nicola Gandolfi: PS9a (female sample, holotype) sample and PS8 sample (male sample, designed as paratype, as defined in art. 9.6, ex. 5 of the International Code of Nomenclature for algae, fungi, and plants: Mcneill, 2012); locality Vohiday forest, Ambositra region (Madagascar); localization S20° 32' E47° 35'. Deposited by Centro Studi Erbario Tropicale, Università degli Studi di Firenze (FT).

Similar to *Piper borbonense* (Miq.) C. DC. but differing since its foliar basis is uneven and roundish instead of cordate. Dioecious. Liana climbing up to 10-15 meters. Leaves alternate. Shape ovate-elliptic, 6.5-8 cm long and 3-5 cm wide. Presence of adventitious roots for climbing at the nodes. Heterophylly: the lower part of the stem showing cordate leaves. Leaf apex acuminate, while the leaf base is uneven and rounded. Inflorescence leaf opposite, cylindrical and erect. Female spikes 3-8 cm long, with a peduncle 1-2 cm long, with small sessile spirally arranged flowers. Single ovary, 3-4 lobed white stigma. Male spikes 6-10 cm long, with a peduncle 2-3 cm long and stamens organized mainly in groups of two. Ripe fruit reddish and oval, 0.4 cm long, fruit pedicel 0.8-1.2 cm. Each fruit gives-off a single rounded-shaped seed.

In Figure 6A the cordate leaves of the lower part of the stem are shown. Figure 6B shows the collection of the plant climbing trees up to 10-12 meters. The fruits are shown in Figures 6C and 6D. The male inflorescence is shown in Figure 6E and the adventitious roots are visible in Figure 6F. The female inflorescence is shown in Figure 7A, the fruits in Figure 7B and the number of stigmas in Figure 7C. Stamens sometimes solitary with two anthers with lateral apertures . Stigmas most frequently three, sometimes four, still visible on the enlarged fertilized ovary. Stigmas are sessile. Inflorescence stem in cross section with two circles of bundles: a group of larger more internal bundles and an external group of smaller bundles. Continuous layer of sclerenchyma enclosing the smaller bundles. Living in humid forest.

4. Discussion

The morphological results show that the description of *P. tsarasotrae* does not overlap with the description of the other species known to be indigenous of Madagascar such as *P. heimii* (quite close to *P. malgassicum*) and *P. pachyphyllum*. *Piper heimii* appears to be very close to *P. malgassicum*, but the first has lanceolate-ovate leaves 12.5 cm long according to the protologue, whereas the second has ovate leaves, 6.5-8 cm long. However, *P. pachyphyllum* and *P. heimii* have not been recently sampled and should be further investigated.

The phylogenetic analysis of the two new species in the context of a subset of the matrix used by Smith et al. (2008) showed that *P. malgassicum* and *P. tsarasotrae* belong to a clade comprising *P. borbonense*, *P. guineense* and *P. caninum*. The same clade was also identified by Smith et al. (2008) with higher Bayesian support with respect to our phylogenetic analysis. Possibly the larger sampling in this group due to the insertion of *P. malgassicum* and *P. tsarasotrae* decreased the robustness (however considerably high, that is 85%). The placement in a monophyletic group formed by *P. malgassicum, P. tsarasotrae* and *P. borbonense* (relatively close to *P. pachyphyllum* in Figure 1) in the phylogenetic analysis is also corroborated by the biogeographical position of these entities, since the first two species are endemic of Madagascar, while *P. borbonense* originates from La Reunion Island and Mauritius (but also present in Madagascar according to De Candolle (1923). The possible presence of *P. borbonense* in Madagascar, not only in cultivated form but also as spontaneous species, also as possible further component of the voatsiperifery pepper, should be ascertained.

The two new entities appear to belong to *Piper* s. s. in the sense of Jaramillo et al. (2008).

Piper malgassicum is probably more closely related to *P. borbonense* and to *P. heimii* than *P. tsarasotrae,* even if *P. malgassicum* appears to be wild in Madagascar, while *P. borbonense* may have been introduced in this Island for spice production. *Piper tsarasotrae* has a completely different ecological niche (creeping on the soil and on the rocks, sometimes lianous, but on low plants) with respect to *P. malgassicum*, which is a more typical forest lianous species of *Piper*. These three species appear to be phylogenetically related to *P. guineense*, endemic of the African mainland. This genetic affinity was already indicated by Jaramillo et al. (2008)· and appears to be more strict between *P. tsarasotrae* and *P. guineense*.

