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New approaches to underground systems in Brazilian *Smilax* species (Smilacaceae)[1]

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Martins, A. R. (Institute of Biology, State University of Campinas – UNICAMP, 13083-970, Campinas, SP, Brazil), N. Pütz, (Division of Biology and Education, University of Vechta, 49377 Vechta, Germany), A. N. Soares, A.B Bombo, and B. Appezzato da Glória (Biological Science Department, Escola Superior de Agricultura 'Luiz de Queiroz', University of São Paulo, 13418-900, Piracicaba, SP, Brazil). J. Torrey Bot. Soc. 137: 220–235. 2010.—New approaches to underground systems in Brazilian *Smilax* species (Smilacaceae). Scientific studies show that the watery extract of the thickened underground stem and its adventitious roots of the genus *Smilax* can act as a therapeutic agent in immunoinflammatory disorders, such as rheumatic arthritis. Brazilians have used this genus of plants in folk medicine, however it is very hard to identify these species, since the morphology of the underground systems is very similar in this group. For better identification of those systems, we studied six species of *Smilax* L. (*S. brasiliensis*, *S. campestris*, *S. cissoides*, *S. goyazana*, *S. oblongifolia* and *S. rufescens*), collected in different regions of Brazil with different physiognomies and soil characteristics. The main purpose is to describe the morpho-anatomy of the underground systems and to analyze if their structure depends on environmental conditions. The underground stem (rhizophore) is of brown color and it is knotty, massive, slender (*S. rufescens*) or tuberous (*S. brasiliensis*, *S. campestris*, *S. cissoides*, *S. goyazana* and *S. oblongifolia*). The tuberization is a result of primary thickened meristem (PTM) activity. The color and thickness of the adventitious roots change during development because the epidermis and outer cortex are disposed of, so the inner cortex becomes the new covering tissue with lignified and dark color cells. There are differences in starch grain shapes in mature roots. The chemical attributes of the soil are very similar in all studied environments and, even when soil characteristics varied, all the species' underground system was distributed close to the soil surface (10 to 15 cm deep). The species exhibited clonal growth hence their underground system functions as storage structures and the axillary buds can sprout into new stems. Only *Smilax rufescens*, collected in sandy soil of Restinga, has vegetative dispersal due to the runners.

Key words: adventitious roots, greenbrier, medicinal plants, morphology, phenolic compounds, rhizophores.

Scientific studies demonstrate that the watery extract of the thickened underground stem (previously called rhizome) and the roots of the genus *Smilax* can act as therapeutic agent in immunoinflammatory disorders, such as rheumatic arthritis (Jiang and Xu 2003). Brazilians have used this genus of plants in popular medicine, but it is very difficult to identify these species, since the morphology of the underground system is very similar and there are about 30 species of this genus in Brazil (Andreata 1997).

A rigorous characterization including morphology and anatomy of underground parts of medicinal species has essential importance for the quality control of the plant material used in phytotherapy research (Ming 1994).

Davis (1891) studying *Smilax glauca* observed that the environment can influence the growth of the underground system. According to the author plants growing in a dry environment had a greater number of tubers when compared to plants of the same species

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that grow in wet conditions. However, there is not enough information about Brazilian species of *Smilax* to develop controlled experiments. In order to do so, the first step is to organize a survey of anatomic and morphologic features considering the environmental conditions to provide a set of characteristics to better understand the kind of growth and the survival strategy adopted by the *Smilax* species.

We studied six species of *Smilax* L. (*S. brasiliensis*, *S. campestris*, *S. cissoides*, *S. goyazana*, *S. oblongifolia* and *S. rufescens*), collected in different regions of Brazil with different physiognomies and soil characteristics. The main purpose is to describe the morpho-anatomy of the underground systems for a better identification and to analyze if their structure depends on the environmental conditions.

Materials and Methods. Plant Material. Adult plant materials of six *Smilax* species were collected in natural populations from different biomes and regions of Brazil (Table 1).

The species *Smilax brasiliensis*, *S. goyazana* and *S. oblongifolia* were collected from open habitat Cerrado (tropical savanna) physiognomies (Figs. 1–3); *S. campestris* in *Campos* (subtropical grasslands); *S. cissoides* in Caatinga and *S. rufescens* in Restinga (sandy coastal plain) (Figs. 4–6). The samples were registered and added to the plant collection of the Herbarium (ESA) of the Escola Superior de Agricultura ''Luiz de Queiroz'' of the Universidade de São Paulo.

Morphology and Anatomy. Underground systems (thickened stem and adventitious roots) of three adult plants were fixed in FAA (formalin-acetic acid-alcohol) (Berlyn and Miksche 1976), dehydrated in ethanol series and stored in 70% ethanol. The morphology of the vegetative organs was analyzed and registered through digital photographs and botanical illustrations.

For the anatomical analysis, cross-sections of different parts of the thickened underground stem were sectioned with a sliding microtome (30–60 μm), cleared in sodium hypochlorite and stained with safranin and astra blue (Bukatsch 1972, Burger and Richter 1991) or crystal violet and orange G (Ma et al. 1993, Purvis et al. 1964) and then dehydrated in a graded ethanol series, and 50% and 100% butyl acetate. Permanent slides were mounted in synthetic resin ''Entellan''.

Young swollen axillary buds from negative gravitropic stems of *Smilax brasiliensis* and adventitious roots of all studied species were fixed as described above, dehydrated in ethanol series and embedded in Historesin (Leica Historesin). Serial sections were cut at 8–10 μm thickness on a rotary microtome (Sass 1951) and stained with toluidine blue O (Sakai 1973). Permanent slides were mounted in synthetic resin.

For histochemical tests, Sudan IV was used to locate lipids (Jensen 1962); zinc chloride iodate for starches (Strasburger 1913); iron chloride for phenolic compounds (Johansen 1940) and ruthenium red for pectin (Johansen 1940).

Images from slides were captured digitally through a Leica DMLB microscope with a video camera attached to a PC, using Leica, IM50 image analysis software.

Soil Analyses. We analyzed soil characteristics according to the procedures described by Raij et al. (1987): Air dried soil samples were sieved (2.0 mm) and analysed for total organic carbon (OM) by spectrophotometry after oxidation with sodium dichromate in presence of sulfuric acid and a subsequent titration with ammonic ferrous sulfate; phosphorus (P) was determined by spectrophotometry after anion exchange resin extraction; exchangeable aluminum (Al) and basic cations (K, Ca, Mg) were extracted with 1 molc L^{-1} KCl, cation exchange resin, and buffer SMP, respectively; the cation exchange capacity (CEC) was determined based on the sum of K, Ca, and Mg; the base saturation (V) was calculated as a percentage of the total CEC; the aluminum saturation (m) was calculated based on effective cation exchange capacity; the sum of bases (SB) was represented as the sum of Ca, Mg, and K; and the pH soil was determined in $CaCl_2$ (0.01 M) solution.

