Leaf anatomical features and their implications for the systematics of dragon's blood, *Croton* section *Cyclostigma* (Euphorbiaceae)

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Establishing species limits in clades in *Croton* using characters from external morphology and common molecular markers has proved cumbersome, especially in *Croton* section *Cyclostigma*, a group of 50 Neotropical arborescent species commonly known as dragon's blood. Given this, we explored leaf and shoot apex anatomical characters for their utility in distinguishing species or groups of species in this section. We analysed 90 specimens belonging to section *Cyclostigma* and 14 specimens from related groups (sections *Adenophylli*, *Cupreati* and *Sampatiki*), recording and describing 45 qualitative leaf characters. These characters were assembled into a matrix and analysed using statistical clustering methods based on similarity. Our results show that trichomes are one of the most diverse and variable anatomical features among the studied specimens. Novel anatomical features for *Croton* include the presence of a hypodermis and two previously unreported types of non-glandular trichomes. Although we did not detect a single anatomical character uniting section *Cyclostigma*, combinations of anatomical characters were useful to establish species limits and taxonomic identities within this section.

ADDITIONAL KEYWORDS: colleters – hypodermis – idioblasts – nectaries – non-articulated laticifers – non-secretory trichomes.

INTRODUCTION

Croton L. is a megadiverse genus with >1200 species (Christenhusz, Fay & Chase, 2017; Haber *et al.*, 2017). The medicinally important dragon's blood trees belong exclusively to *Croton* section *Cyclostigma* Griseb. (Riina, Berry & van Ee, 2009), one of the main Neotropical clades of the genus. About 750 species in the genus occur in the New World, and these are organized into 31 sections in the most recent classification (van Ee, Riina & Berry, 2011). According to ongoing taxonomic studies, section *Cyclostigma* consists of c. 50 species distributed from Mexico to northern Argentina (Riina *et al.*, pers. comm.). All species in this section produce abundant reddish to orange/yellowish latex, which is widely used in many Latin American countries, mainly

as a treatment for skin wounds and burns (Meza, 1999; Borges & King, 2000; Jones, 2003; Salatino, Salatino & Negri, 2007). Dragon's blood trees grow predominantly in moist forests, including riverine, lowland and montane forests, but a few species occur in dry forests (van Ee *et al.*, 2011).

Like the other 30 sections of New World *Croton*, section *Cyclostigma* is monophyletic. However, the resolution of the relationships between and within most sections is still a work in progress. For instance, a monophyletic section *Cyclostigma* was established by excluding several putative species, which were retrieved in seven other clades of *Croton*. The *Cyclostigma* clade is sister to the monotypic section *Cupreati* Riina in the phylogenetic analysis based on *trnL-F* or sister to section *Adenophylli* Griseb. (section *Cascarilla* in Riina *et al.*, 2009) in the ITS tree. In addition, resolution at the species level in the

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Cyclostigma clade was low and many dragon's blood species remained to be sampled (Riina *et al.*, 2009).

The only potential synapomorphy for section *Cyclostigma* is the presence of non-articulated laticifers in wood rays (Wiedenhoeft, Riina & Berry, 2009). On the other hand, all species in the section share a suite of morphological characteristics including arborescent habit, reddish latex, stellate indumentum, conspicuous stipules, acropetiole glands, long thyrsoid inflorescences with bisexual cymes predominantly at the base and staminate flowers with >16 stamens (Riina *et al.*, 2009). However, most of the individual characters from the suite described above are also present in other clades of *Croton* (van Ee *et al.*, 2011).

Although recent taxonomic studies of the dragon's blood group have identified new and morphologically distinct species (Riina, Berry & Cornejo, 2007; Riina, Vigo & Cerón, 2014; Riina *et al.*, 2015; Feio *et al.*, in press), establishing species limits in section *Cyclostigma* has proved difficult due to the prevalent homoplasy in most of the traditional morphological characters and the lack of resolution at the species level with the molecular markers used so far (Riina *et al.*, 2009; van Ee *et al.*, 2011).

Given this scenario, we conducted a comparative analysis of leaf anatomical features using a broad sampling of section *Cyclostigma*, a selection of species from its sister clades from nuclear and plastid phylogenetic analyses (sections *Adenophylli* and *Cupreati*) and a representative of a more distant clade [section *Sampatik* (G.L. Webster) Riina]. Our goals were to identify additional anatomical features that could help to tease apart species or groups in section *Cyclostigma* and to expand the database of characters available for studies of morphological evolution in Euphorbiaceae.

MATERIAL AND METHODS

TAXON SAMPLING

We sampled 104 specimens, of which 90 belong to *Croton* section *Cyclostigma* or have morphological affinities with that section according to ongoing taxonomic studies (R. Riina, pers. comm.), 11 specimens representing six species were from *Croton* section *Adenophylli*, one specimen from the only known species (*C. cupreatus* Croizat) of section *Cupreati* and two specimens of *C. piptocalyx* Müll. Arg. from section *Sampatik*. Specimens were identified to section based on their morphological affinities according to van Ee *et al.* (2011). Plant material for anatomical studies was obtained from herbarium specimens (Appendix 1). Herbarium acronyms follow those of Thiers (2016, continuously updated).

LIGHT MICROSCOPY

Herbarium samples of fully expanded leaves and shoot apex were rehydrated (Smith & Smith, 1942) and stored in 70% ethanol. Subsequently, fragments from the middle and basal portions of the leaf blade (midrib and margin) and the middle and distal portions of the petiole were dehydrated in decreasing ethanol series and were embedded in 2-hydroxyethyl methacrylate resin for sectioning (Historesin Leica®, solutions were prepared according to manufacturer's instructions) following Meira & Martins (2003). All samples were transversally and longitudinally sectioned with a thickness of 3–7 µm, using an automatic rotary microtome (model RM 2265, Leica® Biosystems, Nussloch GmbH, Heidelberg, Germany) using disposable glass knives (Leica®, Biosystems, Nussloch GmbH). Sections were stained with toluidine blue at pH 4.6 (O'Brien, Feder & McCully, 1965) and slides were mounted in resin (Permunt®, Fisher Scientific, NJ, USA).

Some of the samples were also cleared with 5% sodium hydroxide and 20% hypochlorite solutions, stained with 50% ethanol-diluted fuchsin (Shobe & Lersten, 1967) and mounted in glycerinated gelatin (Kaiser, 1880).

Observations and photographic documentation were performed with a light microscope (Model AX70TRF, Olympus Optical, Tokyo, Japan) equipped with a U-Photo system, polarizing filter and digital camera (AxioCam HRC; ©Carl Zeiss, Jena, Germany). Macro images were obtained using a stereomicroscope (Stemi 2000-C®, ©Carl Zeiss Microscopy GmbH, Jena, Germany) with a coupled digital camera (AxioCam ERc5s®, ©Carl Zeiss Microscopy GmbH, Jena, Germany).