The presence of two circles of vascular bundles in the stem of many *Piper* species was defined as polystelic organization by Isnard et al. (2012) and was considered by these authors as a synapomorphy of the family Piperaceae with the exception of genus *Verhuellia*. This character was observed in detail, for instance, in some American species such as *P. amalago* L. (Dos Santos et al., 2015), in which also a continuous layer of sclerenchyma was described as in *P. malgassicum*. This scheme is typical of the investigated species of *Piper* (Dos Santos et al., 2015), but *P. tsarasotrae* shows some difference, since the sclerenchymatic layer is not continuous.

In conclusion, the two new species of *Piper* here described concur to the production of some of the locally produced voatsiperifery pepper, probably together with *P. borbonense,* and are hence of economical importance as spices. The association of species morphological identification with DNA sequences could be useful as a bar coding method for identification of the components of spices and drugs in traditional mixtures (Chaveerach et al., 2006).

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Figure legends

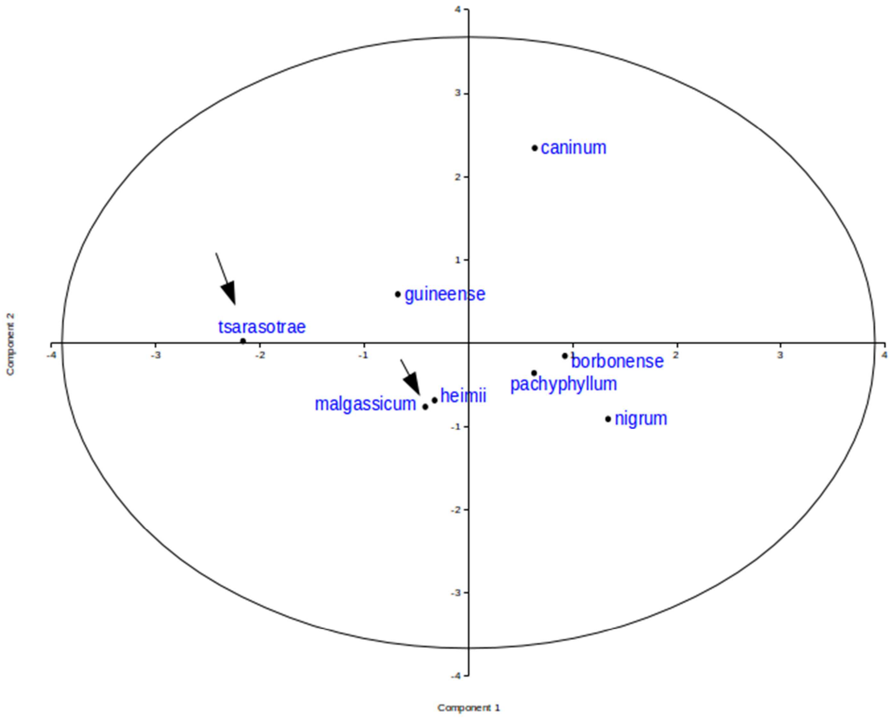

Figure 1. PCA analysis of the two new species of *Piper* together with the more strictly related species. The position of *Piper tsarasotrae* (tsarasotrae in the figure) and *Piper malgassicum* (malgassicum in the figure) are indicated by arrows.