Results. *Smilax rufescens* grows in sandy soils. In the field, the connection between plants is not visible. The disperse distribution of stems can be mistakenly identified as a population of plants. However, analysis of the underground systems makes it clear that all the aerial stems are emitted from an underground stem system distributed horizontally

Table 1. Information about adult plant materials of six *Smilax* species collected in natural populations from different biomes and regions of Brazil.

Species	Locality	Date of collection	Map
Smilax brasiliensis Sprengel	Itapagipe – MG S 19°32′39.6″ W 49°26′36.2″	December 2005	
Smilax campestris Grisebach	Porto Alegre – RS S 30°03′34.2″ W 51°07′31.8″	January 2007	
Smilax cissoides Martius ex Grisebach	Feira de Santana – BA S 12°12′07″ W 38°57′57.4″	July 2006	
Smilax goyazana A. De Candolle	Itapaci – GO S 15°02′42.1″ W 49°48′18.9″	January 2007	
Smilax oblongifolia Pohl ex Grisebach	Distrito de Santo Antônio Leite - Ouro Preto – MG S 20°21′17.4″ W 43°41′13.8″	May 2007	
Smilax rufescens Grisebach	Parque Estadual da Ilha do Cardoso – Cananéia – SP S 25°03′57.7″ W 47°55′06″	March 2006	

close to the soil surface (10 to 15 cm of depth). The underground stem system of *S. rufescens* (Figs. 4–6) forms swollen nodes (knottys) separated from each other by horizontal runners. The runners were formed by elongated internodes and cataphylls of the nodes protected the axillary buds. At regular intervals, some nodes of the runners were swollen. These swollen nodes were able to produce new aerial stems, underground runners, and adventitious roots.

The underground stem of *S. brasiliensis*, *S. campestris*, *S. cissoides*, *S. goyazana* and *S. oblongifolia* was also distributed horizontally close to the surface of the ground (10 to 15cm of depth). It is brownish, woody and measured approximately five to ten centimeters in length. It consisted of swollen nodes (knotty; Figs. 7–11) that can produce negative geotropic stems and adventitious roots. Some axillary buds in the upper nodes of these underground stems had swollen, like the knottys of the original rhizophores (Fig. 7 "2"). These young swollen axillary buds produced stem ramifications (Fig. 7 "1") increasing the underground system complexity. We analyzed the anatomy of these swollen axillary buds to clarify the tuberisation process (Figs. 12–15). In cross section, there was a discontinuous epidermis and cataphylls protecting new buds. The cortex consisted of 10 to 20 layers of parenchyma cells which contained phenolic and raphid idioblasts. Between the cortex and the vascular cylinder, a meristematic area produced new vascular bundles and cortical cells. Thus, the swelling of young swollen axillary buds was a result of the activity of this meristematic area working as a Primary Thickening Meristem (PTM). The anatomy of these swollen axillary buds was similar to the old swollen nodes (rhizophore), as described below and differed only on absence of lignification.

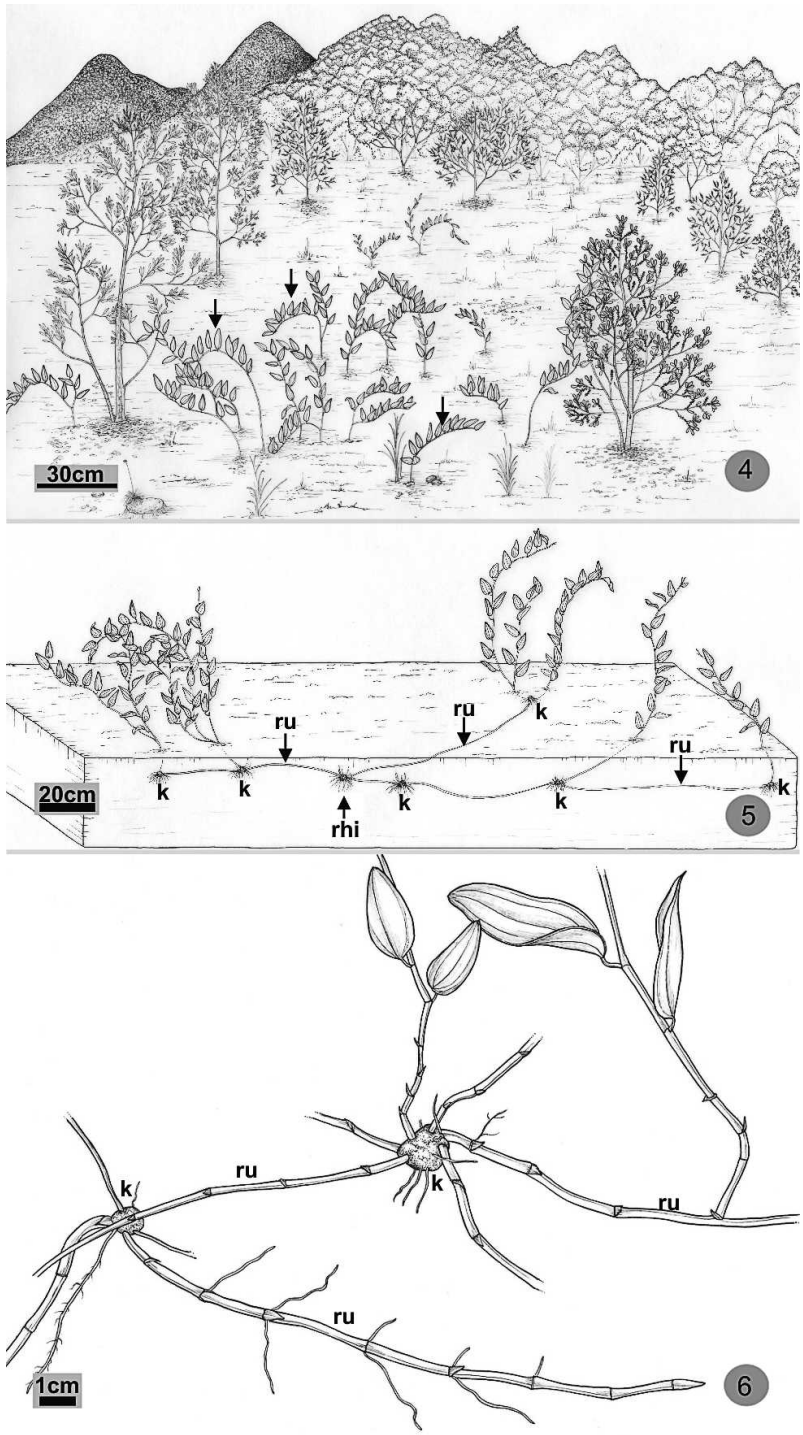
Cross sections of elder swollen nodes in the six species showed natural reddish color (Fig. 16). The anatomical features were similar to younger nodes. There was a loss of the epidermal cells in the swollen nodes and cortical cells were covering the inner structures (Figs. 17 and 18). It is interesting to note that the meristematic area is almost continuous with the endodermis and the pericycle. The vascular cylinder of older swollen nodes was comprised of collateral vascular bundles enclosed by parenchyma cells with thick and lignified walls (Figs. 17 and 19). Phenolic idioblasts were found among the parenchyma cells of the vascular cylinder (Fig. 20). In cross sections without stain, the content of these idioblasts showed a yellow and brown color. During the iron chloride test there was a strong reaction indicating the presence of phenolic compounds. Using zinc chloride, iodated starch grains were detected in parenchyma cells surrounding the vascular bundles.