SCANNING ELECTRON MICROSCOPY

Some of samples stored in 70% ethanol were dehydrated in ethanol and critical point dried with CO₂ (Bozzola & Russel, 1992) in a 020 CPD dryer (Bal-Tec; Balzers, Liechtenstein). They were mounted on aluminium stubs and coated with gold using a FDU 010 sputter coater (Bal-Tec). Examination of specimens and image capture were conducted using a scanning electron microscope Leo 1430VP (Zeiss, Cambridge, UK) at the Centro de Microscopia e Microanálises, Universidade Federal de Viçosa.

DESCRIPTION AND ANALYSIS OF ANATOMICAL CHARACTERS

Anatomical descriptions were based on terms from Metcalfe & Chalk (1979, 1983) and Evert (2006). Trichome terminology followed the classification of

Webster, Del-Arco Aguilar & Smith (1996). Forty-five qualitative characters from leaf and shoot apex were recorded (Table 1). A matrix of specimens by characters was built, in which characters were coded as binary states (Appendix 2). A distance matrix was calculated using the Dice-Sorensen and Jaccard coefficients and similarity dendrograms were generated using the software PAST3 vs. 3.06 (Hammer, Harper & Ryan, 2001). The resulting dendrograms were further edited in FigTree vs.1.4 (Rambaut, 2012) and CorelDRAW X3®.

The taxonomic identity of the specimens was confirmed or modified by integrating information from the resulting clustering pattern, morphology from taxonomic descriptions, field observations, type specimens, habitat and geographical distribution.

RESULTS

LEAF ANATOMY

The matrix of anatomical characters has 1.3% of missing data coded as '?' (Table 2). There were 17 specimens (Table 2), for which we could not evaluate some characters (1–11) because the original herbarium material was too old or poorly preserved. In section *Cyclostigma* the most critical specimens regarding missing data were *C. bogotanus* Cuatrec. (Riina-1591) and *C. mutisianus* Kunth (Barkley-3768), both with the maximum percentage of missing characters (21%) (Table 2).

Of all the surveyed characters, trichomes were the most diverse and variable and may differ even between closely related species (Fig. 1A–O), but no type was exclusive to section *Cyclostigma* or any of the other outgroup species. Non-glandular trichomes were lepidote (Fig. 1A, B), dendritic (Fig. 1C, D), rosulate (Fig. 1E, F), fasciculate cushion-shaped (Fig. 1G, H), stipitate-stellate (Fig. 1I), stipitate-stellate porrect (Fig. 1J), appressed-stellate porrect (Fig. 1K), appressed-stellate (Fig. 1L), multiradiate (Fig. 1M, N) or simple (Fig. 1O). Out of these 16 types, only the rosulate, stipitate-stellate porrect, appressed-stellate porrect and simple types are more typical of section *Cyclostigma*.

Lepidote trichomes [present in two specimens (*C. urucurana* Baill. -1) of section *Cyclostigma* and in sections *Adenophylli* (*C. ruizianus* Müll.Arg.) and *Cupreati* (*C. cupreatus*)], dendritic trichomes [present in *C. vulnerarius* Baill. -2, *C. draco* Schltdl. -1, and *C. celtidifolius* Baill. -1] and fasciculate cushion-shaped trichomes [present in *C. huberi* Steyererm., *C. speciosus* Müll.Arg., *C. medusae* Müll.Arg. -1, *C. medusae*-4 and *C. coriaceus* Kunth -3] were less common.

All specimens present hypostomatic leaves with parasitic stomata (Fig. 2A–C), epidermis with sinuous anticlinal walls on the abaxial side (Fig. 2A, B), straight

to slightly sinuous walls on the adaxial side (Fig. 2C) and a slightly striated cuticle covering the epidermis on both sides (Fig. 2B). In cross section, they present a single-layered epidermis, thin cuticle, and stomata placed at the same level of the ordinary epidermal cells (Fig. 2D, E). In some specimens of section *Cyclostigma* (*C. amentiformis* Riina, *C. bogotanus*, *C. coriaceus*-2, *C. coriaceus*-3, *C. floccosus* B.A.Sm., *C. rimbachii* Croizat) and in section *Cupreati*, we observed a single-layered hypodermis underlying the adaxial epidermis (Fig. 2D).

The mesophyll is dorsiventral in all specimens, with one or two layers of palisade parenchyma and four or five layers of spongy parenchyma (Fig. 2D, E). The vascular system is formed by collateral bundles (Fig. 2E), which possess bundle-sheath extensions only in sections *Cyclostigma* and *Cupreati* (Fig. 2D). Under polarized light, we detected abundant druse crystals in all the studied specimens, but they are found mostly in the ground parenchyma of different regions of the blade (Fig. 2F).

Leaf margins are predominantly revolute (Fig. 2G, inset); however, in section *Cyclostigma* there are specimens with a slightly involute margin (Fig. 2H, inset) as in *C. bogotanus*, *C. draco*-2, *C. echinocarpus* Müll.Arg., *C. erythrochilum* Müll.Arg., *C. erythrochyloides* Croizat, *C. floccosus* and Riina-1416. Leaf margins can also present either continuous (Fig. 2G, H) or discontinuous (Fig. 2I) palisade parenchyma.

The midrib is biconvex (Fig. 2J) in sections *Cyclostigma*, *Cupreati* and *Sampatik* and *C. abutilifolius* Croizat and *C. gracilipes* Baill. (section *Adenophylli*) and uniconvex with the adaxial side flat to slightly concave in *C. conduplicatus* Kunth and *C. ruizianus* (section *Adenophylli*) (Fig. 2K). Sections *Cyclostigma* and *Adenophylli* possess a midrib with five to ten layers of angular-annular collenchyma (Fig. 2J, L) and a continuous palisade parenchyma (Fig. 2K, L), but the palisade parenchyma is not continuous in species of sections *Cupreati* and *Sampatik*. In all the studied species, except *C. aequatoris* Croizat and *C. conduplicatus* in section *Adenophylli*, the vascular system consists of collateral bundles arranged in a main open arch and one to five smaller dorsal bundles and all bundles are surrounded by fibres (Fig. 2J, L, M). In contrast, *C. aequatoris* and *C. conduplicatus* are the only species with a single main vascular bundle (Fig. 2K).