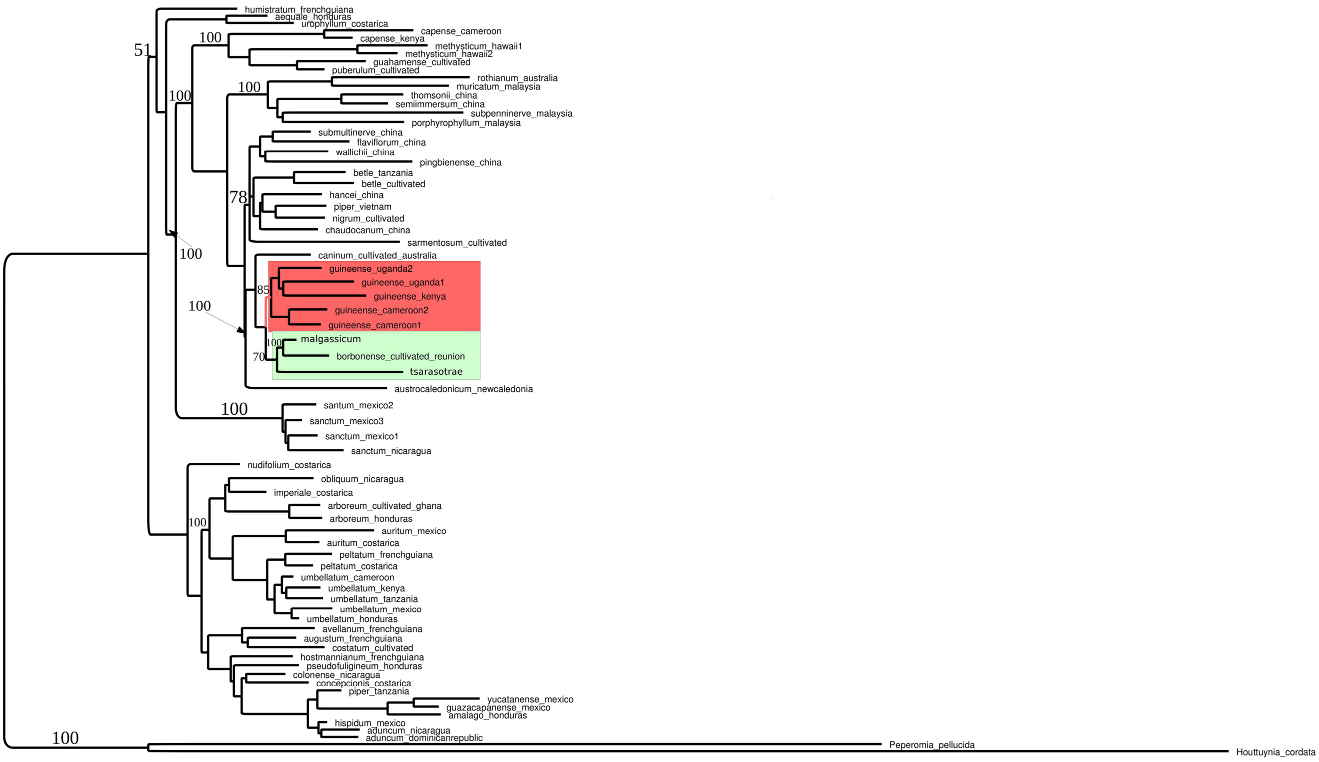

Figure 2. Phylogenetic analysis with maximum likelihood based on trnL intron, ndhF and G3pdH genes. Bayesian support reported on branches. The position of *Piper tsarasotrae* (SPN Tsarasotra in the figure) and *Piper malgassicum* (SPN Vohiday in the figure) are evidentiated in green, together with the genetically strictly associated P. *borbonense*. The phylogenetically close *P. guineense* accessions are evidentiated in red. All the names refer to species of genus *Piper* L. with the exception of *Peperomia pellucida* and *Hottuynia cordata*, whose names are reported entirely (together with the genus name) with the provenence on the right.