Finally, the adventitious roots were studied in all six species (Figs. 21–28). These roots were rather long, sometimes more than one meter, distributed mainly horizontally at depths between 10 and 30 cm (occasionally 100 cm). We observed roots in two stages of development: young roots, with soft texture and white color and rigid roots of brown color and smaller diameter. In longitudinal section of the root apex, all six species studied had an apical open-type organization which was protected by the root cap (Figs. 21). Analyses of the cross sections of the root apex showed a uniseriate protodermis, the single layered exodermis and the cortex divided into an inner and an outer part (Figs. 21–28). Outer cortical cells were isodiametric (many times the cells were under division) with large intercellular spaces and contained idioblasts. The inner cortex was constituted of 2 or 3 layers of small, quadrangular and juxtaposed cells. The endodermis was fully developed (Fig. 22).

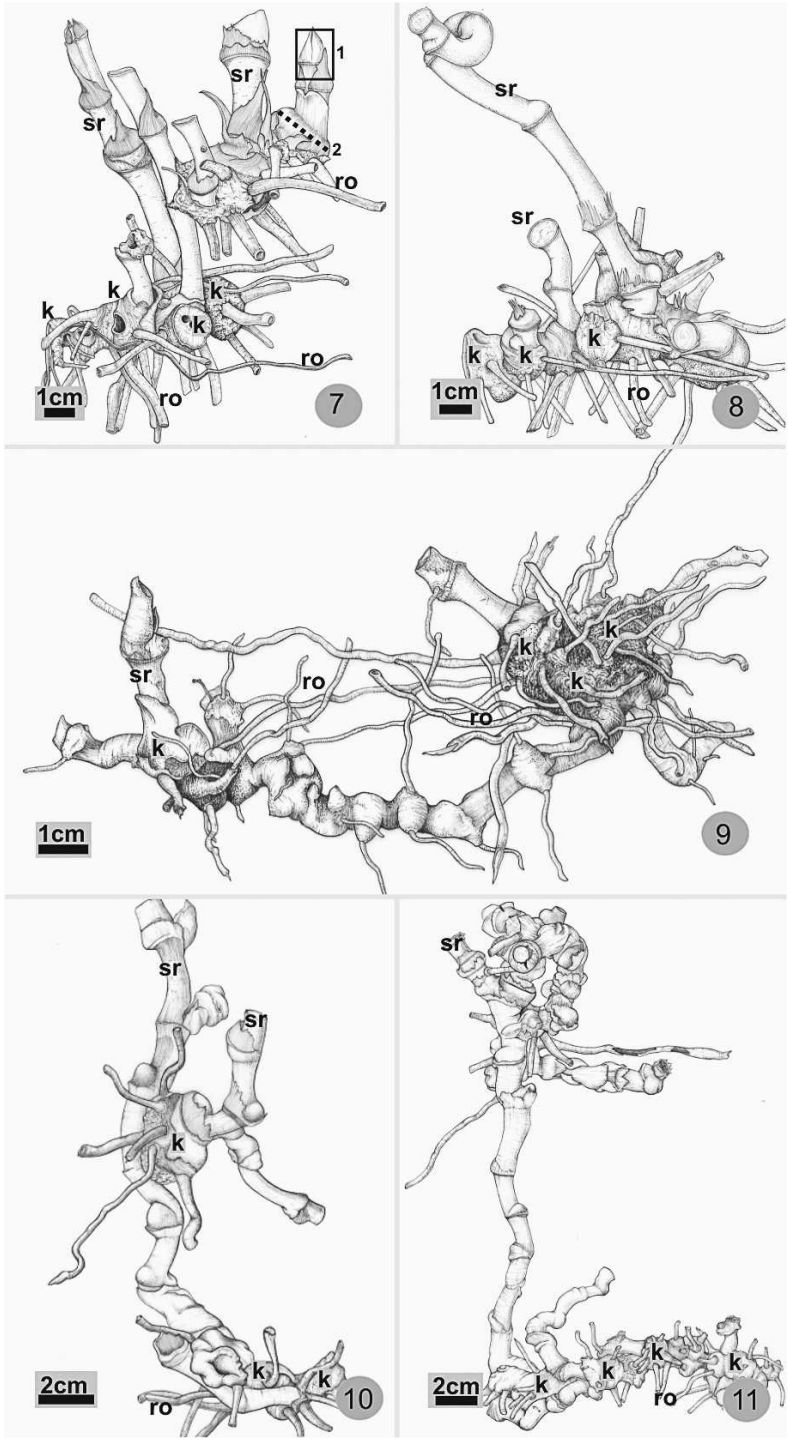
The soft white root was covered by the root epidermis (Figs. 23–25). The cortex was composed of a single-layered exodermis with thick-walled cells, several layers (18–20) of large, isodiametric parenchyma cells (outer cortex) and two or three layers of small, juxtaposed cells (inner cortex; Fig. 22). The outer cortex contained phenolic and crystal idioblasts. The endodermis is distinguishable despite the absence of an identifiable Casparian band in the cells. The vascular cylinder is polyarch, with wide parenchymatous pith and is surrounded by a two-layered pericycle of thin-walled cells.

