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Original article

Extrafloral nectaries of an Atlantic Forest conservation area in Southeastern Brazil

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ABSTRACT

Although extrafloral nectaries (EFNs) are reported as extremely important to understand ecological interactions, the diversity of EFN-bearing plants remains underappreciated. In Brazil, studies are concentrated in Cerrado and extremely poorly known in other phytogeographic domains, such as Atlantic forest. Thus, this study provides one of the first checklists of angiosperms with EFNs in a protected area of the Brazilian Atlantic forest, bringing information about the richness, location of EFNs on the plant's organs, as well as plant's habit and conservation status. A total of 93 EFN-bearing species belonging to 61 genera and 29 families is reported, which corresponds to 16.5 % of the local flora. The vast majority has not been evaluated for their conservation status, and twenty species are endemic to the Brazilian Atlantic forest. Vines and trees are the predominant plant habits and EFNs are mostly found on leaves. More than half of the species (53.7 %) comprises new additions to the world list of EFN-bearing plants, including the first record in Dilleniaceae. Our findings show a large number of EFN-bearing plant species from a single and small protected area in the Brazilian Atlantic forest. It reinforces the lack of studies of this nature, where further investigations are strongly recommended.

Keywords: ant-plant interactions, checklist, nectar, plant morphology, taxonomy, 'restingas'

Introduction

Nectaries are plant structures where the nectar, an aqueous solution compounded by sugar, water, and amino acids, is produced, secreted, and offered to interacting animals (Pacini *et al.* 2003; Heil, 2011; Nepi *et al.* 2018). Nectaries are usually classified based on their location on the plant as well as on the ecological role they play. For instance, floral nectaries are found on flowers and generally have a nuptial function (*i.e.*, pollination), while extrafloral nectaries (hereafter EFNs) occur on other organs aboveground (Elias 1983) and are unrelated to pollination (Delpino 1874; Bentley 1977a; b; Heil 2011). EFNs can be found in different lineages of angiosperms with high diversity in

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their phylogenetic and biogeographic distribution, plant location, morphology, and ecology (Marazzi *et al.* 2013).

For taxonomic and floristics purposes, the presence of EFN-bearing plants works as a reliable feature to determine the composition and the functional diversity of biological communities. Furthermore, EFNs also provide important ecological information once it highlights putative local inter- or intraspecific interactions between organisms (Heil 2011), which are primordial for modeling conservation strategies (Wang *et al.* 2015).

However, EFN-bearing plants richness is weakly reported and likely remains underappreciated (Marazzi *et al.* 2013), once most of the detailed studies are focused on animal-plant ecology (Almeida & Figueiredo 2003; Schoereder *et al.* 2010; Lay *et al.* 2011; Lange *et al.* 2013; Câmara *et al.* 2017), morphology and anatomy (Machado *et al.* 2008; Melo *et al.* 2010a; b; Aguirre *et al.* 2013; Pires *et al.* 2017), phylogeny (Weber & Keller 2013), or phylogeography (Schupp & Feener 1991; Del-Claro *et al.* 2013; Krosnick *et al.* 2013; Marazzi *et al.* 2013; Weber & Keeler 2013). Nevertheless, extremely important, broader, and thorough ecological questions are strictly dependent on a solid floristic database and a correct taxonomic determination; otherwise, the answer proposed in such studies might not be validated. In this sense, knowing the geographical distribution of plants with EFNs in different vegetation types can provide a backbone that validates and might allow further ecological, evolutionary, and conservation studies.

EFNs can be widely found on plants in tropical and temperate zones, but available information is more commonly cited for the former (Oliveira & Leitão-Filho 1987; Morellato & Oliveira 1991; Koptur, 1992; Fiala & Lisenmair 1995; Rico-Gray & Oliveira 2007; Schoereder *et al.* 2010). In tropical regions, plants bearing EFNs are remarkably rich and well distributed (Oliveira & Leitão-Filho 1987; Koptur 1992; Rico-Gray & Oliveira 2007; Schoereder *et al.* 2010; Weber & Keeler 2013). Nevertheless, in Brazil, most of EFNs records are given from Cerrado areas, where anatomical and ecological studies were vastly performed (Oliveira & Leitão-Filho 1987; Oliveira & Pie 1998; Del-Claro & Santos 2000; Santos & Del-Claro 2001; Machado *et al.* 2008; Nascimento & Del-Claro 2010; Schoereder *et al.* 2010; Anjos *et al.* 2017; Pires *et al.* 2017).

Even though plants bearing EFNs comprises a considerable fraction of plant species in tropical ecosystems (Schupp & Feener 1991; Fiala & Linsenmair 1995; Morellato & Oliveira 1991; 1994; Blüthgen *et al.* 2000), few studies surveyed EFN-bearing plants distribution in the Amazonian forest (Morellato & Oliveira 1991; 1994; Blüthgen *et al.* 2000; Nogueira *et al.* 2020), Caatinga (*e.g.* Melo *et al.* 2010a; b), and Atlantic forest (*e.g.* Almeida & Figueiredo 2003; Câmara *et al.* 2017; Dutra 2019), a hotspot for conservation priorities (Myers *et al.* 2000). Under this point of view, this study focused on providing one of the first checklists of angiosperms with EFNs in a protected area of the Atlantic forest, bringing information about the taxonomic richness, their location on the plant's organs as well as plant's habit and conservation status.

Material and methods

The study was carried out in the State Park of Itaúnas (PEI), managed by 'Instituto Estadual de Meio Ambiente e Recursos Hídricos' (IEMA), located in the municipality of Conceição da Barra, on the shore region of northern Espírito Santo State, Brazil (Fig. 1). It has an area of approximately 3,500 ha, mean annual temperature ranges between 21.7º-26.7ºC, total annual precipitation of approximately 1,310 mm, and Köppen's classification 'Aw' climate with rainy months during summer (Souza *et al.* 2016). Most vegetation coverage is represented by 'restingas' (vegetation on sandy soils) and dunes, however, wetlands, 'Tabuleiro' forest (flat relief and soil from the tertiary formation), and mangroves are also recorded.

The list herein provided is based on the previous angiosperm checklist published by Souza *et al.* (2016). Vouchers were analyzed by observation of EFNs in order to investigate the taxonomic richness of EFN-bearing plants in the study area. In addition, field expeditions for collecting fresh material were performed from July/2017 to August/2019. Only species with visible secreting structures or secretions visited and defended by ants (not sampled) were included and, following Elias (1983) and Delpino (1874), both extrafloral and extranuptial nectaries were considered. All fertile specimens were observed, collected, photographed, and processed according to traditional methods in plant taxonomy (Peixoto & Maia 2013) for further incorporation in the Federal University of Espírito Santo herbarium, VIES (acronym follows Thiers (2021)). All species with EFNs were thoroughly analyzed regarding characteristics such as plant habit, EFN location, and conservation status. After analyzing all samples, a careful literature search aiming to bring more information about EFN-bearing species was carried out. The World List of plants with EFNs (Weber *et al.* 2015) was consulted and the list of specimen vouchers can be found in Table 1.