In all specimens, the petiole in cross section is rounded with a slight depression in the central region of the adaxial side (Fig. 2N). The epidermis is single-layered with tiny cells (Fig. 2O) and covered by a thin cuticle. In the cortical region, nine or ten layers of angular collenchyma are present underlying the epidermis, followed by seven to ten layers of

Table 1. Qualitative anatomical characters used in cluster analysis coded as present (1) or absent (0) in the matrix

Number	Character and states
1	Secretory idioblasts
2	Hypodermis
3	Continuous palisade parenchyma in the midrib
4	Bundle-sheath extension
5	Margin with continuous palisade parenchyma
6	Colleters on the leaf margin
7	Basilaminar/acropetiolar colleters
8	EFNs basilaminar
9	EFNs acropetiolar
10	EFNs dispersed on the blade
11	EFNs marginal
12	EFNs sessile
13	EFNs stipitate
14	EFNs with concave surface
15	EFNs with convex surface
16	EFNs with flat surface
17	Lepidote trichomes
18	Rosulate trichomes
19	Dendritic trichomes
20	Fasciculate cushion-shaped trichomes
21	Stipitate-stellate trichomes
22	Stipitate-stellate porrect trichomes
23	Appressed-stellate porrect trichomes
24	Appressed-stellate trichomes
25	Multiradiate trichomes
26	Fasciculate trichomes
27	Simple trichomes
28	Hypostomatic leaf
29	Amphistomatic leaf
30	Paracytic stomata
31	Secretory idioblasts only in the abaxial epidermis
32	Secretory idioblasts only in the adaxial epidermis
33	Secretory idioblasts in both abaxial epidermis and palisade parenchyma
34	Secretory idioblasts in the palisade parenchyma
35	Secretory idioblasts dispersed in the mesophyll
36	Non-articulated branched laticifers
37	Articulated laticifers
38	Unistratified epidermis
39	Dorsiventral mesophyll
40	Biconvex midrib
41	Midrib uniconvex, with flat to slightly concave adaxial face
42	Midrib with collateral bundles arranged in an open arch-like with one to five dorsal bundles
43	Midrib with collateral bundles arranged in an open arch-like without dorsal bundles
44	Revolute margin
45	Involute margin

parenchyma. The collateral vascular system presents horseshoe-shaped and two accessory bundles, with fibres in the perivascular region (Fig. 2O). In the adaxial face, adjacent to the main vascular bundle, there are two accessory bundles surrounded by fibres (Fig. 2P).

SECRETORY STRUCTURES

We identified four types of secretory structures with different topology and histological organization distributed in all studied specimens. These were idioblasts, laticifers, colleters and extrafloral nectaries (EFNs).

Table 2. Specimens with missing data in the leaf character matrix and their respective numbers and percentage. Total number of characters coded is 45, and total number of cells in the matrix is 5408 (45 characters × 104 specimens)

Specimens (Voucher)	# missing characters	% missing characters
<i>C. aequatoris</i> (Riina-1434)	6	12
<i>C. bogotanus</i> (Riina-1591)	11	21
<i>C. bonplandianus</i> (Riina-1517)	10	19
<i>C. conduplicatus</i> (Riina-1296)	6	12
<i>C. hibiscifolius</i> (Breteler-3446)	4	8
<i>C. hibiscifolius</i> (Contreras-042)	2	4
<i>C. mutisianus</i> -2 (Barkley-3768)	11	21
<i>C. piptotocalyx</i> (Bortoluzzi-379)	2	4
<i>C. pseudopopulus</i> (Mota-2276)	1	2
<i>C. pseudopopulus</i> (Mota-2284)	1	2
<i>C. pseudopopulus</i> (Mota-2291)	1	2
<i>C. rimbachii</i> (Riina-1402)	3	6
<i>C. rusbyi</i> (Riina-1479)	1	2
<i>C. rusbyi</i> (Riina-1481)	1	2
<i>C. urucurana</i> -1 (Leitão-Filho-1603)	3	6
<i>C. urucurana</i> -1 (Pollito-VA 001)	5	10
<i>C. vulnerarius</i> -1 (Forero-8148)	2	4
Total (entire matrix)	70	1.3

Secretory idioblasts were common in all specimens, and consist of large cells, sometimes strongly stained due to the secretion accumulated inside the vacuole. They were located in the abaxial epidermis (Fig. 3A) and scattered in the mesophyll (Fig. 3B) in most specimens of section *Cyclostigma* and in all specimens of sections *Adenophylli* and *Sampatik*. In section *Cupreati*, idioblasts were present both in the abaxial epidermis and palisade parenchyma (Fig. 3C), and present only in the palisade parenchyma (Fig. 3D) in *C. pseudopopulus* Baill., *C. floccosus*, Riina-1416 and *C. urucurana*-1. Some idioblasts were situated on the stipe of trichomes (Fig. 3E) in some specimens from all sampled sections, but the presence of these structures in specimens with stipitate trichomes did not show a consistent pattern within species.

The shoot meristems of all specimens examined presented laticifers dispersed in ground tissues of leaves, which exhibited a 'Y' branching pattern (Fig. 3F–H) and evident secretory activity. These laticifers appear to be non-articulated, although the anatomical preparations from the herbarium material used did not allow a clear enough visualization of the pattern of cellular division to be totally confident about the laticifer type.

Colleters occurred exclusively along the leaf margin of all analysed specimens, and they were of the standard type (Fig. 3I), non-vascularized, with a secretory epidermis with a single-layered, radially arranged palisade and a thin cuticle. They have a slightly constricted base and a central axis composed by fundamental parenchyma where secretory idioblasts, druse crystals and laticifers can be present. In fully

expanded and mature leaves, colleters eventually fall off with age and they are regarded as deciduous.

Extrafloral nectaries were also present in all specimens. They were located either at the base of the leaf blade (basilaminar) (Fig. 3J) or at the distal portion of the petiole (acropetiole) (Fig. 3K, L). Acropetiole or basilaminar EFNs were usually in one pair. There were EFNs dispersed on the blade (Fig. 3M) and along the leaf margin (Fig. 3N, inset) only in *C. piptotocalyx* (section *Sampatik*). The only specimens in section *Cyclostigma* with more than two acropetiole and basilaminar EFNs were *C. redolens* Pittier, *C. pseudopopulus* and *medusae*-1 (Carvalho-3789) (Fig. 3O, P). EFNs varied among and within species from sessile (Fig. 3L–O) to stipitate (Fig. 3J, K, P), and their secretory surface can be concave (Fig. 3Q, R), convex (Fig. 3S) or flat (Fig. 3T). *Croton piptotocalyx* was the only species with EFNs scattered over the abaxial side of the blade near the margins (Fig. 3M), the apex of the petiole and alternating with colleters along the margins (Fig. 3N). Basilaminar and acropetiole EFNs were similar in their structure and were present in all species sampled (Fig. 3Q–T). They consist of a single-layered palisade secretory epidermis, a dense protoplast and a smooth and thick cuticle. Underlying the secretory epidermis, there were ten to 12 layers of nectariferous parenchyma of isodiametric cells with dense cytoplasm, containing druse crystals, secretory idioblasts and laticifers. EFNs were vascularized by bundles originating from the blade and/or the petiole and consisting of both xylem and phloem, but with the latter being more abundant (Fig. 3Q, T).