During root maturation there was secondary thickening of the cell walls, and the outer cortex broke down. The inner cortex cells and the endodermis exhibit cell wall thickening. The inner cortex cells became brown in color due to the deposition of tannins in the walls. The pericycle and the vascular parenchyma also became thickened (Figs. 26–28). The older roots were thinner and brown in color

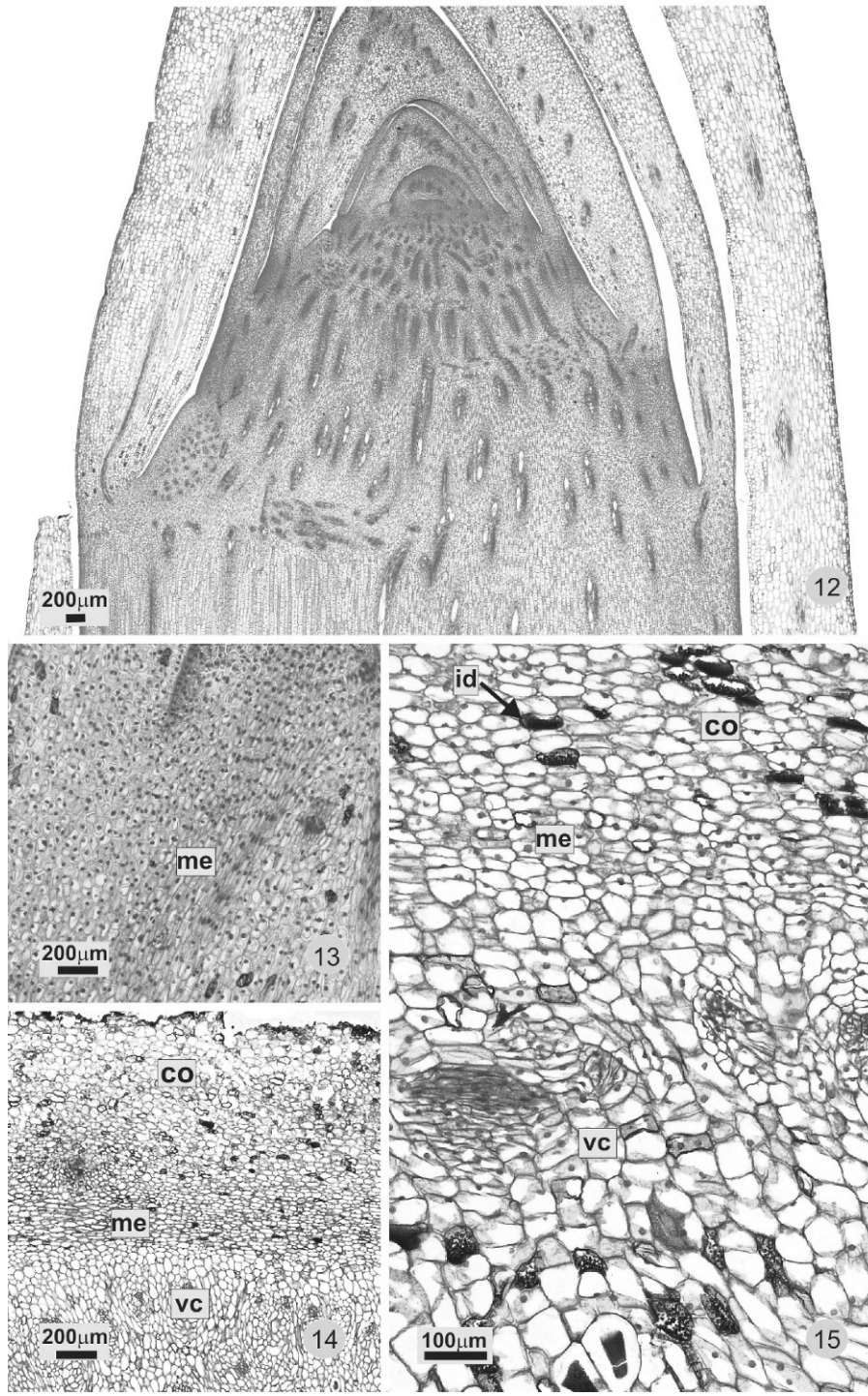
FIGS. 1–3. The species *Smilax brasiliensis* (FIG. 1) and *S. goyazana* (FIGS. 2–3) collected from open habitat Cerrado (tropical savanna) physiognomies. FIG. 1. The characteristic environment with predominance of herbs and dispersal trees. FIGS. 2–3. Stony soil and vegetation composed by herbs.



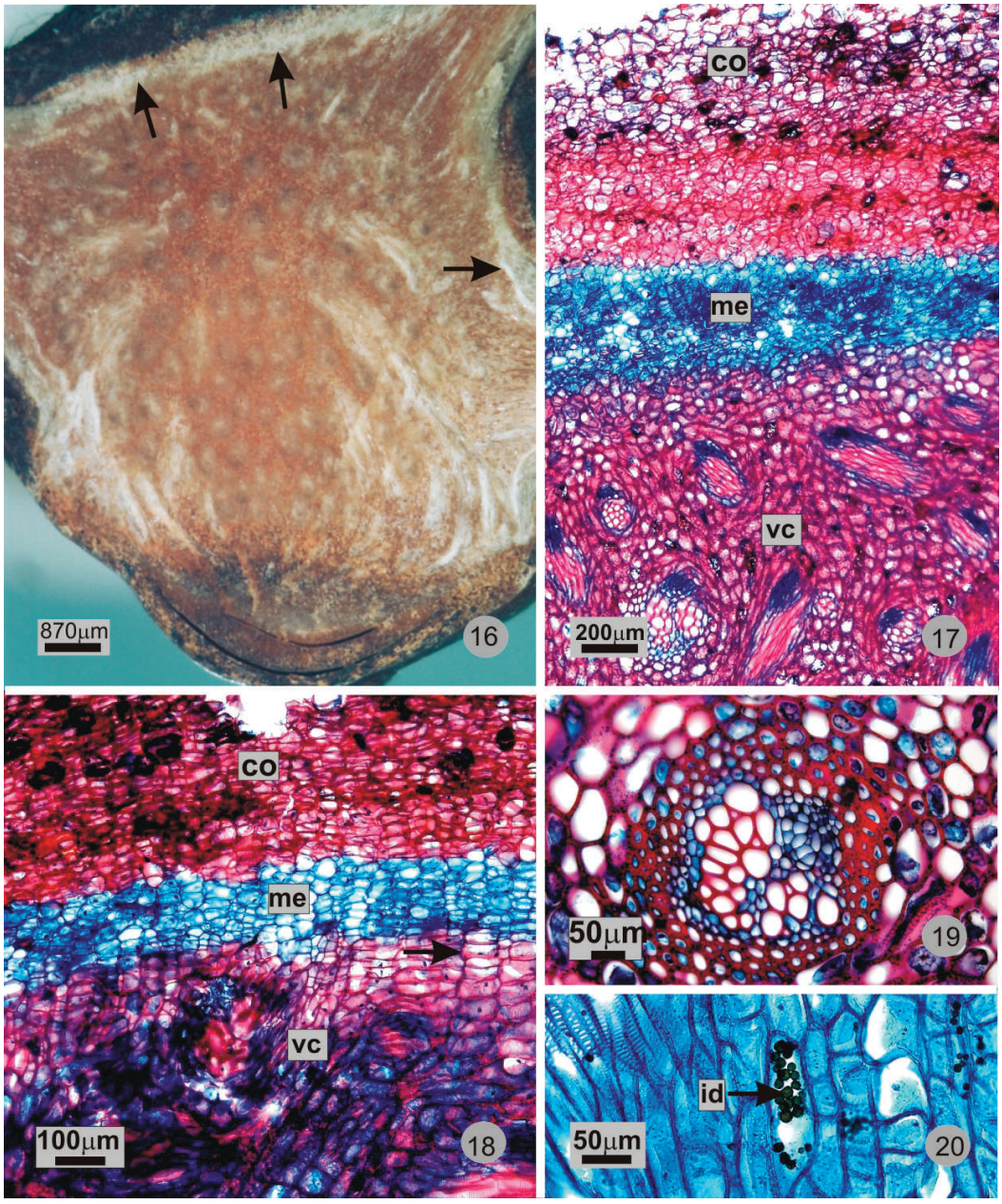
FIGS. 4–6. *S. rufescens* in Restinga environment (sandy coastal plain). FIG. 4. General view of the environment. One plant appears like a population because of the branch system (arrows). FIG. 5. The underground distribution of the runners (ru) and knottys (k). FIG. 6. Detail of underground system. rhi = rhizophore.