Family names followed the Angiosperm Phylogeny Group (APG IV (2016)) with some exceptions such as Passifloraceae and Turneraceae, herein recognized as different families. Author names follow the International Plant Names Index (IPNI), and conservation status was established according to the Centro Nacional de Conservação da Flora - CNCFlora (2021).

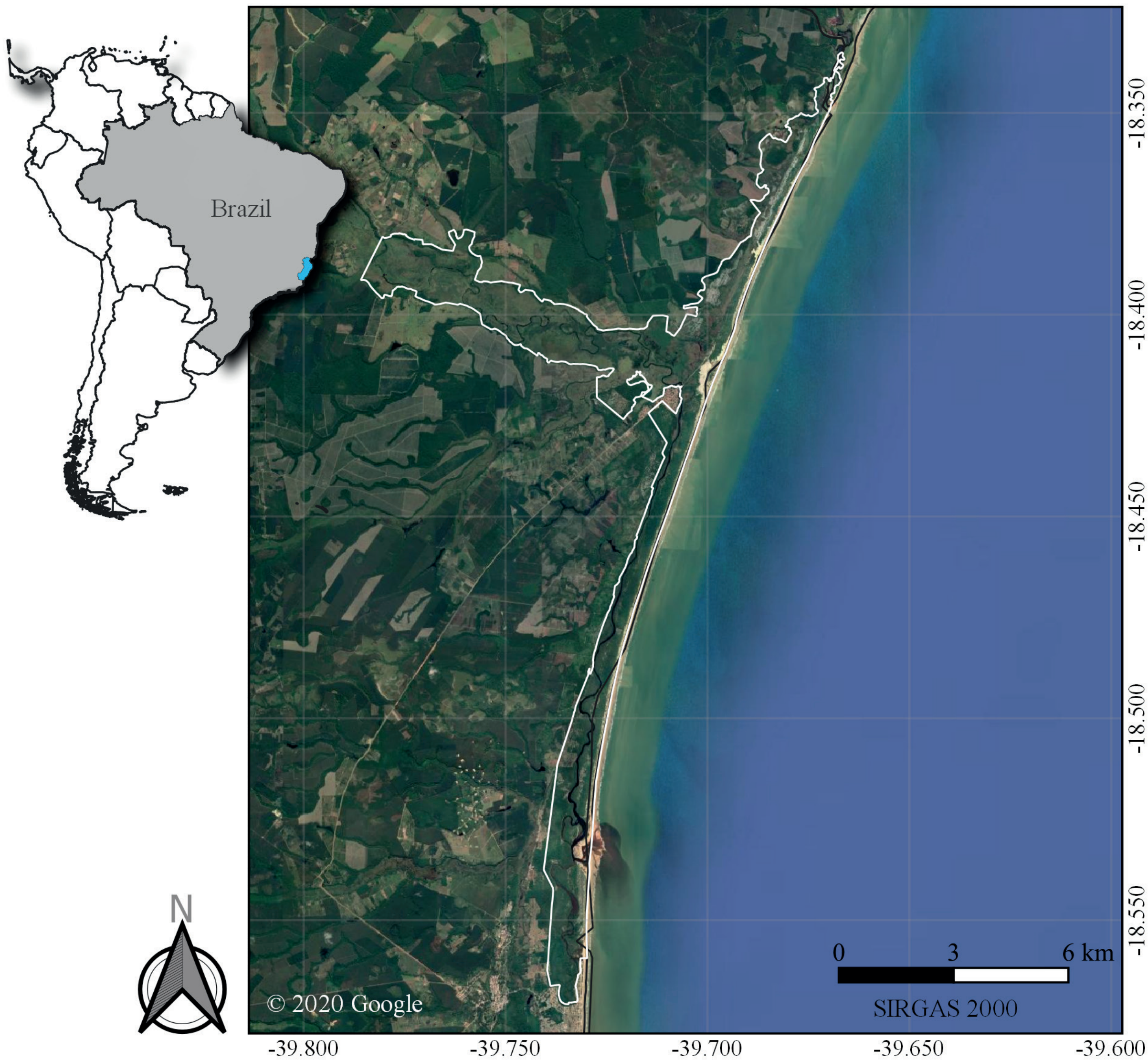

Figure 1. Study area. Geographic location of the State Park of Itaúnas.

Results

Of the 562 species previously surveyed in the study area, 93 species belonging to 61 genera and 29 families have EFNs (Tab. 1). Most of them (82 spp.) has no extinction risk assignments (NE - not evaluated), eight are categorized as Least Concern (LC), while *Davilla cf. macrocarpa* as Vulnerable (VU), *Inga exfoliata* as Nearly Threatened (NT), and *Humiriastrum spiritu-sancti* as Critically Endangered (CR) (Fig. 2A). Eudicotyledons are represented by 81 species, while 11 are monocotyledons and a single one is a basal angiosperm (*Annona glabra*). Moreover, 20 out of 93 species (21.5 %) are endemic to the Brazilian Atlantic forest, but none of them exclusive to the study area (Fig. 2B).

Most of the species was recorded in the 'restingas' formations (90 spp.), however *Inga exfoliata*, *Laguncularia racemosa*, and *Ipomoea pes-caprae* were exclusively found in the 'Tabuleiro' forest, mangroves, and dunes, respectively. The list brings a total of 50 species not previously cited as having EFNs, which represents new records for the World checklist of EFN-bearing plants.

The top five richest families and genera (Fig. 2C-D) hold more than half (54.8 %) and almost one third (30 %) of the species richness in the checklist, respectively. Considering plant habit, vines (woody or herbaceous) are predominant (Fig. 2E) with *Passiflora* (Passifloraceae) (nine spp.) and *Smilax* (Smilacaceae) (five spp.) as the top richest genera (Tab.1). On the other hand, herbs are the less species-rich group where *Philodendron fragrantissimum* and *P. pedatum* (Araceae) are hemiepiphytes, and *Ctenanthe glabra* (Marantaceae) and *Epidendrum denticulatum* (Orchidaceae) are terrestrial.