Figure 1. Diversity of non-glandular trichomes in *Croton*. A, B. Lepidote trichomes; A. *C. cupreatus*; B. *C. urucurana*-1; C, D. Dendritic trichomes in *C. vulnerarius*; E, F. Rosulate trichomes in *C. floccosus*; G, H. Fasciculate cushion-shaped in *C. speciosus*; I. Stipitate-stellate trichomes in *C. alchorneicarpus*; J. Stipitate-stellate porrect trichomes in *C. bonplandianus*; K. Appressed-stellate porrect trichomes in *C. redolens*; K, L. Appressed-stellate trichomes in *C. piptocalyx*; M, N. Multiradiate trichomes in *C. hibiscifolius*; O. Simple trichomes in *C. coriaceus*. Bars: 60 μ m (L); 100 μ m (E, G, I, M, O); 150 μ m (F, H); 200 μ m (J, K); 300 μ m (A–C, N); 500 μ m (D).

CLUSTER ANALYSIS OF ANATOMICAL CHARACTERS

The topologies of dendrograms obtained using the Dice-Sorensen or Jaccard similarity indices were identical in their branching and clustering pattern, and we will use the dendrogram based on the widely used Jaccard's similarity index (Fig. 4) for describing and discussing our main results below. The cluster analysis performed on the 45 characters and 104 specimens examined yielded 44 groups (Fig. 4). These groups were established using similarity values greater or equal to 0.8 ($J \geq 0.8$) based on their anatomical similarities (a value of 1 indicates 100% similarity among specimens). We assigned the most appropriate specific taxonomic identity integrating anatomical similarity, shown in the cluster analysis, with information on external morphology, ecological knowledge and geographical data. Thus, of the 44 determined and/or confirmed species, 36 in fact belong to section *Cyclostigma*, and only six to section *Adenophylli*, one to section *Sampatik* and one to section *Cupreati* (Fig. 4). Some specimens belonging to the same species of *Cyclostigma* did not group together (e.g. *Croton draco*, *C. coriaceus*, *C. vulnerarius*), so we assigned them a serial number (e.g. *draco*-1, *draco*-2). To better explain the overall similarity pattern among specimens, we divided the dendrogram from its base to the tips according to the beginning of the main clusters at different levels. This resulted in three major levels (L1, L2, L3) indicated by dotted vertical lines (Fig. 4). These levels correspond to 0.57, 0.60 and 0.66 similarity values, respectively.

Specimens of sections *Sampatik*, *Cupreati* and a group of *Adenophylli* (*C. abutilifolius*, *C. gracilipes*) appear more similar anatomically to section *Cyclostigma* than specimens of *Adenophylli* g1 and g2. However, in contrast with the position of the *Sampatik* and *Cupreati* clusters, the two species of *Adenophylli* are nested well in the *Cyclostigma* group (Fig. 4). Thirteen specimens, marked with a star (Fig. 4), could not be assigned to any known species, but they all have morphological affinities to members of section *Cyclostigma*. They represent about 11 undescribed species of section *Cyclostigma*. Some

of these undetermined specimens, which appear in four different separate clusters in the dendrogram, were assigned to an informal taxonomic group (the 'medusae' group) based on leaf anatomy and external morphology (Fig. 4).

Subgroups g1 and g2 in *Adenophylli* were further divided in four subgroups (g6, g7, g8 and g9) based on characteristics, such as acropetiolar EFNs (g6: *C. conduplicatus*), basilaminar EFNs and appressed-stellate trichomes (g7: *C. bonplandianus* Baill.), appressed-stellate porrect trichomes and absence of associated bundles in the midrib (g8: *C. aequatoris*), and presence of one to five associated bundles in the midrib (g9: *C. ruizianus*). The other group of section *Adenophylli* (*C. abutilifolius*, *C. gracilipes*) and most species of section *Cyclostigma* were clustered together essentially because the presence of secretory idioblasts on the abaxial epidermis and stipitate-stellate porrect trichomes (Fig. 4).

DISCUSSION

SIGNIFICANCE OF LEAF ANATOMICAL FEATURES FOR *CROTON* SYSTEMATICS

Anatomical characters were useful for the taxonomic identification of 36 species of section *Cyclostigma* and for the detection of either intraspecific variation or wrongly identified specimens. Among the character states exclusive to section *Cyclostigma* and useful to separate species, were the presence of hypodermis, fasciculate cushion-shaped trichomes (both present in five species), dendritic trichomes (restricted to two species) and the absence of nectaries from leaf margin and blade (in all species of section *Cyclostigma*). On the other hand, some characters states (presence of hypostomatic leaves, paracytic stomata, dorsiventral mesophyll, collateral bundles, druse crystals, basilaminar/acropetiolar extrafloral nectaries, colleters, non-articulated laticifers and stellate trichomes) were common to all *Croton* spp. sampled. Several studies, focused on one or a couple of species of section *Cyclostigma*, have also reported

Figure 2. General anatomical features. A–C. Frontal view; D–P. Cross-sections; A. Abaxial epidermis with sinuous anticlinal walls in *C. celtidifolius*; B. Detail of abaxial epidermis, showing paracytic stomata and cuticle with attenuated stretch in *C. macrobothrys*; C. Adaxial epidermis with straight to slightly sinuous walls in *C. macrobothrys*; D. Dorsiventral mesophyll with unistratified epidermis in *C. amentiformis*, note: hypodermis (arrowhead), bundle-sheath extension (arrow); E. Dorsiventral mesophyll with collateral bundles (arrow) in *C. ruizianus*; F. Druse crystals under polarized light in the longitudinal section of petiole in *C. alchorneicarpus*; G, H, I. Leaf margin, G. Revolute in *C. conduplicatus*, note inset in *C. amentiformis*; H. Involute in *C. bogotanus*, Arrowhead: continuous palisade parenchyma; I. Discontinuous palisade parenchyma in *C. lechleri*; J. Biconvex midrib in *C. lechleri*; K. Midrib with flat to slightly concave adaxial side in *C. conduplicatus*; L. Vascular system of midrib with dorsal bundles in *C. vulnerarius*; M. Vascular system of midrib without dorsal bundles in *C. aequatoris*; N, O, P. Petiole of *C. charaguisis*; N. General view of petiole with round shape, horseshoe-shaped vascular system and two accessory bundles (arrows); O. Detail of petiole showing epidermis, cortical region and part of vascular system; P. Detail of adaxial side of petiole with two accessory bundles. Pp: palisade parenchyma, Sp: spongy parenchyma, Bars: 100 μ m (B); 150 μ m (D, E, F, I); 200 μ m (A, C); 250 μ m (M); 300 μ m (G, H, K, L, O); 600 μ m (insets Fig. G and H, P); 700 μ m (J); 800 μ m (N).

these characters (Rudall, 1994; Webster *et al.*, 1996; Sá-Haiad *et al.*, 2009; Vitarelli *et al.*, 2015; Feio, Riina & Meira, 2016).

