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COMPOUND SPECIFIC TRENDS OF CHEMICAL DEFENCES IN *Ficus* ALONG AN ELEVATIONAL GRADIENT REFLECT A COMPLEX SELECTIVE LANDSCAPE

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39 **Abstract** – Elevational gradients affect the production of plant secondary metabolites through
40 changes in both biotic and abiotic conditions. Previous studies have suggested both elevational
41 increases and decreases in host-plant chemical defences. We analysed the correlation of
42 alkaloids and polyphenols with elevation in a community of nine *Ficus* species along a
43 continuously forested elevational gradient in Papua New Guinea. We sampled 204 insect
44 species feeding on the leaves of these hosts and correlated their community structure to the
45 focal compounds. Additionally, we explored species richness of folivorous mammals along the
46 gradient. When we accounted for *Ficus* species identity, we found a general elevational increase
47 in flavonoids and alkaloids. Elevational trends in non-flavonol polyphenols were less
48 pronounced or showed non-linear correlations with elevation. Polyphenols responded more
49 strongly to changes in temperature and humidity than alkaloids. The abundance of insect
50 herbivores decreased with elevation, while the species richness of folivorous mammals showed
51 an elevational increase. Insect community structure was affected mainly by alkaloid
52 concentration and diversity. Although our results show an elevational increase in several groups
53 of metabolites, the drivers behind these trends likely differ. Flavonoids may provide figs with
54 protection against abiotic stressors. In contrast, alkaloids affect insect herbivores and may
55 provide protection against mammalian herbivores and pathogens. Concurrent analysis of
56 multiple compound groups alongside ecological data is an important approach for
57 understanding the selective landscape that shapes plant defences.

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60

61 **Key Words** – Coleoptera, folivorous mammals, herbivory, Lepidoptera, New Guinea,
62 phenanthroindolizidine alkaloids, polyphenols, possum, tannins.

63

64

65 INTRODUCTION

66 Elevational gradients lead to local adaptations and differential selection on traits, rapid turnover
67 in community composition, and changing interaction networks (Segar et al. 2016; Toussaint et
68 al. 2013). As a result, long wet elevational gradients in the tropics are often among the most
69 diverse places on earth in terms of both species richness and functional diversity (Perrigo et al.
70 2019). In plants, elevational gradients can drive significant changes in the production of
71 secondary metabolites in response to changes in both biotic and abiotic conditions (Defossez et
72 al. 2018; Moreira et al. 2018). These changes in plant chemistry have cascading effects on the
73 associated organisms, as plant secondary chemistry underpins patterns of diversity across
74 multiple trophic levels (Richards et al. 2015; Volf et al. 2019).

75 Plants might be expected to invest progressively less into chemical defences with increasing
76 elevation because insect abundance and herbivory generally decrease towards higher elevations
77 (Garibaldi et al. 2011; Pellissier et al. 2014; Sam et al. 2019b). However, the costs of
78 compensating for biomass lost to herbivores show a strong elevational increase too. This may
79 favour a higher investment into defences at the expense of growth by plants at higher elevations
80 (Defossez et al. 2018; Givnish 1999; Salgado et al. 2016). Elevational trends in anti-herbivore
81 defences can be further modified by changes in herbivore communities that normally show a
82 strong turnover with elevation (Novotny et al. 2005). As different herbivores respond to
83 different plant defences (Volf et al. 2015; Volf et al. 2018), such changes in insect community
84 composition can modify the relative importance of individual defensive traits along elevational
85 gradients. Furthermore, while studies have typically focused on elevational trends in insect
86 herbivory, the abundance of plant pathogens and other groups of herbivores, such as folivorous
87 mammals, also show pronounced elevational trends (Brown and Vellend 2014; Geml et al.
88 2014; Tallowin et al. 2017). Thus, the plant chemotype observed is a result of multiple biotic
89 drivers operating over both ecological and evolutionary scales.