EFNs are more commonly found exclusively on the leaf blades (or leaflet blades + rachises) (65 species), followed by exclusively on the petioles with 20 species, and exclusively

Table 1. List of species of Angiosperms with extrafloral nectaries (EFNs) of the State Park of Itaúnas, Espírito Santo, Brazil. Legend: *=new records for the world list of plants bearing EFNs; Δ = endemic species of Atlantic Forest; Bd=buds; Br/Brt=bracts/bracteols; Cx=calyx; Fr=fruit; Hhe=herb hemiepiphyte; Lb/Llb=leaf blade/leaflet blade; Lws=lower surface; Mg=margin; Pd/Flb=pedicel/flower bud; Pt=petioles; Rcs=rachis; S=shrub; St=stipules; T=tree; Th=terrestrial herb; Ups=upper surface; V=vine; CS=conservation status; CR= critically endangered; LC=least concern; NE=not evaluated; NT=nearly threatened; VU=vulnerable.

Taxon	Habit	CS	Location											Voucher
			Bd	St	Pt	Lb/Llb				Br/Brt	Pd/Flb	Cx	Fr	
						Rcs	Mg	Ups	Lws					
Anacardiaceae R.Br.														
Anacardium occidentale L.	T	NE	•						•			•	•	Machado, J. 101 (VIES)
Annonaceae Juss.														
Annona glabra L.	T	LC											•	Souza, W. 186 (VIES)
Araceae Juss.														
Philodendron fragrantissimum (Hook.) G.Don	Hhe	NE			•				•					Marcarini, L. 74 (SAMES)
Philodendron pedatum (Hook.) Kunth	Hhe	NE			•				•	•				Pereira, O. 4433 (VIES)
Bignoniaceae Juss.														
Adenocalymma validum L.G.Lohmann*	V	NE										•		Monteiro, M. 84 (SAMES)
Amphilophium frutescens (DC.) L.G.Lohmann Δ	V	NE							•			•		Miranda, V.S. 376
Anemopaegma chamberlaynii (Sims) Bureau & K.Schum.	V	NE										•		Hatschbach, G. 69212 (HUFU)
Bignonia corymbosa (Vent.) L.G.Lohmann	V	NE	•						•					Nunes, T. 1712 (HUEFS)
Fridericia conjugata (Vell.) L.G.Lohmann	V	NE			•				•					Monteiro, M. 200 (SAMES)
Fridericia subincana (Mart.) L.G.Lohmann Δ	V	NE			•				•					Giaretta, A. 430 (SAMES)
Jacaranda bracteata Bureau & K.Schum.* Δ	T	NE							•					Hatschbach, G. 68362 (MBML)
Jacaranda puberula Cham.	T	LC							•					Monteiro, M. 100 (SAMES)
Lundia corymbifera (Vahl) Sandwith	V	NE			•				•					Monteiro, M. 36 (SAMES)
Lundia longa (Vell.) DC.*	V	NE		•	•									Nunes, T. 1705 (HUEFS)
Pyrostegia venusta (Ker Gawl.) Miers	V	NE							•					Silva-Castro, M. 1243 (HUEFS)
Tabebuia roseoalba (Ridl.) Sandwith	T	NE							•					Giaretta, A. 762 (SAMES)
Capparaceae A.Juss.														
Cynophalla flexuosa (L.) J.Presl.*	S	NE	•		•									Miranda, V.S. 387 (VIES)
Chrysobalanaceae R.Br.														
Chrysobalanus icaco L.	S	NE							•					Souza, W. 19 (VIES)
Hirtella corymbosa Cham. & Schltdl.* Δ	S	NE							•					Souza, W. 22 (VIES)
Combretaceae R.Br.														
Laguncularia racemosa (L.) C.F.Gaertn.	T	NE			•									Machado, J. 186 (VIES)
Convolvulaceae Juss.														
Ipomoea cairica (L.) Sweet	V	NE			•							•		Machado, J. 161 (VIES)

Table 1. Cont.

Taxon	Habit	CS	Location											Voucher
			Bd	St	Pt	Lb/Llb				Br/Brt	Pd/Flb	Cx	Fr	
						Rcs	Mg	Ups	Lws					
Ipomoea fimbriosepala Choisy	V	LC										•		Machado, J. 164 (VIES)
Ipomoea pes-caprae (L.) R.Br.	V	NE			•							•		Thomaz, L. 639 (VIES)
Dilleniaceae Salisb.														
Davilla cf. macrocarpa Eichler* Δ	V	VU										•		Miranda, V.S. 368 (VIES)
Dioscoreaceae R.Br.														
Dioscorea laxiflora Mart. ex Griseb.	V	NE										•		Souza, W. 212 (VIES)
Ericaceae Juss.														
Gaylussacia brasiliensis (Spreng.) Meisn.*	S	NE							•					Souza, W. 341 (VIES)
Euphorbiaceae Juss.														
Alchornea triplinervia (Spreng.) Müll.Arg.	T	NE						•						Pereira, O. 6156 (VIES)
Aparisthmium cordatum (A.Juss.) Baill.	T	NE						•	•					Hatschbach, G. 75133 (CESJ)
Cnidoscolus urens (L.) Arthur*	S	NE			•			•						Souza, W. 66 (VIES)
Croton lundianus (Didr.) Müll.Arg.*	S	NE			•									Souza, W. 196 (VIES)
Dalechampia micromeria Baill.	V	NE			•									Machado, J. 224 (VIES)
Joannesia princeps Vell.*	T	LC			•			•						Souza, W. 280 (VIES)
Microstachys serrulata (Mart.& Zucc.) F.Dietr*	S	NE						•				•		Amorim, B.S. 1957 (UFP)
Plukenetia serrata (Vell.) L.J.Gillespie* Δ	V	NE						•						Souza, W. 65 (VIES)
Fabaceae Lindl.														
Abarema filamentosa (Benth.) Pittier* Δ	T	LC			•	•		•						Machado, J. 140 (VIES)
Canavalia rosea (Sw.) DC.	V	NE	•		•									Simonelli, M. 317 (VIES)
Chamaecrista ensiformis (Vell.) H.S.Irwin & Barneby	T	NE				•								Ribeiro, M. 55 (VIES)
Chamaecrista flexuosa (L.) Greene	S	NE			•									Machado, J. 35 (VIES)
Chamaecrista ramosa (Vogel) H.S.Irwin & Barneby	S	NE			•									Machado, J. 34 (VIES)
Crotalaria incana L.	S	NE		•							•			Machado, J. 157 (VIES)
Inga exfoliata T.D.Penn. & F.C.P.Garcia* Δ	T	NT				•								Ribeiro, M. 31 (VIES)
Inga laurina (Sw) Willd.	T	LC				•								Pereira, O. 6074 (VIES)
Inga subnuda Salzm. ex Benth.* Δ	T	NE				•								Souza, W. 115 (VIES)
Inga thibaudiana DC.*	T	NE				•								Pereira, O. 5696 (VIES)
Leptolobium bijugum (Spreng.) Vogel* Δ	T	NE								•	•			Lima, H. 2972 (RB)
Piptadenia adiantoides (Spreng.) J.F.Macbr.	T	NE			•									Miranda, V.S. 378 (VIES)
Rhynchosia phaseoloides (Sw.) DC.*	S	NE			•									Giaretta, A. 116 (SAMES)

Table 1. Cont.