We identified ten types of non-glandular trichomes and confirmed that section *Cyclostigma* presented all types described by Webster *et al.* (1996) plus two additional types (fasciculate cushion-shaped and appressed-stellate porrect trichomes), which are here described for the first time in *Croton*. Previous research about comparative anatomy of *Croton* (Webster *et al.*, 1996; Senakun & Chantaranothai, 2010; Liu, Deng & Liao, 2013) or *Crotoneae* (Sá-Haiad *et al.*, 2009; Vitarelli *et al.*, 2015) showed that trichome types are useful for taxonomy mostly at the species level. On the other hand, we noted trichome morphology seems to be plastic among the studied species, sometimes even within the same individual. Moreover, we demonstrated that simple trichomes may sometimes correspond to immature stages of a more complex type. Thus, trichome characters should be used with caution for taxonomic purposes in *Cyclostigma*, and potentially in all other sections of *Croton*.

In addition, the absence of lepidote trichomes has been used as a character state to distinguish section *Cyclostigma* from other clades of *Croton* such as sections *Cupreati*, *Lasiogyne* (Klotzsch) Baill. and *Lamprocroton* (Müll.Arg.) Pax in Engl. & Prantl (van Ee *et al.*, 2011). However, such trichomes were recently reported in *C. macrobothrys* Baill. and *C. urucurana* (Sá-Haiad *et al.*, 2009; Caruzo *et al.*, 2016; Feio, 2016). Here we confirmed that, indeed, all species of section *Cyclostigma* studied here typically lack these trichomes, except for two specimens of *C. urucurana* (Pollito-VA-001 and Leitão-Filho-1603), and their presence may vary between populations. Thus, we recommend that these two species be confirmed with additional sampling, given that both taxa have rather large geographical ranges in the Brazilian Atlantic Rain Forest (Caruzo *et al.*, 2016; Santos, Riina & Caruzo, 2017).

The presence of a hypodermis in six of the 36 species of section *Cyclostigma* examined and in *C. cupreatus* is a new report for *Croton*. Species of section *Cyclostigma* occur along mountain creeks, edges and gaps of forests, river sides and along roads (Riina *et al.*, 2009; van Ee *et al.*, 2011). Despite the high humidity present in

some of these habitats (e.g. cloud forest, river sides), these *Croton* spp. are exposed to high luminosity and the presence of a hypodermis is clearly advantageous. In fact, all species with a hypodermis occur in montane forest at high elevation in the Andes, but they are not the only species in section *Cyclostigma* occupying these habitat types.

SYSTEMATIC IMPLICATIONS OF SECRETORY STRUCTURES

Previous studies of *Crotoneae* have reported idioblasts producing only lipophilic secretion (e.g. Sá-Haiad *et al.*, 2009; Vitarelli *et al.*, 2015). However, in a detailed study of two species of section *Cyclostigma*, Feio *et al.* (2016) also detected polysaccharides, phenolic compounds, alkaloids and proteins, suggesting a richer and more complex nature of the secretion of idioblasts in members of that section.

Vitarelli *et al.* (2015) described articulated laticifers in leaves and shoots apices in most species of *Crotoneae* they analysed [i.e. *Croton* sections *Cleodora* (Klotzsch) Baill. (12 species) and *Lamprocroton* (Müll.Arg.) Pax (11 species) and the related genera *Astraea* Klotzsch (one species) and *Brasiliocroton* P.E.Berry & Cordeiro (one species)] and suggested that the articulated type might be widespread in subfamily *Crotonoideae*. In contrast, non-articulated laticifers were reported by Rudall (1994) for seven *Croton* spp. (*C. antisiphiliticus* Mart. ex Müll.Arg., *C. conduplicatus*, *C. heteropleurus* Urb., *C. megalobotrys* Müll.Arg., *C. occidentalis* Müll. Arg., *C. sagraeanus* Müll.Arg., *C. sylvaticus* Hochst. and four undetermined species) belonging to three different clades of *Croton* [sections *Geiseleria* (A.Gray) Baill. and *Adenophylli* and the Old World *Croton* clade]. Non-articulated laticifers were also found in wood rays of section *Cyclostigma* (Wiedenhoeft *et al.*, 2009) and additionally in leaves and flowers of two representatives of this section, *C. echinocarpus* and *C. urucurana* (Feio *et al.*, 2016). This evidence and our own results suggest that both types of laticifers are probably present in the subfamily, although additional studies including a comprehensive taxon sampling is needed to determine which type is more common in *Crotonoideae*.

Figure 3. Diversity of secretory structures in *Croton*. A–E. Secretory idioblasts (black arrows); F–H. Laticifers (white arrows); I. Colleter; J–T. Extrafloral nectaries (EFN); A. Idioblasts in the abaxial epidermis in *C. alchorneicarpus*; B. Idioblasts scattered in the mesophyll in *C. rimbachii*; C. Idioblasts in the epidermis and palisade parenchyma in *C. cupreatus*; D. Idioblasts in the palisade parenchyma in *C. pseudopopulus*; E. idioblasts situated at the base of the trichomes in *C. lechleri*; F–H. Non-articulated branched laticifers in the shoot apical meristem (white arrows); F. *C. amentiformis*; G, H. *C. celtidifolius*; I. Non-vascularized colleter of the standard type in *C. bogotanus*; J. Stipitate EFN basilar in *C. macrobothrys*; K, L. EFN acropetiole; K. Stipitate EFN in *C. celtidifolius*; L. Sessile EFN in *Riina-1520*; M. Dispersed EFN on the blade in *C. piptocalyx*; N. Sessile EFN on the leaf margin in *C. piptocalyx*; O, P. EFN in both acropetiole and basilar; O. Sessile EFN in *C. redolens*; P. Stipitate EFN in *C. pseudopopulus*; Q, R. EFN with concave surface in *Riina-1592* and *C. aequatoris*, respectively; S. EFN with convex surface in *C. gossypifolius*; T. EFN with flat surface in *C. cupreatus*. Bars: 50 µm (G, H); 150 µm (A–E); 250 µm (F, I); 300 µm (R); 800 µm (Q, S, T); 0.5 mm (M–O); 1 mm (J–L, P).