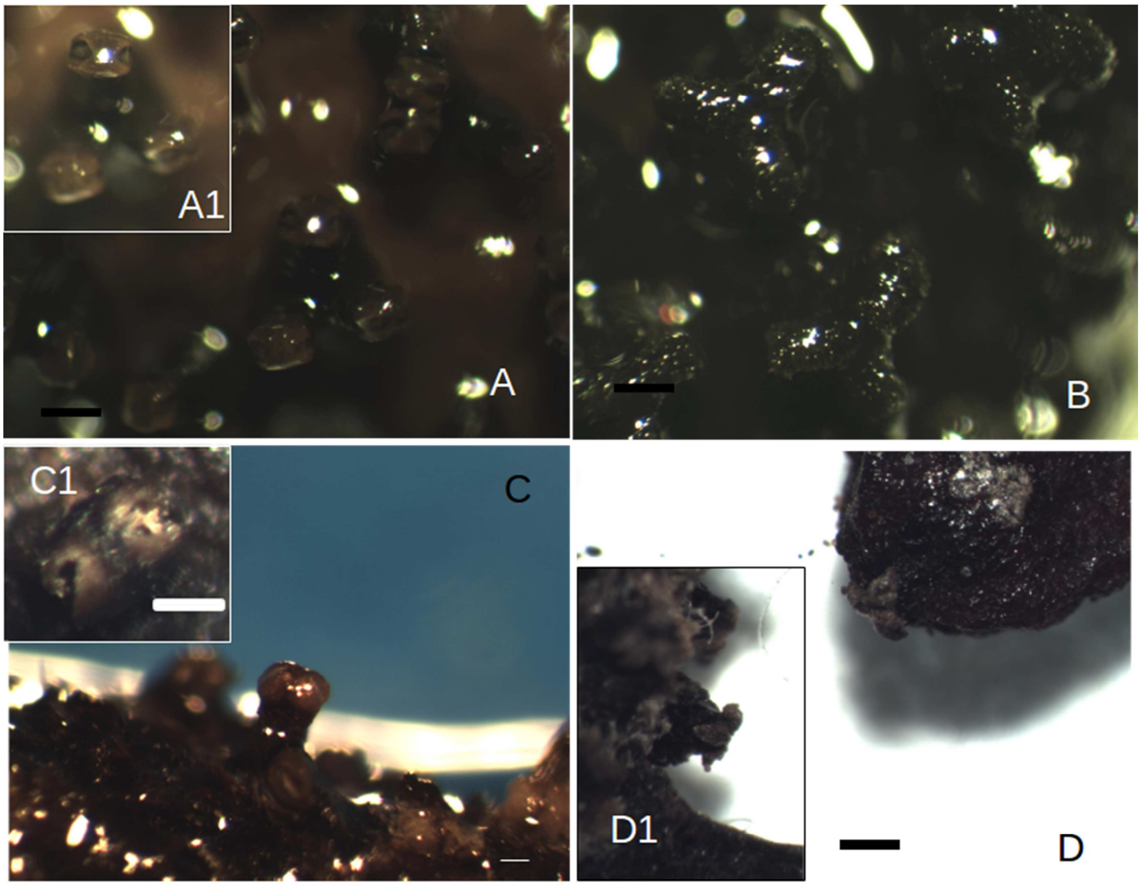

Figure 3 – Observation with stereomicroscope. *Piper tsarasotrae.* A: A group of 3 stamens is visible. In A1 a detail of the anthers. B: Shape of the stigmas. The surface appears to be covered by appendages. *Piper malgassicum.* C: Stamen in lateral view. C1: detail of the anthers. D: Stigmas on an already grown ovary. D1: lateral view of an ovary with three stigmas.