Taxon	Habit	CS	Location											Voucher
			Bd	St	Pt	Lb/Llb				Br/Brt	Pd/Flb	Cx	Fr	
						Rcs	Mg	Ups	Lws					
Senna appendiculata (Vogel) Wiersema*	S	NE				•								Souza, W. 84 (VIES)
Zygia latifolia (L.) Fawc. & Rendle*	T	NE			•									Pereira, O. 6065 (VIES)
Humiriaceae A.Juss.														
Humiria balsamifera Aubl.*	T	NE							•					Nepomuceno, A. 791 (VIES)
Humiriastrum spiritu-sancti Cuatrec.* Δ	T	CR						•						Nepomuceno, A. 792 (VIES)
Vantanea bahiaensis Cuatrec.* Δ	T	NE						•						Luz, A. 342 (CVRD)
Malpighiaceae Juss.														
Heteropterys coleoptera A.Juss.*	V	NE					•							Ribeiro, M. 54 (SAMES)
Heteropterys nordestina Amorim* Δ	V	NE			•		•							Hatschbach, G. 51424 (INPA)
Peixotoa hispidula A.Juss.* Δ	S	NE							•					Miranda, V.S. 381 (VIES)
Stigmaphyllon blanchetii C.E.Anderson*	V	NE						•						Hatschbach, G. 75129 (CEPEC)
Stigmaphyllon ciliatum (Lam.) A.Juss* Δ	V	NE						•						Pereira, O. 6060 (VIES)
Stigmaphyllon paralias A.Juss.*	V	NE						•						Miranda, V.S. 386 (VIES)
Tetrapterys phlomoides (Spreng.) Nied.*	V	NE							•					Hatschbach, G. 75073 (HCF)
Malvaceae Juss.														
Talipariti pernambucense (Arruda) Bovini	T	NE							•			•		Souza, W. 156 (VIES)
Urena lobata L.	S	NE							•					Souza, W. 267 (VIES)
Marantaceae R.Br.														
Ctenanthe glabra (Körn.) Eichler	Th	NE							•					Monteiro, M. 93 (SAMES)
Meliaceae Juss.														
Guarea macrophylla Vahl*	T	NE			•			•	•				•	Giaretta, A. 266 (SAMES)
Moraceae Gaudich.														
Ficus bahiensis C.C.Berg & Carauta*	T	NE						•						Vinha, P. 1257 (VIES)
Ficus crocata (Miq.) Miq.*	T	NE						•						Giaretta, A. 155 (SAMES)
Ficus gomelleira Kunth & C.D.Bouché*	T	NE			•									Pereira, O. 4425 (VIES)
Ochnaceae DC.														
Ouratea bahiensis Sastre* Δ	S/T	NE		•										Miranda, V.S. 383 (VIES)
Ouratea parvifolia (A.St.-Hil.) Engl.*	S/T	LC		•										Machado, J. 89 (VIES)
Orchidaceae Juss.														
Epidendrum denticulatum Barb.Rodr.*	Th	NE									•			Machado, J. 01 (VIES)
Vanilla planifolia Jacks. ex Andrews	V	NE									•			Machado, J. 193 (VIES)

Table 1. Cont.

Taxon	Habit	CS	Location											Voucher
			Bd	St	Pt	Lb/Llb				Br/Brt	Pd/Flb	Cx	Fr	
						Rcs	Mg	Ups	Lws					
Passifloraceae Juss. ex Roussel														
Passiflora alata Curtis	V	NE			•									Souza, W. 102 (VIES)
Passiflora edulis Sims	V	LC			•			•						Milward, M. 43 (RB)
Passiflora haematostigma Mart. ex Mast.	V	NE			•									Pereira, O. 5701 (VIES)
Passiflora misera Kunth	V	NE							•					Souza, W. 211 (VIES)
Passiflora mucronata Lam.	V	NE			•		•							Souza, W. 31 (VIES)
Passiflora ovalis Vell. ex M. Roem.* ∆	V	NE			•									Hatschbach, G. 75071 (CEPEC)
Passiflora pentagona Mast. ∆	V	NE			•									Milward, M. 93 (RB)
Passiflora rhamnifolia Mast. ∆	V	NE			•									Souza, W. 45 (VIES)
Passiflora silvestris Vell.*	V	NE			•									Giaretta, A. 970 (SAMES)
Polygalaceae Hoffmanns. & Link														
Securidaca lanceolata A.ST.-Hil. & Moq.*	V	NE			•									Giaretta, A. 494 (SAMES)
Rubiaceae Juss.														
Tocoyena formosa (Cham. & Schltdl.) K.Schum.	S/T	NE											•	Souza, W. 07 (VIES)
Simaroubaceae DC.														
Simarouba amara Aubl.	T	NE							•					Ribeiro, M. 28 (SAMES)
Smilacaceae Vent.														
Smilax brasiliensis Spreng.*	V	NE							•					Machado, J. 02 (VIES)
Smilax elastica Griseb.*	V	NE							•					Martinelli, G. 9720 (RB)
Smilax fluminensis Steud.*	V	NE							•					Hatschbach, G. 75092 (RB)
Smilax rufescens Griseb.*	V	NE							•					Farney, C. 4734 (RB)
Smilax syphilitica Humb. & Bonpl. ex Willd.*	V	NE							•					Hatschbach, G. 75062 (RB)
Turneraceae Kunth ex DC.														
Turnera hermannioides Cambess.*	S	NE			•									Miranda, V.S. 483 (VIES)
Turnera subulata Sm.	S	NE			•									Machado, J. 36 (VIES)
Verbenaceae J. St.-Hil.														
Stachytarpheta cayennensis (Rich.) Vahl*	S	NE							•					Souza, W. 151 (VIES)
Stachytarpheta schottiana Schauer* ∆	S	NE							•					Miranda, V.S. 496 (VIES)
Vitaceae Juss.														
Cissus erosa Rich.*	V	NE			•									Miranda, V.S. 375 (VIES)
Cissus spinosa Cambess.*	V	NE			•									Machado, J. 03 (VIES)

on calyx (five species). They are less frequently present on axillary buds, stipules, bracts or bracteoles, pedicels or flower buds, and fruits (Figs. 2F, 3, 4, 5). Some species show EFNs in different plant organs and not only in a restricted location, such as in *Anacardium occidentale* (Anacardiaceae), *Philodendron* species (Araceae), *Canavalia rosea* and *Crotalaria incana* (Fabaceae), and *Guarea macrophylla* (Meliaceae) (Tab.1). *Tocoyena formosa* (Rubiaceae) has an ordinary nectary, forming a disk on the ovary apex that secretes nectar for pollination process, however it continues to exudate during fruit development.