Figure 4. Dendrogram of the 104 specimens analysed using the Jaccard's similarity coefficient. Taxonomic identities assigned to each group are next to specimens. *Note:* specimens without species name belong to *Croton* section *Cyclostigma* (star). Labels L1–3 and g1–14 correspond to levels of similarity and groups (see text).

Colleters have been previously reported in vegetative (leaves) and reproductive organs in three species of section *Cyclostigma* (Riina *et al.*, 2015; Feio *et al.*, 2016) and representatives of four other *Croton* sections, namely *C. glandulosus* L. (section *Geisleria*) (Machado *et al.*, 2015) and sections *Alabamenses* B.W.van Ee, *Lamprocroton*, and *Cleodora* (Vitarelli *et al.*, 2015). This last study also reported colleters in two other genera, *Brasiliocroton* and *Astraea*, which are closely related to *Croton*. There is no consensus about the importance of vascularization in colleters. Carlquist (1969) pointed out that the presence of vascularization is directly related to the size of a structure and not necessarily to its state of development. On the other hand, Thomas (1991) suggested that the vascularization of colleters was more related to the derivation or not of vascular bundles from the organs to which colleters are attached, regardless the size of the structure (Arekal & Ramakrishna, 1980; Appezzato-da-Glória & Estelita, 2000). In all studied species, colleters on leaves were non-vascularized and sometimes deciduous (on mature leaves). Deciduous, non-vascularized colleters have been previously reported on the leaves of other *Croton* spp. (Riina *et al.*, 2015; Vitarelli *et al.*, 2015; Feio *et al.*, 2016). Colleter-deciduousness on mature leaves could be related to the lack of a continuous nourishment in these secretory structures, but this hypothesis needs further investigation.

Structural variation and location of extrafloral nectaries were useful features distinguishing group of species at different levels in the cluster analysis. For example, species of section *Adenophylli* were separated in three subgroups: one group with sessile EFNs and convex surface (g1); one with stipitate EFNs and concave surface (g2) and one (*C. abutilifolius*, *C. gracilipes*) clustering with members of section *Cyclostigma*. The presence of nectaries scattered on the blade and along the leaf margin (alternating with colleters) confirmed the classification of Riina *et al.* (2009), which removed *C. piptocalyx* from section *Cyclostigma sensu* Webster (1993).

DELIMITATION OF GROUPS AND TAXONOMIC IMPLICATIONS

Specimens of sections *Sampatik*, *Cupreati* and a group of *Adenophylli* (*C. abutilifolius*, *C. gracilipes*) appear more similar anatomically to section *Cyclostigma* than specimens of *Adenophylli* g1 and g2. This gives an indication of the homoplasious nature of the anatomical characters used in this study, which is similar to the pattern found with external morphological characters (Riina *et al.*, 2009). The morphoanatomical characters alone will not be enough to separate groups at the sectional level in the genus *Croton*. However, our results show that the use of anatomical characters could facilitate taxonomic delimitation at the species

level in sections of *Croton*, and it is possible that using anatomical and morphological characters in combination could be even more useful for establishing species limits. There is a recent study establishing species limits in *Croton* occurring in a region of the northern Andes (Luján, León & Riina, 2015), but these authors only analysed vegetative morphological characters.

Specimens *Soto-414* and *Soto-430* are from the same locality in Bolivia. They are morphologically identical and were initially identified based on external morphology as *C. aff. urucurana*. These two specimens clustered with *Riina-1498*, which was initially identified as *C. tyndaridum* Croizat. It is possible that these three specimens belong to the same species given their high similarity (100%) in anatomical characters. *Riina-1498* is problematic because it comes from a cultivated plant in the city of Lima (Peru) and there were no records of its provenance, and its identification as *C. tyndaridum* was always questionable, since *C. tyndaridum* is only known from the type locality (Rio Perené, Junín, Peru). Because *Soto-414* and *Soto-430* did not group with any of the *C. urucurana* groups, and the uncertainty regarding the identity and origin of *Riina-1498*, we did not assign any name to this group and consider it as an unknown and probably undescribed species.

Croton draco-1 (Berry-7595), despite being originally identified as *C. draco* based on morphology, clustered with *C. rusbyi* Britton ex Rusby and not with *C. draco-2* (Marquez-558 and Martínez-6054). *Croton draco-1* has a bundle-sheath extension, dendritic trichomes and revolute margin, which are features absent in *C. draco-2*. *Croton draco* is another widespread and variable species occurring from Mexico to Panama (Webster & Burch, 1968; Webster & Huft, 1988) and, given its wide geographical range, it would have been desirable to have a better sampling of it. Our results show that there might be at least two morphotypes that could be easily separated by anatomical characters. This group obviously merits further investigation using anatomy, morphology and molecular data and a better sampling across its entire range.

Croton mutisianus-1 and *C. mutisianus-2* also did not cluster together as expected. Instead, *C. mutisianus-2* clustered with *C. quadrisetosus* Lam. It is possible that this grouping is an artefact since *C. mutisianus-2* has the highest percentage of missing data (21%) in our matrix. In fact, *C. mutisianus-2* (Barkley-3768) was a poor sample and it was not possible to obtain information about EFN and stem apex to characterize the type of laticifers. External morphology does not support this grouping either, since *C. mutisianus-2* and *C. quadrisetosus* have many differences in floral morphology (e.g. flowers with long pedicels, and stigmas bifid and terete in *C. mutisianus* vs. flowers sessile to subsessile and stigmas multifid

with expanded and flattened tips in *C. quadrisetosus*). In addition, *C. mutisianus* is widespread in montane forests of Colombia, and *C. quadrisetosus* is only known from montane forests of Peru (Berry *et al.*, 2015).

Unexpected grouping patterns, similar to those discussed above, were found for specimens previously identified using external morphology as *C. celtidifolius*, *C. vulnerarius* and *C. coriaceus*. Specimens from these three species show relatively low levels of similarity in anatomical characters appearing in separate groups of the dendrogram. It is possible that in some cases the species are indeed more polymorphic in relation to anatomical characters than to characters from external morphology. However, the limited sampling prevents us from using anatomical data alone to further subdivide such species, especially because the external morphology does not support the observed clustering pattern based on anatomy. All these taxa need further investigation using a broader sampling across their geographical range and integration of anatomy and morphology in the evaluation of species limits.