FIGS. 7–11. General view of the morphology of the rhizophores of the six species of *Smilax*. FIG. 7. *Smilax brasiliensis*. FIG. 8. *Smilax cissoides*. FIG. 9. *Smilax campestris*. FIG. 10. *Smilax goyazana*. FIG. 11. *Smilax oblongifolia*. k = knotty; ro = roots; sr = stem ramification.



FIGS. 12–15. Longitudinal (FIGS. 12–13) and cross sections (FIGS. 14–15) of shoot apex of *Smilax brasiliensis*. FIG. 12. General view of the area shown in FIG. 7 '1'. The intercalary meristem was not observed. FIG. 13. Detail of the PTM (Primary Thickening Meristem) closer to the axillary buds formation. FIG. 14. General view of the swollen area showed in Fig. 7 '2'. FIG. 15. Detail of the PTM activity forming new vascular bundles and cortical cells. co = cortical cells; id = phenolic idioblasts; me = meristem layers; vc = vascular cylinder.



FIGS. 16–20. Cross sections of the older swollen nodes (rhizophore). FIG. 16. *Smilax campestris*. FIGS. 17–19. *Smilax cissoides*. FIG. 20. *Smilax brasiliensis*. FIG. 16. Cross section of swollen nodes in the six species exhibited a natural reddish color. The arrows indicate the meristematic layers. FIG. 17. General view of the cortical area and of the vascular cylinder. FIG. 18. The cortical cells covered the structure. The meristematic cells can originate new vascular bundles and cortical cells (arrow). FIG. 19. Vascular bundles of the collateral type. FIG. 20. Phenolic idioblasts. Co = cortical area, me = meristematic cells, vc = vascular cylinder, id = idioblasts.

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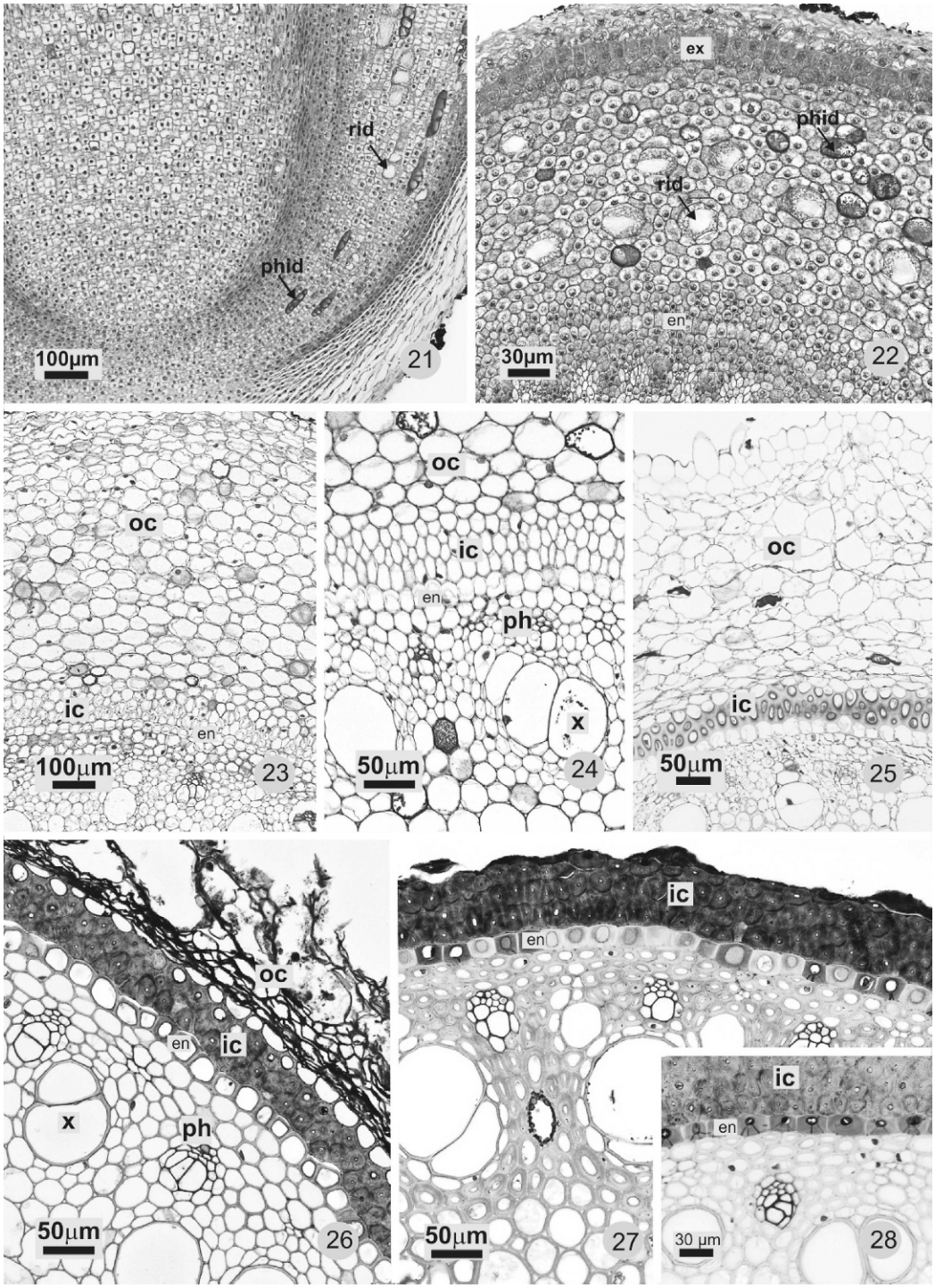
Figs. 21–28. FIGS. 21–22. Longitudinal (21) and cross (22) sections of the root apex of *Smilax campestris*. FIG. 21. Apical tip showing open-type organization which is protected by the root cap. FIG. 22. Detail of the uniseriate protodermis and the single-layered exodermis. FIGS. 23–25. Cross sections of the white root. FIG. 23. *Smilax brasiliensis*. FIG. 24. *Smilax cissoides*. FIG. 25. *Smilax campestris*. FIGS. 23–24. The cortex divides