Discussion

EFNs taxonomic distribution

The species with EFNs in PEI corresponds to 16.5 % of the local flora which is somehow expected when compared to other studies, where the percentage values for EFN-bearing plants varies from 15 to 31 % depending on the type of vegetation (*e.g.* Madison 1979; Oliveira *et al.* 1987; Morellato & Oliveira 1991, 1994; Del-Claro *et al.* 1996; Oliveira & Pie 1998; Blüthgen *et al.* 2000; Del-Claro & Santos 2000; Machado *et al.* 2008; Schoereder *et al.* 2010; Nascimento & Del-Claro 2010; Del-Claro *et al.* 2013; Lange *et al.* 2013; Assunção *et al.* 2014; Campos & Camacho 2014; Lange & Del-Claro 2014; Anjos *et al.* 2017; Dutra 2019). Specifically concerning our study site, located in the Atlantic forest, despite absolute richness is much higher compared to previous studies - 93 against 23 (Câmara *et al.* 2017) and 46 species (Dutra 2019) - the relative values is approximate to the previously encountered, ranging between 12.2-17.1 % (*e.g.* Câmara *et al.* 2017; Dutra 2019). However, unlike Dutra (2019), Câmara *et al.* (2017) included only trees in their analysis and the percentage likely would change if considered non-tree species.

Depending on environment, the habit predominance varies and, despite showing different phytophysiognomies, the proportion of EFN-bearing plants may converge. For instance, 'restingas' vegetation in PEI and semideciduous seasonal tropical forest (Dutra 2019), both in the Atlantic forest domain, are represented by vines as the richest habit of EFNs-bearing plant, followed by trees and shrubs. This pattern is different compared to other phytogeographic domains, such as the Cerrado and the Amazon forest where shrubs and lianas/epiphytes are the most common elements (Oliveira & Leitão-Filho 1987; Oliveira & Oliveira-Filho 1991; Pires 2015; Blüthgen *et al.* 2000). Non-tree plants represent almost 70 % of the PEI's flora with EFNs, however, they consist of a commonly neglected group of plants in broader surveys and the EFNs richness is possibly underestimated in several areas. According to Gentry & Dodson (1987), herbs and shrubs, together, contribute with 33 to 52 % of the local diversity in tropical ecosystems. Thus, even with ephemeral life cycles, herbs have high importance in the vegetation dynamics (Lima 2011) with EFNs and visitors establishing key ecological interactions.

The four richest families in PEI are in accordance with previous studies that listed them as the most common elements regardless phytophysiognomy and plant community composition (Oliveira & Leitão-Filho 1987; Morellato & Oliveira 1991; Díaz-Castelazo *et al.* 2005; Machado *et al.* 2008; Melo *et al.* 2010b; Urbanetz *et al.* 2010; Weber *et al.* 2015). Floristics comparisons are fundamental, however most of the plant checklists are essentially based on general information with no further biological aspects, such as the presence of EFNs themselves. By performing similarity analysis, for instance using those checklists, the results may not reflect the real richness of EFN-bearing plants by the inclusion of the well-known species only. More detailed data about the predominant plant habit, composition, proportion of EFN-bearing plants, and/or local phytophysiognomy may explain the distributional patterns of the vegetation. Even so, this issue has not been properly studied yet and available studies do not bring this specific data.

Although PEI has a huge vegetational heterogeneity (see Souza *et al.* 2016), the 'restingas' are predominant and hold over 96 % of local EFN-bearing plant richness. These results corroborate the higher richness values for floras from open areas (Schupp & Feener 1991; Fiala & Lisenmair 1995; Blüthgen & Reifenrath 2003). The structure of the vegetation affects the distribution patterns and, according to Câmara *et al.* (2017) and Díaz-Castelazo *et al.* (2020), shaded habitats have a different pattern when compared to the open ones.

More than half of the herein listed species (53.7 %) comprises new additions to the world list of plants with EFNs, including the first record in the Dilleniaceae (see Weber *et al.* 2015), and 21.5 % of them are endemic to the Atlantic forest. However, despite its importance as a basis for further ecological studies, especially on ant-plant interactions, information about species conservation status is vastly lacking with few assignments to a given CNCFlora's category.

EFNs location and ecological insights

In PEI, most of EFNs (around 90 %) are found on leaves which is a common pattern (Melo *et al.* 2010a; b; Pscheidt & Cordeiro 2012; Silva 2015; Weber *et al.* 2015; Dutra 2019). Those leaf EFNs are classified as both extrafloral and extranuptial, however, there are examples of floral and nuptial nectaries with extranuptial function (Santos & Del-Claro 2001). For example, *Tocoyena formosa* (Rubiaceae) and *Epidendrum denticulatum* (Orchidaceae) present floral nectaries with singular extranuptial activity (Santos & Del-Claro 2001; Almeida & Figueiredo 2003; Sousa-Lopes *et al.* 2020). *Tocoyena formosa* has a nectar gland surrounding the apex of the ovary during blooming and, after pollination, the nectariferous disk on young