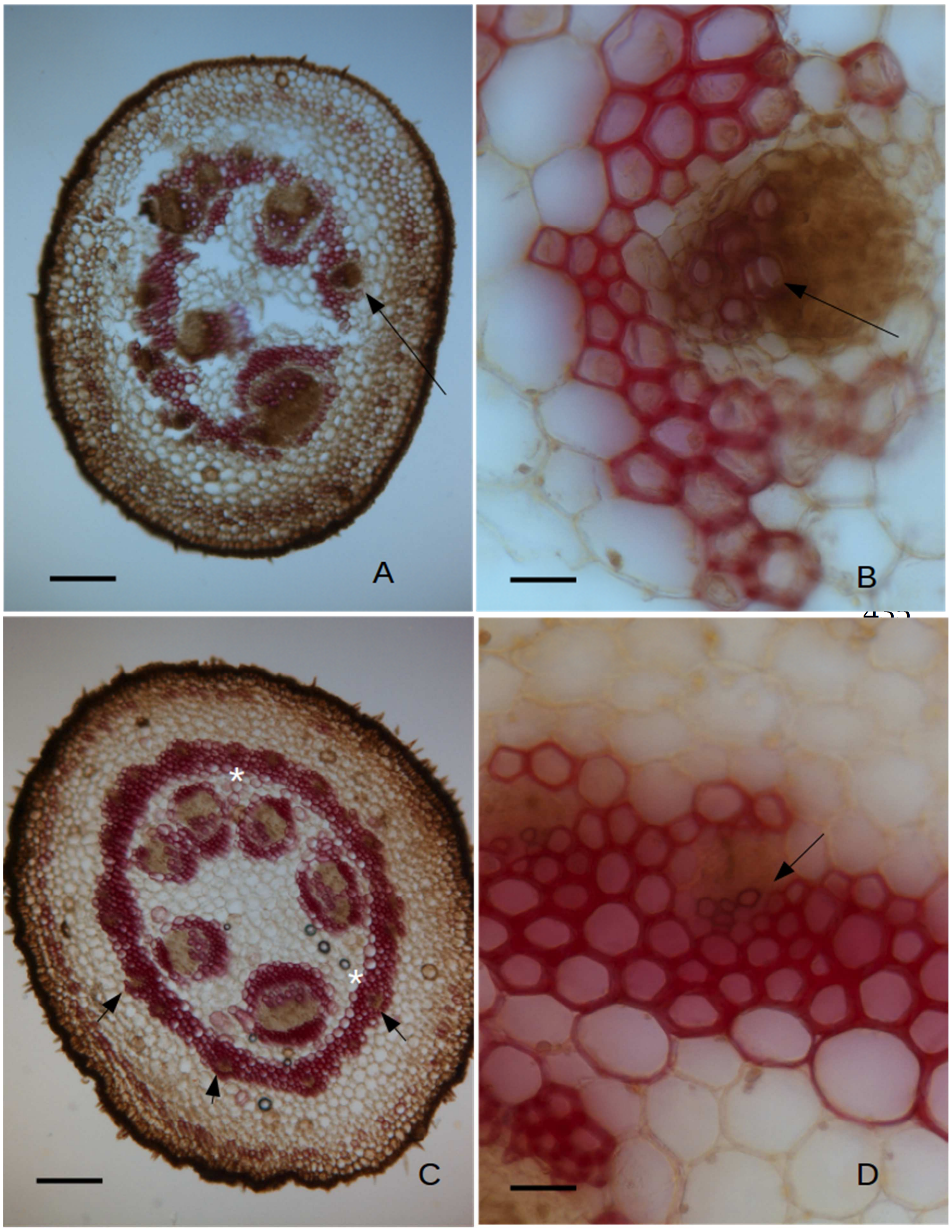

Figure 4 - *Piper tsarasotrae*. Cross section of the inflorescence stem. A: general aspect of the eustele with a group of larger more internal bundles and an external group of smaller bundles. The arrow indicates the smaller bundle of Figure 7B. Bar = 400цm. B: Detail of Figure 7A. One of the smaller bundles. The arrow indicates one of the tracheal elements. Bar = 50цm. *Piper malgassicum.* Cross section of the inflorescence stem. C:

general aspect of the eustele with a group of larger more internal bundles and an external group of smaller bundles. The arrows indicate the smaller bundles. The white asterisks indicate a continuous layer of sclerenchymatic cells. 7D. Bar = 400ɥm. D: Detail of Figure 7C. One of the smaller bundles. The arrow indicates one of the tracheal elements. Bar = 50ɥm.