Our study provides additional characters that can contribute to clarify the taxonomic affinities of species in section *Cyclostigma*, and it gives support to the taxonomic position of the section adopted by Riina *et al.* (2009) and van Ee *et al.* (2011). As with external morphology (Riina *et al.*, 2009), section *Cyclostigma* can also be characterized by a suite of anatomical characters and so far only one anatomical wood character (secondary xylem rays containing non-articulated laticifers) has been identified as a possible synapomorphy for the section (Wiedenhoeft *et al.*, 2009).

CONCLUSIONS AND FUTURE WORK

Although we did not find an anatomical character present in all members of section *Cyclostigma*, the use of a suite of characters was useful to group specimens based on their similarities and to confirm and/or to determine taxonomic identities at the species level in most cases. However, we also recognize the limitation of these characters in several taxa for which taxonomic resolution was not achieved. The presence of different types of stellate trichomes, non-articulated laticifers, absence of marginal EFNs and the lack of lepidote trichomes can be used in combination to define section *Cyclostigma* anatomically. Three new anatomical features were described for *Croton*: hypodermis, fasciculate cushion-shaped trichomes and appressed-stellate porrect trichomes. Our results do not fully support the hypothesis of a widespread occurrence of articulated laticifers in Crotonoideae, but future studies for re-evaluating laticifers in this subfamily are necessary and should include analyses of their ontogeny and the nature of their secretions. Finally, we highlight

the partial utility of anatomical characters at the species level and the need of using them along with other characters in an integrative taxonomic framework.

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Appendix 1. List of species with their most recent taxonomic identification (previous to this study) and voucher information. Herbarium acronyms in parentheses according to Thiers (2016).

Species	Specimen number, collector and collection number (herbarium)
<i>Croton</i> section <i>Cyclostigma</i>	
<i>C. alchorneicarpus</i> Croizat	1: Riina 1526 (MICH); 2: Barreto 206 (MG); 3: Riina 1531 (MICH); 4: Occhioni 8208 (MBM)
<i>C. amentiformis</i> Riina	5: Jorgensen 97 (MO)
<i>C. bogotanus</i> Cuatrec.	6: Riina 1591 (MICH)
<i>C. aff. celtidifolius/pseudopopulus</i>	7: Cavalcante 1166 (MG)
<i>C. celtidifolius</i> Baill.	8: Catharino 640 (MG); 9: Riina 1520 (MICH); 10: Riina 1530 (MICH); 11: Shepherd 12205 (MG)
<i>C. charaguensis</i> Standl.	12: Riina 1513 (MICH)
<i>C. churutensis</i> Riina & Cornejo	13: Cornejo 7590 (MICH)
<i>C. coriaceus</i> Kunth	14: Berry 7603 (MICH); 15: Riina 1403 (MICH); 16: Riina 1417 (MICH)
<i>C. aff. coriaceus</i> Kunth	17: Berry 7620 (MICH)
<i>C. draco</i> Schltdl.	18: Berry 7595 (MICH); 19: Marquez 558 (MBM); 20: Martínez 6054 (MBM)
<i>C. echinocarpus</i> Müll.Arg.	21: Riina 1316 (MICH); 22: Riina 1519 (MICH); 23: Batista 01 (VIC)
<i>C. erythrochyloides</i> Croizat	24: Riina 1503 (MICH)
<i>C. fastuosus</i> Baill.	25: Costa s/n (BHCB)
<i>C. floccosus</i> B.A.Sm.	26: Riina 1405 (MICH); 27: Riina 1406 (MICH); 28: Riina 1407 (MICH)
<i>C. glaziovii</i> Müll.Arg.	29: Riina 1521 (MICH)
<i>C. gossypifolius</i> Vahl	30: Riina 1261 (MICH); 31: Riina 1303 (MICH)
<i>C. hibiscifolius</i> Kunth ex Spreng.	32: Breteler 3446 (MG); 33: Contreras 042 (MICH); 34: Riina 1413 (MICH); 35: Riina 1414 (MICH)
<i>C. aff. hibiscifolius</i>	36: Riina 1592 (MA); 37: Orsini 2013-13 (MYF)
<i>C. huberi</i> Steyererm.	38: Riina 1276 (MICH); 39: Steyermark s.n. (MG)
<i>C. lechleri</i> Müll.Arg.	40: Riina 1443 (MICH); 41: Riina 1449 (MICH); 42: Riina 1496 (MICH); 43: Riina 1497 (MICH)
<i>C. aff. lechleri</i> Müll.Arg.	44: Riina 1453 (MICH)
<i>C. macrobothrys</i> Baill.	45: Barros 2014 (MG); 46: Custodio Filho 1905 (MG); 47: Mori s.n. (MG); 48: Riina 1522 (MICH)
<i>C. aff. medusae</i> Müll.Arg.	49: Pirani 4982 (SPF)
<i>C. aff. mutisianus</i> Kunth	50: Riina 1590 (UEN, MA)
<i>C. mutisianus</i> Kunth	51: Barkley 3768 (MBM)
<i>C. perspicuosus</i> Croizat	52: Riina 1435 (MICH); 53: Riina 1441 (MICH); 54: Riina 1444 (MICH)
<i>C. pilulifer</i> Rusby	55: Riina 1500 (MICH); 56: Riina 1508 (MICH)
<i>C. aff. pilulifer</i> Rusby	57: Riina 1461 (MICH); 58: Riina 1462 (MICH); 59: Riina 1483 (MICH)
<i>C. plagiograptus</i> Müll.Arg.	60: Carvalho 3789 (MBM)
<i>C. pseudopopulus</i> Baill.	61: Mota 2276 (VIC); 62: Mota 2284 (VIC); 63: Mota 2291 (VIC)
<i>C. quadrisetosus</i> Lam.	64: Daza 4051 (E)
<i>C. redolens</i> Pittier	65: Riina 1848 (MICH); 66: Riina 1850 (MICH); 67: Webster 23688 (MICH)
<i>C. aff. redolens</i> Pittier	68: Webster 23620 (MICH)
<i>C. rimbachii</i> Croizat	69: Riina 1402 (MICH); 70: Riina 1422 (MICH); 71: Riina 1440 (MICH)
<i>C. rusbyi</i> Britton ex Rusby	72: Riina 1479 (MICH); 73: Riina 1481 (MICH)
<i>C. sp1</i>	74: Daza 4572 (E)
<i>C. sp2</i>	75: Riina 1426 (MICH)
<i>C. sp3</i>	76: Riina 1416 (MICH)
<i>C. speciosus</i> Müll.Arg.	77: Berry 7590 (MICH); 78: Riina 1262 (MICH); 79: Riina 1278 (MICH)
<i>C. tyndaridum</i> Croizat	80: Riina 1498 (MICH)
<i>C. urucurana</i> Baill.	81: Heringer 18473 (MG); 82: Leitão-Filho-1603 (MG); 83: Pollito-VA-001 (MG)
<i>C. aff. urucurana</i> Baill.	84: Cordeiro 3325 (SP); 85: Soto 430 (USZ)
<i>C. aff. urucurana/lechleri</i>	86: Soto 414 (USZ)
<i>C. vulnerarius</i> Baill.	87: Cordeiro 345 (MG); 88: Forero 8148 (MG); 89: Saran 07 (MG); 90: Silva 1242 (VIC)