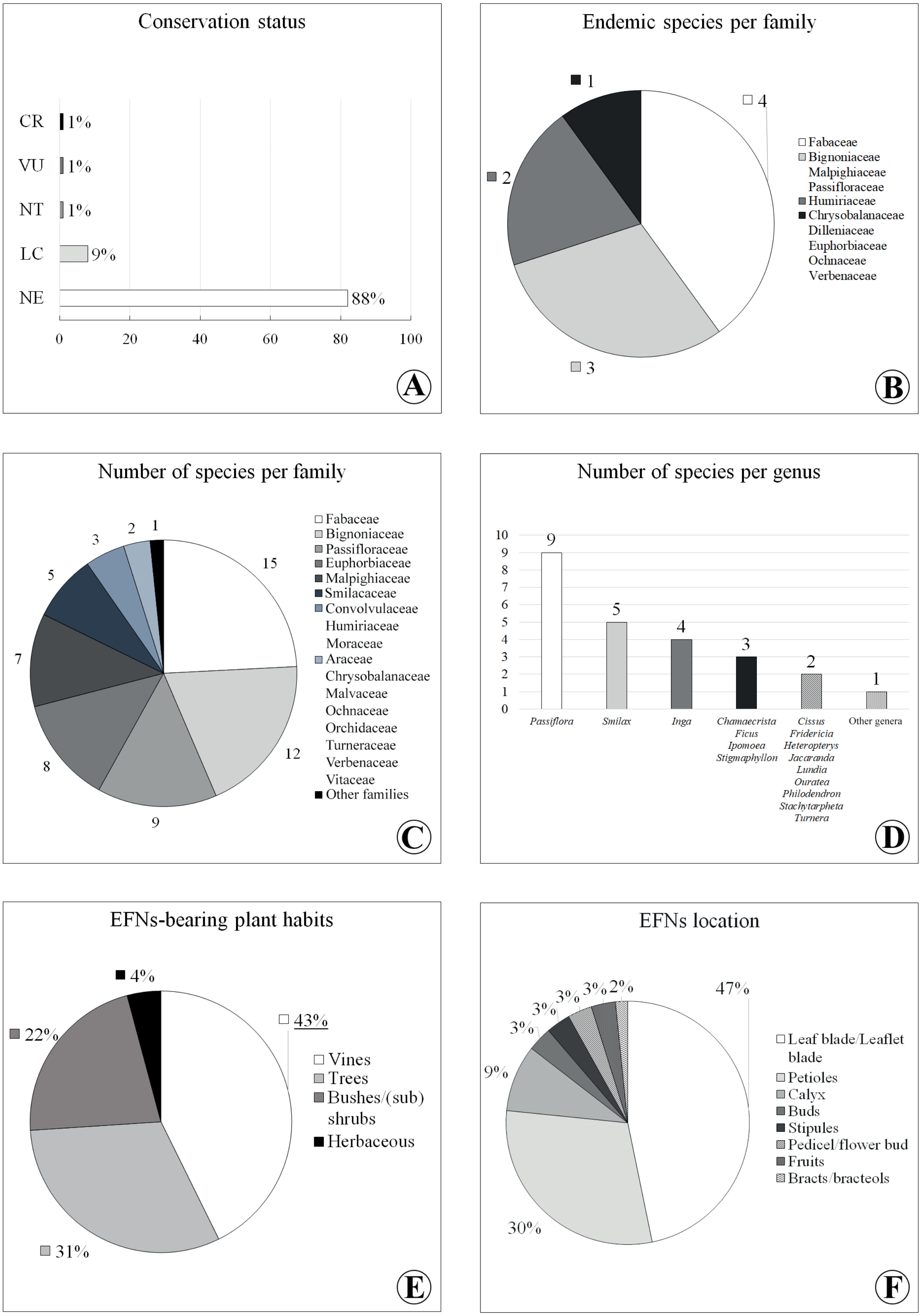

Figure 2. EFNs-bearing plants of the State Park of Itaúnas, Espírito Santo State, Brazil. **A.** Conservation status based on CNCFlora (2021). **B.** Number of endemic species per family. **C.** Number of species per family. **D.** Number of species per genus. **E.** Percentage of plant habits**.** **F.** Percentage of EFNs location on plant's body. CR= critically endangered; LC=least concern; NE=not evaluated; NT=nearly threatened; VU=vulnerable.