into an inner and an outer part. FIG. 25. The secondary thickening of endodermal cell walls and the progressive degeneration of the outer cortex. FIGS. 26–28. The inner cortex cells become brown in color due to deposition of tannins in the walls. The pericycle and the vascular parenchyma also becomes thickened. FIG. 27. The endodermis exhibits O-shaped cell wall thickenings. en = endodermis; ep = root epidermis, ex = exodermis; ic = inner cortex; oc = outer cortex; ph = phloem; phid = phenolic idioblasts; rid = raphid idioblasts; x = xylem.

because they were covered by the inner cortical cells. These roots posses a large amount of starch grains in the pith parenchyma (Figs. 29–35). These starch grains may be: simple (isolated) and spherical in *S. goyazana* and *S. brasiliensis* (Figs. 29, 31, 33), or polyhedral and grouped in *S. oblongifolia*, *S. campestris* and *S. cissoides* (Figs. 30, 32, 34, 35).

It is interesting to note that we found fungus infecting the rhizophore (Fig. 36), and ground parasites infecting the meristematic area of the root apex (Fig. 37). In infected rhizophores, there was always a great concentration of phenolic and raphid idioblasts surrounding the infected area (Figs. 36–37).

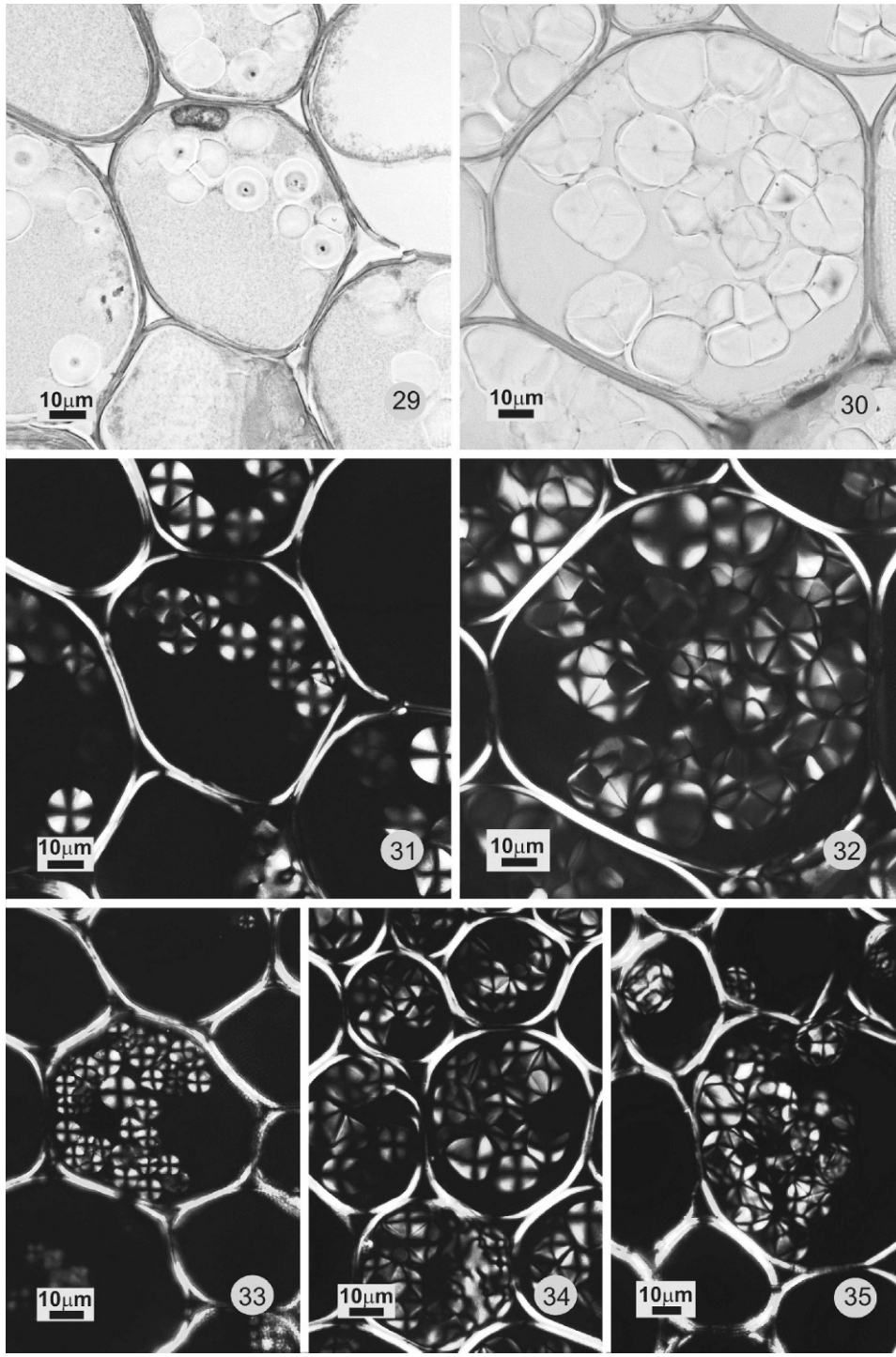
The chemical attributes of the soil were very similar in all studied environments. The soil analyses (Table 2) showed typical characteristics of the Cerrado environment, a very acid pH, high concentration of aluminum in most cases, low level of phosphorus and low capacity of cation exchange. In all collected areas, the organic matter content was higher at 0–20 cm soil depth. Our field observations revealed similarities in soil surface (for comparison see Figs. 1–6), in contrast with *Smilax goyazana*, collected from a stony soil (Fig. 2). However, the underground system morphology and distribution was very similar to *S. brasiliensis* collected in a non stony soil (Fig. 1).