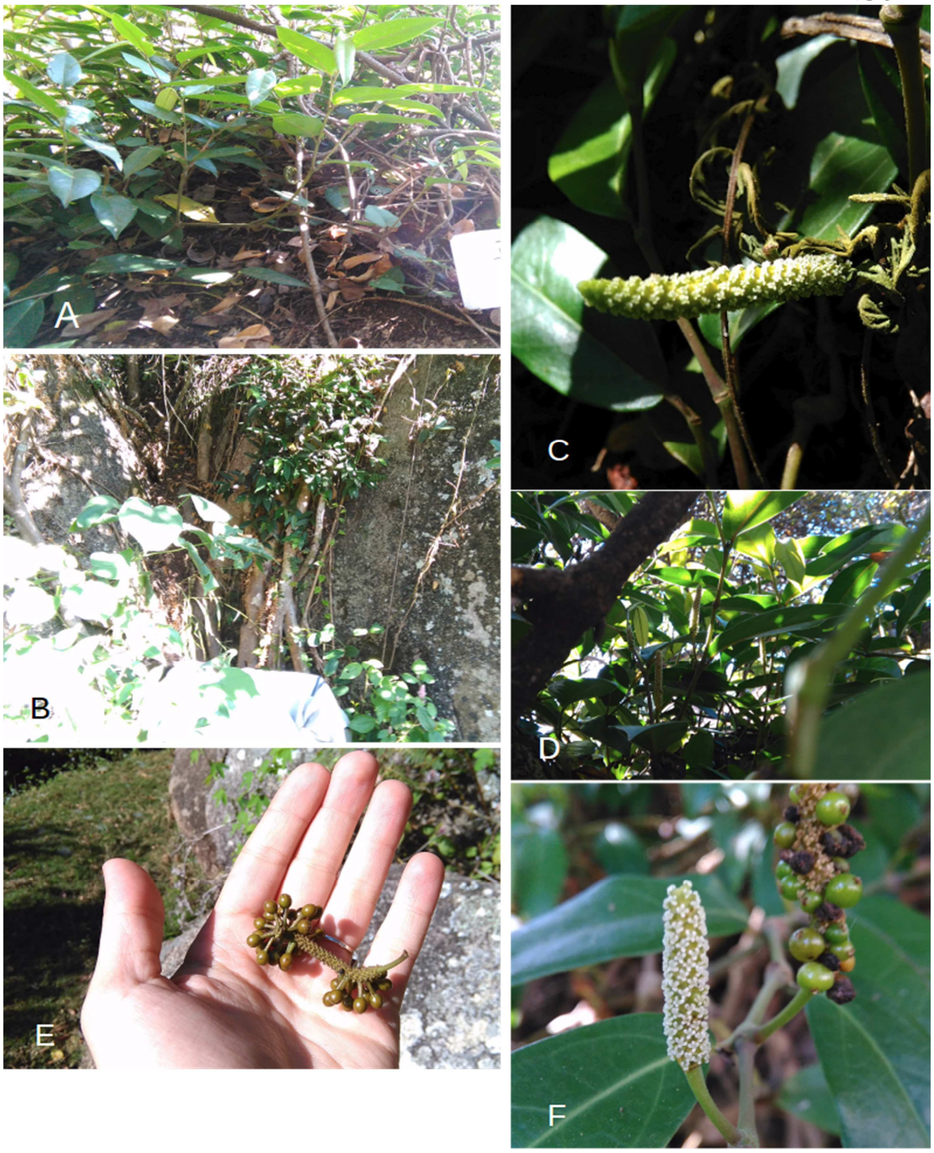

Figure 5 - *Piper tsarasotrae.* A: General habitus of the species. B: typical environment of the species.

FC: emale cones, with details of the stigmas. D: Position of the female cones on the female plant. E: Ripe fruits. F: both the female inflorescence and the fruits at different level of ripeness can be observed on the same individual.

Figure 6 - *Piper malgassicum.* A: the cordate leaves of the lower part of the stem are shown. B: Method of collection of the fruits from plants climbing trees (up to 10-12

meters). C: Ripe fruits in the context of the plant. D: Infructescence with fruits at various stages of ripeness. E: Detail of the male inflorescence. F: Adventitious roots growing from nodes.

Figure 7 - *Piper malgassicum.* A: Female inflorescence on the plant. B: fruits at various stages of ripeness. C: Female inflorescences at various stages of maturation with a detail of the stigmas.

Table 1 – Morphological characters obtained from herbarium samples coded for the Principal Component Analysis (PCA). Characters used in the table and codification of character states: 1) stem nodes: swollen = 1; not swollen = 0; 2) habitat: harid forest = 1; humid forest = 1; shady forest = 0; 3) leaf shape: lance-ovate = 1; ovate = 0; cordate = 2; 4) leaves of the low part of the stem: presence of cordate leaves = 0; never presence of cordate leaves = 1; 5) leaf length in cm: miminum 6 cm = 1; minimum 6,5 = 0; 6) maximum leaf length: less than 10 = 0; more than 10 = 1; 7) Minimal leaf width in cm: less than 3 = 0; more = 1; 8) Maximal leaf width in cm: less than 6.5 = 0; more = 1; 9) leaf apex: not acuminate = 0; acuminate = 1; 10) leaf base: Iniqual narrowly cuneate = 0; iniqual cuneate = 1; cordate = 2; 11) leaves: alternate = 0; non alternate = 1; 12) leaf petiole: max length <= 2,5 = 0; more = 1; 13) petiole minimal length in cm: < 1 cm = 0; more or equal than 1 = 1; 14) leaf nerves: palmate = 0; pinnate = 1; 15) minimum number of stigmas: 2 = 2; 3 = 3; 4 = 4; 16) flower color: red = 1; not red = 0; 17) flower spike dimension: max length less than 5 = 0; more = 1; 18) fruit spike dimension in cm: maximum less than 4 cm = 0; more than 4 = 1; 19) flower spikes opposite to leaves: yes = 0; no = 1; 20) dioicy: dioecious = 0; not dioecious = 1; 21) growth form: liane = 0; shrub = 1; 22) vegetative dimension m: more than 6 = 0; up to 6 = 1. The interrogative mark “?” means either that the character is variable in the species or that the character state is not known. Characters obtained through direct measurements for the first two species; from Verdcourt (1996) for species 3 and 4; De Candolle (1869 C. DC. Prodromus Systematis Naturalis Regni Vegetabilis 16(1): 339. 1869) for species 5; De Candolle (1911 C. DC. 1911. Notul. Syst. (Paris) 2: 51) for species 6; Baker (1885 Baker J. G. (1885) Further controbutions to the Flora of Central Madagascar. - Second and final part. Journal of the Linnean Society, Botany 21: 436. 1885.) for species 7 and Blume (1826 C. L. Blume (1826 Monographie der Oost-indische Pepersoorten/diir. Verh. Batav. Genootsch. Kunst. 11: 214, f. 26) for species 8. Botanical nomenclature after Simpson (2010).