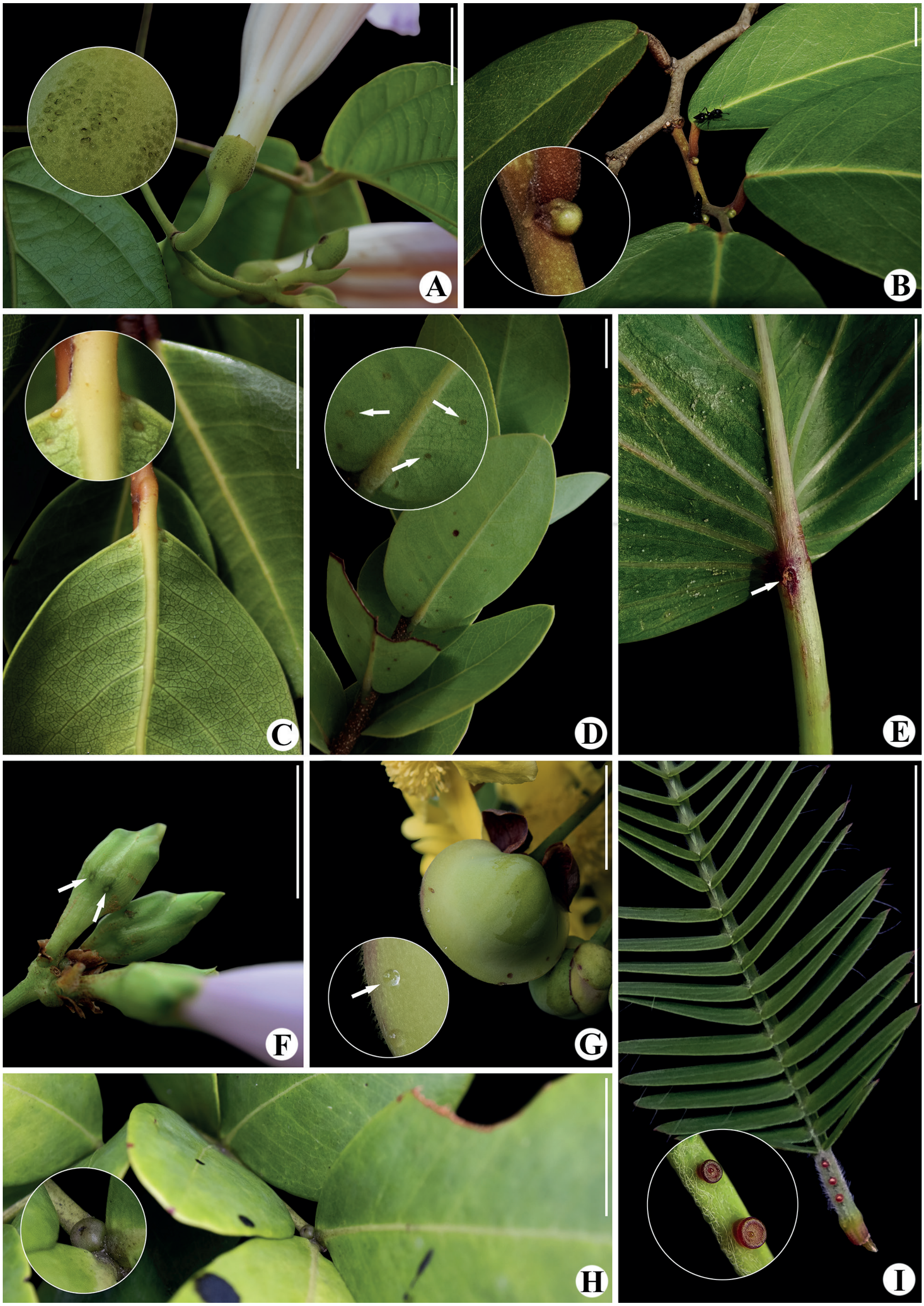

Figure 3. EFNs of the State Park of Itaúnas, Espírito Santo State, Brazil. **A.** *Amphilophium frutescens*. **B.** *Cynophalla flexuosa*. **C.** *Chrysobalanus icaco*. **D.** *Hirtella corymbosa*. **E.** *Ipomoea pes-caprae*. **F.** *Ipomoea fimbriosepala*. **G.** *Davilla cf. macrocarpa*. **H.** *Abarema filamentosa*. **I.** *Chamaecrista flexuosa*. Bars= 1cm; EFNs= extrafloral nectaries; white arrows= EFNs (Photos A-D and G-H by Rafael S. Mathielo).

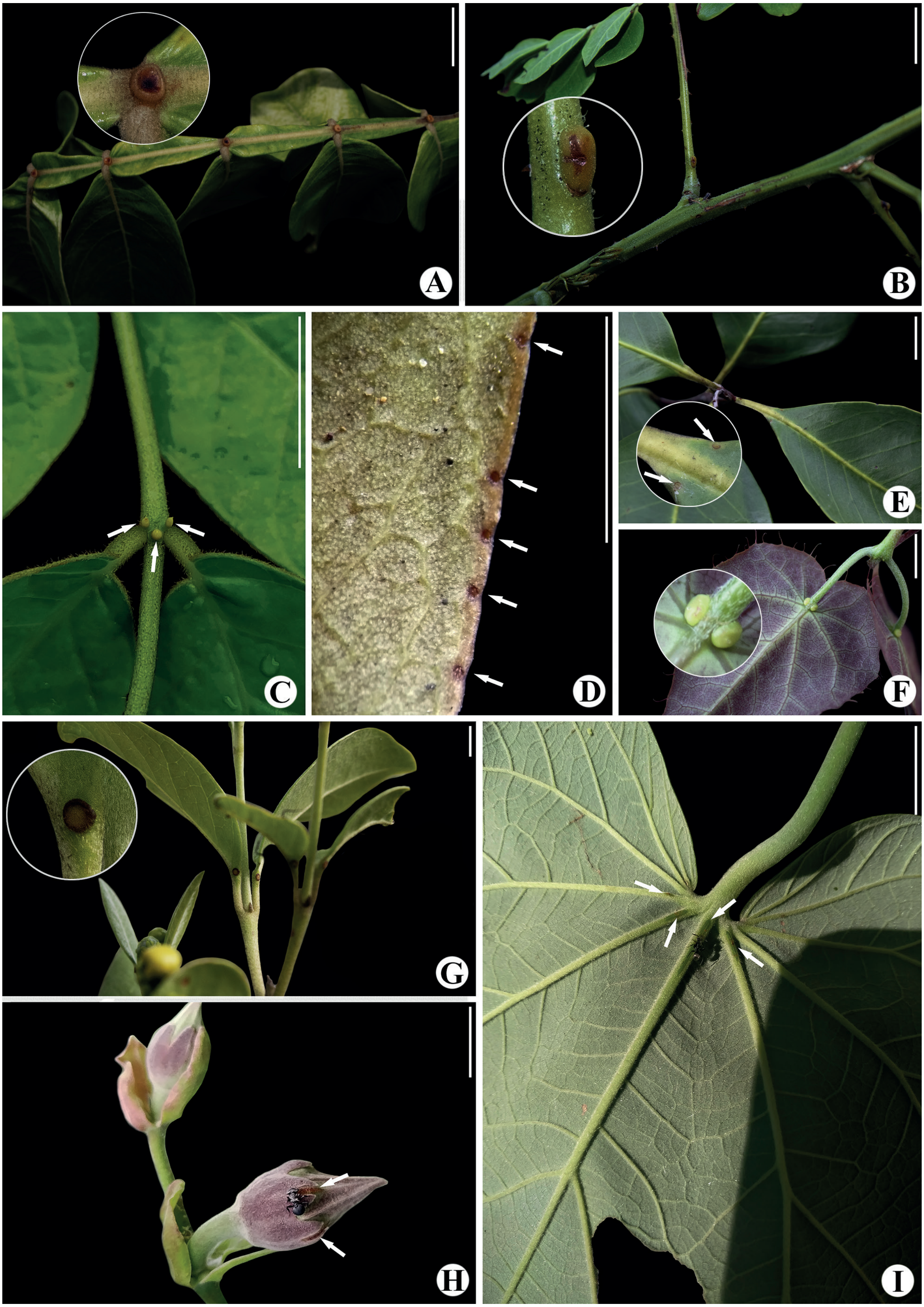

Figure 4. EFNs of the State Park of Itaúnas, Espírito Santo State, Brazil. **A.** *Inga subnuda*. **B.** *Piptadenia adiantoides*. **C.** *Senna appendiculata*. **D.** *Humiria balsamifera*. **E.** *Humiriastrum spiritu-sancti*. **F.** *Stigmaphyllon ciliatum*. **G.** *Stigmaphyllon paralias*. **H-I.** *Talipariti pernambucensis*. Bars= 1cm; EFNs= extrafloral nectaries; white arrows= EFNs (Photos A-B and D-G by Rafael S. Mathielo)