Discussion. The first classification as rhizophore of the underground system of the *Smilax* genus was adopted by Andreata (1997) and Andreata and Menezes (1999). These authors described the underground system consisting of two stem branching systems: one aerial (plumule origin) and one subterranean (cotyledonary bud origin). This underground vegetative system is called rhizophore (Andreata 1997). In our study with adult plants of *Smilax polyantha* we also adopt this classification (Martins and Appezzato-da-Gloria 2006). In contrast, Holm (1929) reported the rhizome originates from the plumule and constitutes the only vegetative stem branching system of the plant. However, most authors do not study the origin of underground systems from the seedling, and thus, called the underground system of *Smilax* as rhizome (Davis 1891, Holm 1890, Caponetti and Quimby 1956, Oliveira et al. 1973, Gatuso 1995, Ju and Jia 1992, Du et al. 2005, Ooi et al. 2004).

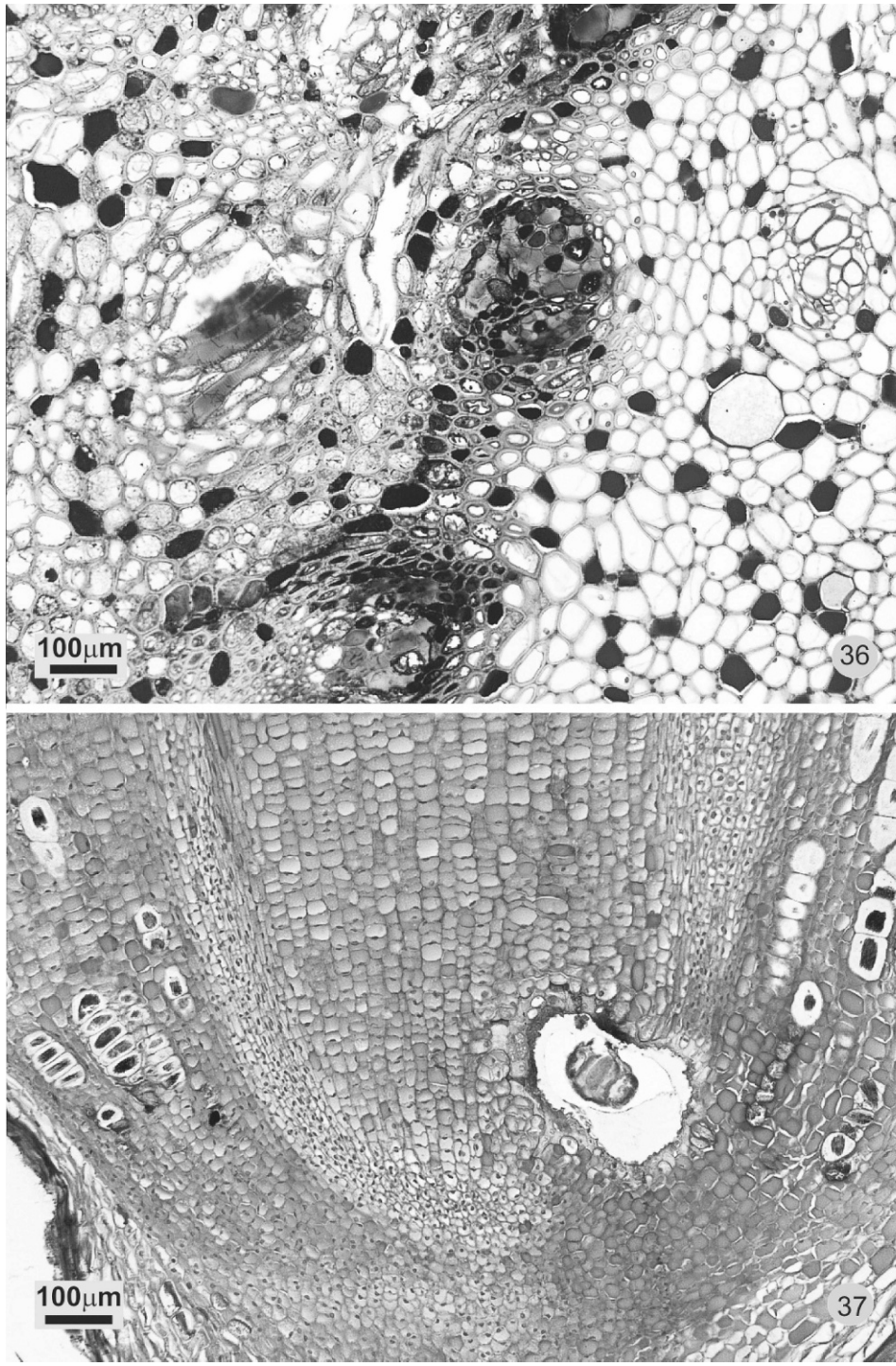
The sympodial system of the rhizophore is originated from the seedling. In *Smilax* it forms by a set of swollen axillary buds connected by sympodial growth. In most cases the swollen buds are very close together and form a short thickened underground stem. The *Smilax* rhizophores are very similar to other tuberous-stems rhizomes (for example *Scrophularia nodosa*, Hegi 1943).

However, classification is difficult, because the description of underground stems has its basis mostly on a snapshot. Usually, one digs out some individuals and categorizes them with terms like rhizophore, rhizome, stolon and so on. But, the behavior of a geophilous plant starts with the seedling, and is characterized by different developmental phases, in which the fulfillment of different functions is required. When analyzing the subterranean organ systems, development within a holistic-temporal context is necessary. In this case, the three-step model of the geophilous development (Pütz 2006) offers an ideal basic pattern. Geophilous systems are dynamic and mobile. They are dynamic, because they can adapt their morphological configuration to the changing functional requirements and they are mobile, because their position within the soil is marked by continuous movement. This has been emphasized by several ecological researches (Bell and Tomlinson 1980, Pütz 1994, Meyer and Schmid 1999, Brock et al. 2000). In the present study no examination on the dynamic development of *Smilax* rhizophores was made, but the underground system distribution (0–20 cm soil depth) in all species coincides with higher contents of organic matter whether the soil being stony or not. All of them exhibited clonal growth hence their underground system functions as storage structures and the axillary buds can sprout into new stems. Only *Smilax rufescens*, collected in sandy soil of Restinga, presents vegetative dispersal due to the runners.

Our anatomical examination has clarified some details. Caponetti and Quimby (1956) describe the presence of a periderm which covers the underground system. We cannot verify a periderm in any of the six species. However, covering of the rhizophores is constituted by some remaining epidermal cells or by the cortex. The cortex with the function of covering has been observed in *Smilax*



FIGS. 29–35. Cortical cells of young roots of *Smilax goyazana*. (FIGS. 29, 31), *Smilax oblongifolia* (FIGS. 30, 32), *Smilax brasiliensis* (FIG. 33). *Smilax campestris* (Fig. 34) and *Smilax cissoides* (FIG. 35). FIGS. 29, 31, 33. Isolated starch grains are simple and spherical. FIGS. 30, 32, 34, 35. Grouped starch grains are of the polyhedral type.