Appendix 1. *Continued*

Species	Specimen number, collector and collection number (herbarium)
<i>Croton</i> section <i>Adenophylli</i>	
<i>C. abutilifolius</i> Croizat	91: <i>Riina</i> 1505 (MA)
<i>C. aequatoris</i> Croizat	92: <i>Riina</i> 1434 (MA)
<i>C. bonplandianus</i> Baill.	93: <i>Riina</i> 1517 (LPB)
<i>C. conduplicatus</i> Kunth	94: <i>Riina</i> 1266 (VEN); 95: <i>Riina</i> 1302 (VEN); 96: <i>Riina</i> 1296 (MICH); 97: <i>Riina</i> 1837 (MA)
<i>C. gracilipes</i> Baill.	98: <i>Riina</i> 1501 (MA)
<i>C. ruizianus</i> Müll.Arg.	99: <i>Riina</i> 1386 (MICH); 100: <i>Riina</i> 1486 (MICH); 101: <i>Riina</i> 1487 (MICH)
<i>Croton</i> section <i>Sampatiki</i>	
<i>C. piptocalyx</i> Müll. Arg.	102: <i>Bortoluzzi</i> 379 (VIC); 103: <i>Bortoluzzi</i> 381 (VIC)
<i>Croton</i> section <i>Cupreati</i>	
<i>C. cupreatus</i> Croizat	104: <i>Riina</i> 1408 (MA)

Specimen Anatomical characters

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Appendix 2. Continued

Specimen	Anatomical characters																																																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45				
40	1	0	0	1	1	1	0	1	0	0	0	1	0	0	1	0	0	1	0	0	0	1	1	0	0	0	0	1	1	0	0	0	1	1	0	1	1	0	1	1	0	0	1	0	0	1			
41	1	0	0	1	0	1	0	1	0	0	0	1	0	0	1	0	0	1	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0	1	1	0	1	1	0	1	0	1	0	0		
42	1	0	0	1	0	1	0	1	0	0	0	1	0	0	1	0	0	1	0	0	0	1	1	0	0	0	0	1	1	0	0	0	0	1	1	0	1	1	0	1	1	0	1	0	1	0	0		
43	1	0	0	1	0	1	0	1	0	0	0	1	0	0	1	0	0	1	0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	1	1	0	1	1	0	1	1	0	1	0	1	0	0		
44	1	0	0	1	0	1	0	0	1	0	0	1	0	0	1	0	0	1	0	0	0	1	1	0	0	0	0	0	1	1	0	0	0	1	1	0	1	1	0	1	1	0	1	0	1	0	0		
45	1	0	0	1	1	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	1	1	0	0	0	1	1	0	1	1	0	1	1	0	1	0	1	0	0		
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47	1	0	0	1	1	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	1	1	0	1	1	0	1	1	0	1	0	1	0	0		
48	1	0	0	1	1	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	1	1	0	1	1	0	1	1	0	1	0	1	0	0		
49	1	0	0	1	1	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	1	1	0	1	1	0	1	1	0	1	0	1	0	0		
50	1	1	0	1	1	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	1	1	0	1	0	1	0	0	1	0		
51	1	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
52	1	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0	0	1	1	0	1	1	0	1	1	0	1	0	1	0	?	?	
53	1	0	0	1	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	1	1	0	1	1	0	1	0	1	0	0	
54	1	0	0	1	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	1	1	0	1	1	0	1	0	1	0	0	
55	1	0	0	1	1	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	1	1	0	1	1	0	1	0	1	0	0	
56	1	0	0	1	1	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	1	1	0	1	1	0	1	0	1	0	0	
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59	1	0	0	1	1	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	1	1	0	1	1	0	1	0	1	0	0	
60	1	0	0	1	1	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	1	1	0	1	1	0	1	0	1	0	0	
61	1	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
62	1	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
63	1	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
64	1	0	0	1	1	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	1	1	0	1	1	0	1	0	1	0	0	
65	1	0	1	1	1	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	1	1	0	1	1	0	1	0	1	0	0	0
66	1	0	1	1	1	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	1	1	0	1	1	0	1	0	0	0	0	
67	1	0	1	1	1	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	1	1	0	1	1	0	1	0	0	0	0	
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69	1	1	0	1	1	1	0	0	1	0	0	0	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
70	1	1	0	1	1	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	1	1	0	1	1	0	1	0	1	0	0	
71	1	1	0	1	1	0	0	1	0	0	0	0	1	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	1	1	0	1	1	0	1	0	1	0	0	
72	1	0	0	1	1	1	0	0	1	0	0	0	1	0	1	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
73	1	0	0	1	1	1	0	0	1	0	0	0	1	0	1	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	
74	1	0	0	1	1	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	1	1	0	0	0	0	1	1	0	1	1	0	1	1	0	1	0	0	0		
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76	1	1	0	1	0	1	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	0	0	0	0	1	0	1	1	0	1	1	0	1	0	0	0	0	

Appendix 2. *Continued*

Specimen	Anatomical characters																																														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45		
777	1	0	0	1	0	1	0	0	1	0	0	0	1	0	1	0	0	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	0	1	0	1	0		
778	1	0	0	1	0	1	0	0	1	0	0	0	1	0	1	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	0	1	0	1	0		
779	1	0	0	1	0	1	0	0	1	0	0	0	1	0	1	0	0	0	0	1	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	0	1	0	1	0		
780	1	0	1	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	0	1	0	0	0		
781	1	0	1	1	1	0	0	1	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	0	1	0	0		
782	1	0	0	1	1	?	0	0	1	0	0	?	?	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	1	0	1	0			
783	1	0	0	1	1	?	0	0	1	0	0	?	?	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	1	0	0	0			
784	1	0	1	1	0	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	1	0	0	0		
785	1	0	1	1	1	0	1	0	0	0	0	0	1	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	0	1	0	0	0		
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787	1	0	1	1	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	0	1	0	0	0		
788	1	0	1	1	?	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	1	?	1	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	1	1	0	1	0	1	0	
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792	?	0	1	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	1	0		
793	0	0	?	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
794	1	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
795	1	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
796	?	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?	?
797	1	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
798	1	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
799	1	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
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1010	1	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
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1030	1	0	0	0	0	1	0	0	1	1	1	0	1	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1040	1	1	0	1	1	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0