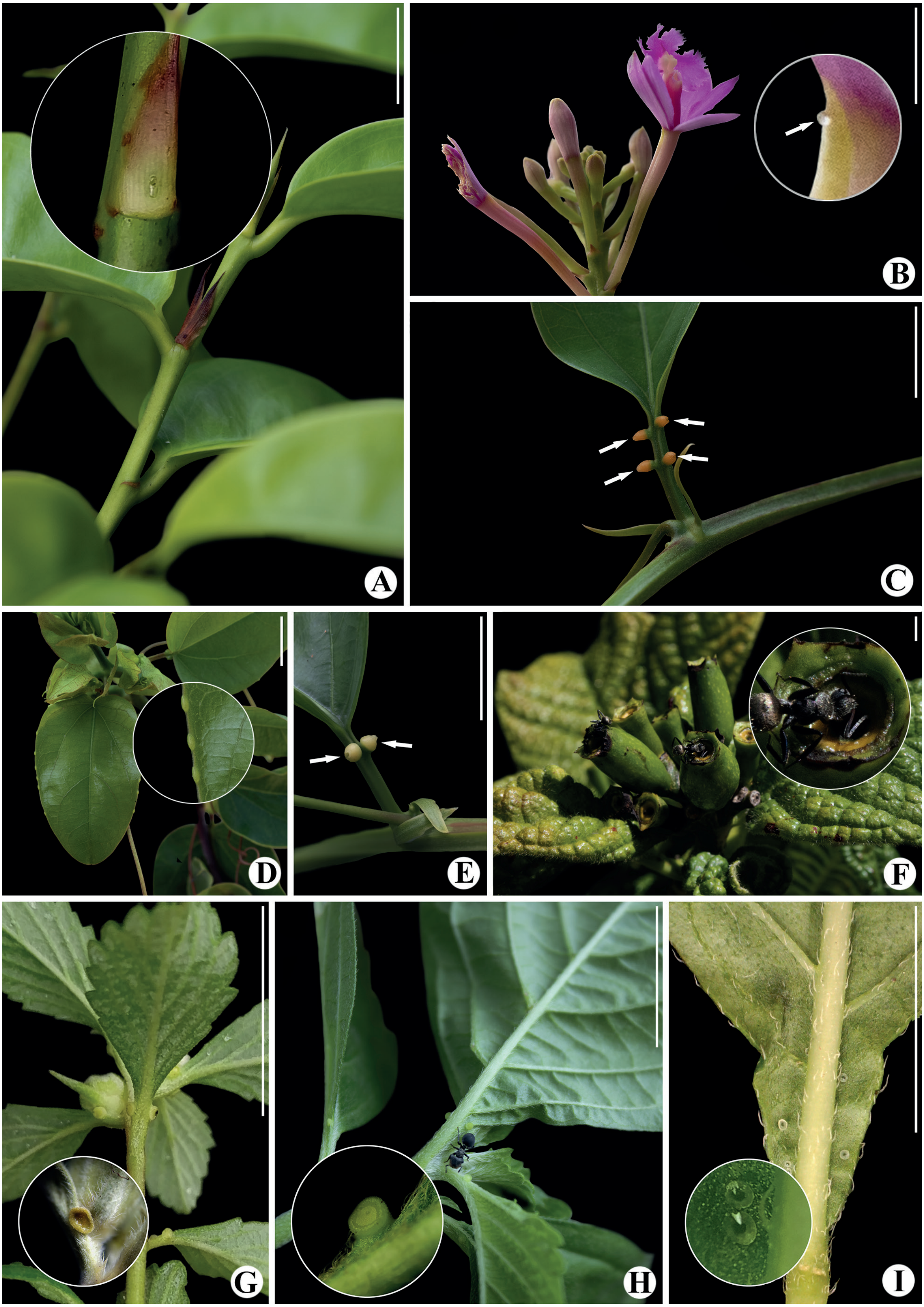

Figure 5. EFNs of the State Park of Itaúnas, Espírito Santo State, Brazil. **A.** *Ouratea bahiensis*. **B.** *Epidendrum denticulatum*. **C.** *Passiflora alata*. **D-E.** *Passiflora mucronata*. **F.** *Tocoyena formosa*. **G.** *Turnera hermannioides*. **H.** *Turnera subulata*. **I.** *Stachytarpheta cayennensis*. Bars= 1cm; EFNs= extrafloral nectaries; white arrows= EFNs (Photos A-F and H by Rafael S. Mathielo)

fruits are fully functional and produce an exudate which is attractive for ants. *Epidendrum denticulatum* produces nectar on the apex of the peduncle on buds, flowers, and fruits that, by attracting ants, provide an efficient protection against herbivory (Calvo 1990; 1993; Almeida & Figueiredo 2003; Ballarin *et al.* 2019).

Five species belonging to *Smilax* (Smilacaceae) have inconspicuous EFNs only noticed due to ant visitation and secreted drops on the petiole. Our results provide new records for the genus adding up to the world list of plants with EFNs. This secreting activity was previously noticed to a native species from Cerrado, *Smilax polyantha* Griseb. which has EFNs composed of few cell layers with no external structure (Pires *et al.* 2017). On the other hand, *Ouratea bahiensis* and *O. parvifolia* (Ochnaceae) have conspicuous EFNs on the stipules of the young branches (Fig. 5A). Those stipules are frequent and intensively visited by ants (*pers. observ.*) for a short time during the branch development, just before they fall off. After stipule' senescence, signs of herbivory were noticed on mature leaves (*pers. observ.*).

In this study, we provide a relevant update for the world list of angiosperms with EFNs. Most importantly, it consists of a massive number of EFN-bearing plant species from a single and small protected area where we found high values of richness. It reinforces the lack of studies of this nature in the Brazilian Atlantic forest, where further investigations are strongly recommended. We realized that studies about ant-plant interactions have usually been the main source of detecting EFN-bearing plants (Calixto *et al.* 2018) and, thus, they modulated our knowledge case-to-case about plant richness (*e.g.* Calixto *et al.* 2020; 2021). EFNs are relevant mediators of the ant-plant mutualism (Calixto *et al.* 2018), which can be an important enhancer of ecosystem biodiversity (Heil 2011). So, by knowing the richness of EFN-bearing flora, the chances of ensuring ecosystem conservation are greater. These results are part of ongoing studies on EFNs in areas of the Atlantic forest involving mainly cataloging of biodiversity. Here, we contribute with a thoroughly investigated EFN-bearing plant database that will allow the academic community to perform further studies regarding botanical, ecological, and evolutionary issues. A novel research avenue that could also be explored in future studies is the investigations of the functional attributes of EFN in plant communities. For example, two recent studies suggest that nectar volume and concentration in EFN are strongly related to ant-plant community structure and defensive behavior in ants that visit EFNs (Ballarin *et al.* 2019; Calixto *et al.* 2021). These studies point out that other EFN traits (*e.g.*, nectar volume and concentration) could be relevant for future surveys since they are closely related to ecological processes and patterns. Therefore, we point that not only novel inventories must be made in underappreciated ecosystems, but also meticulous studies of EFN traits should help novel studies to be developed.

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