FIGS. 36–37. FIG. 36. Cross section of a swollen node (knotty) of *Smilax goyazana* with fungus infection of the cortical cells of the rhizophore. FIG. 37. Longitudinal section of the root apex of *Smilax campestris* showing the meristematic area infected with ground parasites. A great concentration of phenolic and raphid idioblasts surrounding the infection areas in both cases were observed.

Table 2. Soil chemical properties at two different depths. (OM = organic matter, P = phosphorus, K = potassium, Ca = calcium, Mg = magnesium, Al = exchangeable aluminum, V% = base saturation, m% = aluminum saturation, total of bases (SB), total cation exchange capacity calculated (CEC).

Place	Sample depth (cm)	pH	H+Al	Al	K	Na	OM	Ca	Mg	P	SB	CEC	V	m
Units			$mmol_c\ dm^{-3}$	$mmol_c\ dm^{-3}$	$mmol_c\ dm^{-3}$	$mmol_c\ dm^{-3}$	$g\ dm^{-3}$	$mmol_c\ dm^{-3}$	$mmol_c\ dm^{-3}$	$mg\ dm^{-3}$	$mmol_c\ dm^{-3}$	$mmol_c\ dm^{-3}$	%	%
Porto Alegre	0–20	4.13	98.67	17.17	2.44	10.08	34.72	19.67	6.67	6.88	38.86	137.52	30.56	29.29
	20–40	3.93	133.33	30.63	1.07	9.45	22.63	11.67	5.33	2.92	27.52	160.86	18.87	51.43
Itapaci	0–20	4.13	40.00	7.90	1.14	1.89	17.67	4.33	2.00	3.15	9.36	49.36	19.41	45.54
	20–40	4.20	27.00	7.17	0.60	1.26	8.06	1.67	1.00	1.28	4.53	31.53	14.25	61.49
Itapagipe	0–20	4.62	31.67	4.73	1.24	2.52	23.25	10.33	7.67	3.62	21.76	53.43	39.06	21.90
	20–40	4.61	31.67	7.00	0.75	1.89	17.67	7.43	5.67	3.27	15.74	47.41	30.99	37.46
Feira de Santana	0–20	4.59	19.67	0.77	1.33	6.93	18.60	17.30	9.00	5.60	34.56	54.22	63.80	2.22
Ilha do Cardoso	0–20	4.61	11.00	1.93	0.36	3.15	8.68	0.00	1.67	2.22	5.18	16.18	31.54	28.61
	20–40	4.76	9.33	1.60	0.31	3.78	5.58	0.00	2.00	1.87	6.09	15.43	39.53	20.78
Ouro Preto	0–20	5.08	16.67	1.67	0.90	1.89	19.53	8.10	5.33	3.15	16.23	32.89	42.88	21.76
	20–40	4.98	16.00	2.67	0.53	1.26	16.12	6.73	4.00	2.80	12.53	28.53	37.10	35.60

polyantha (Martins and Appezzato-da-Glória 2006) and in *Raphonticum carthamoides* (Lotocka and Geszprych 2004).

Cunha (1940) and Caponetti and Quimby (1956) suggested that the type of thickening of the endodermis could be used like a distinctive anatomical criterion for the identification of the species. However, for the six species studied and for *S. polyantha* (Martins and Appezzato-da-Glória 2006) the type of thickening of the endodermis (type "O") is not a distinctive character.

The anatomy of young swollen axillary buds in underground parts of stem ramification of *Smilax* species examined here is very similar to the anatomy of the swollen nodes of the rhizophore, differing only in the degree of lignification. These observations indicate the formation of the rhizophore is by a set of swollen axillary buds present in the base of each new sympodial stem.

The Primary Thickening Meristem (PTM) found in swollen nodes of the underground parts of new ramifications is similar in position to the meristematic area observed in the rhizophore of the six species of *Smilax* studied here.

Estelita and Rodrigues (2007) described the characteristics between stem and scape in the studies with Cyperaceae. It can be argued that aerial stems of *Smilax* are possibly scapes (flower stem). The presence of an intercalary meristem and absence of PTM are two characteristics for the differentiation of a stem and a scape (Estelita and Rodrigues 2007). However, we did not observe an intercalary meristem in the species studied, so there is no possibility of the aerial stem of *Smilax* to be a floral scape.

The alternative medicinal use of the roots and rhizophore of the *Smilax* genus was registered in the first edition of the Brazilian Pharmacopeia of 1929 (Silva 1929). In this edition of the Pharmacopoeia the medicinal species of *Smilax*, whose popular name was 'salsaparrilha', identified by the way roots were packed, the diameter of the root and its coloration. The six species studied here show that these criteria are untenable. The six species studied show as well that this criterion is untenable. The roots of the studied species are anatomically very similar, and the difference in coloration and thickness depends on the development phase of the root. Roots with white coloration and higher diameter are

simply younger, and roots with brown coloration and lesser diameter are older ones. The roots have turned brown due to deposition of tannins in the walls of cells external to the endodermis. A similar condition has been reported by McKenzie and Peterson (1995) in *Pinus banksiana* and *Eucalyptus pilularis*.

The change in root coloration indicates an interesting anatomical development. The diameter decreases by the loss of the area of the outer cortex. Cunha (1940) and Stellfeld (1940) also observed the loss of cortical parenchyma in some Brazilian species of *Smilax*. We found that the brown covering tissue in older roots is formed by the inner cortex with highly lignified cell walls that can prevent the invasion of microorganisms (Sexton and Roberts 1982).

The early development of raphides idioblasts and phenolic idioblasts in the apex of the roots of the species can be associated with protection. There was a presence of a higher number of phenolic idioblasts surrounding parasite infection in the root apex of *S. campestri* as well as fungal infection in the rhizophore of *S. goyazana*. Sawidis et al. (2005) showed that raphides seem to be vital for the protection of the root tuber parenchyma of *Asphodelus aestivus* (Asparagales) from herbivores. The large amount of raphides idioblasts found in the underground system of *Smilax* can also be attributed to the protection.

We conclude that observing the anatomy of the vegetative organs does not allow distinguishing between the six species studied. Species still need to be identified by the morphology of its leaves. However, the data presented in this study will be able to assist in distinguishing *Smilax* species from the other genera of medicinal plants that are also called popularly of 'salsaparrilha', such as the genus *Herreria* (Cunha 1937, Stellfeld 1940).

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