Ref.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
tsarasotrae	0	2	1	1	1	0	0	0	1	0	0	0	0	0	3	?	0	?	?	0	0	0
malgassicum	0	1	0	0	0	0	0	0	1	1	0	0	0	0	3	?	1	?	?	0	1	1
nigrum	0	1	0	?	0	1	1	1	1	1	0	0	1	1	4	?	1	?	?	1	1	1
guineense	?	0	0	?	1	1	0	1	1	?	0	1	0	0	3	?	0	1	yes	0	0	0
borbonense	?	0	0	?	0	1	1	0	1	1	0	0	1	1	3	?	?	?	?	1	0	0
heimii	?	?	1	?	0	1	1	0	?	0	0	0	0	1	4	?	0	?	?	0	?	?
pachyphyllum	?	?	0	?	0	1	1	0	1	1	0	0	0	1	3	1	?	?	?	yes	?	1
caninum	?	1	2	?	0	1	1	1	1	2	0	1	1	?	2		0	?	?	?	?	?

Table 2 – Geographical coordinates of the samples collected for DNA extraction. Genbank accession numbers of the corresponding g3pdH, trnL and ndhF are reported on the right side of each accession. All the samples of *P. tsarasotrae* come from the locality Tsarasotra, Ambositra region (Madagascar) while all the samples of *P. malgassicum* come from the Vohiday forest, Ambositra region (Madagascar). Latitude and longitude of collection places are indicated underneath the species name.

***P. tsarasotrae* (Tsarasotra)**		Genbank g3pdh	Genbank trnL	Genbank ndhf	***P. malgassicum* (Vohiday)**		Genbank g3pdh	Genbank trnL	Genbank ndhf
PNSt1	S20° 26.716' E47° 11,157'	MH234634	MH234638	MH234636	PNSv1	S20° 31.899' E47° 27.492'	MH234633	MH234637	MH234635
PNSt2	S20° 27.146' E47° 10.948'	not variable	not variable	not variable	PNSv2	S20° 32.278' E47° 35.298'	not variable	not variable	not variable
PNSt3	S20° 27.150' E47° 10,961'	not variable	not variable	not variable	PNSv3	S20° 32.310' E47° 35.281'	not variable	not variable	not variable
PNSt4	S20° 27.165' E47° 10,999'	not variable	not variable	not variable	PNSv4	S20° 32.367' E47° 29.198'	not variable	not variable	not variable
PNSt5	S20° 27.165' E47° 10,999'	not variable	not variable	not variable	PNSv5	S20° 32.615' E47° 35.498'	not variable	not variable	not variable
PNSt6	S20° 27.169' E47° 10.993'	not variable	not variable	not variable	PNSv6	S20° 32.661' E47° 35.301'	not variable	not variable	not variable
PNSt7	S20° 27.941' E47° 11,401'	not variable	not variable	not variable	PNSv7	S20° 32.704' E47° 35.146'	not variable	not variable	not variable
PNSt8	S20° 27.941' E47° 11,401'	not variable	not variable	not variable	PNSv8	S20° 32.896' E47° 27.492'	not variable	not variable	not variable
PNSt9	S20° 95.647' E47° 11,456'	not variable	not variable	not variable	PNSv9	S20° 32.963' E47° 35.403'	not variable	not variable	not variable
PNSt10	S20° 98.747' E47° 11,392'	not variable	not variable	not variable	PNSv10	S20° 45.224' E47° 28.428'	not variable	not variable	not variable