90 While herbivores and pathogens are important drivers of secondary metabolite diversity, abiotic
91 factors also play an important role. Temperature, and in most cases resources, decrease with
92 elevation and this can impair some of the metabolic pathways responsible for producing
93 secondary metabolites. This is largely true in the alpine zone, above the tree line, where plants
94 are exposed to extreme abiotic conditions (Pellissier et al. 2014). On the other hand, secondary
95 metabolites involved in protection against low temperatures and UV irradiation, such as various
96 flavonoids, should increase in concentration with elevation (Rasmann et al. 2014). This increase
97 in specific metabolite groups stimulated by abiotic conditions can secondarily affect insect
98 herbivores that also respond to the changing environmental conditions themselves (Escobar-
99 Bravo et al. 2017).

100 Indeed, it is the interaction between biotic and abiotic factors that drives elevational trends in
101 host plant defences (Defossez et al. 2018). Given the complexity of these interactions,
102 elevational gradients do not generate a simple directional change in the overall intensity of
103 chemical defences. Instead they act to modify the relative importance of individual groups of
104 secondary metabolites and forms of plant defence (Defossez et al. 2018; Moreira et al. 2018;
105 Rasmann et al. 2014). Quantification of herbivore or pathogen communities and environmental
106 variables is necessary for the correct interpretation of trends in host-plant defences (Moreira et
107 al. 2018).

108 Here we focus on the compound specific leaf chemistry of figs (*Ficus*; Moraceae) along one of
109 the world's most diverse elevational gradients, the New Guinean Central Range. *Ficus* has a
110 pantropical distribution and is an extraordinarily species rich genus of woody plants, containing
111 over 800 species, of which ca 150 occur in Papua New Guinea (PNG) (Berg and Corner 2005;
112 Cruaud et al. 2012). *Ficus* is a keystone plant genus. It supports diverse communities of
113 herbivorous insects and several groups of frugivorous and herbivorous birds and mammals
114 (Kanowski et al. 2003; Novotny et al. 2005; Shanahan et al. 2001). The insect herbivores

115 associated with the genus can typically feed on multiple con-generics which is thought to have
116 contributed to the chemical divergence among *Ficus* species (Volf et al. 2019; Volf et al. 2018).
117 The majority of the mammalian herbivores feeding on *Ficus* in the New Guinean region are
118 possums, cuscuses or tree mice (Flannery 1995). *Ficus* is over-represented amongst plant
119 species with wide elevational ranges (Novotny *et al.*, 2005) and in PNG, elevational gradients
120 have probably played an important role in the speciation within the genus. Parapatric speciation
121 has likely generated distinctive lowland/highland populations, sister species, and communities
122 (Segar et al. 2016; Souto-Vilarós et al. 2019).

123 Fig leaves contain a variety of secondary metabolites, including alkaloids, polyphenols, and
124 terpenoids (Volf et al. 2018). Phenanthroindolizidine alkaloids are among the most important
125 alkaloid groups in *Ficus*. They have a rather restricted distribution among plants and are
126 typically produced by species of Moraceae, Apocynaceae, and Caricaceae (Damu et al. 2005;
127 Han et al. 2013; Konno et al. 2004). Phenanthroindolizidine alkaloids exhibit a pronounced
128 cytotoxicity and inhibit the enzymes involved in the synthesis of DNA (Stærk et al. 2000). They
129 are strong antifeedants for generalist herbivores (Miller and Feeny 1983). In contrast, some
130 specialized and highly adapted insect herbivores feeding on *Ficus*, such as moths from the
131 genus *Asota*, are probably able to sequester these metabolites (Sourakov and Emmel 2001).
132 Some phenanthroindolizidine alkaloids, such as antofine, also show anti-pathogen activities,
133 being effective inhibitors of bacteria and fungi (Mogg et al. 2008). Polyphenols are a diverse
134 group of secondary metabolites with a broad variety of functions. Their anti-herbivore function
135 against insects results from at least three factors: (1) oxidative activation mediated by the high
136 pH of the insect gut, or by plant polyphenol oxidases release by cell lysis, (2) binding and
137 precipitation of nutritive proteins at the low to neutral pH present at the oral cavity or in the gut
138 of some insect species, and (3) activity resulting from degradation/hydrolysis products of
139 polyphenols that may be accelerated by high pH or microbe action (Salminen 2014; Salminen

140 and Karonen 2011). Importantly, the high pH found especially in the gut of lepidopteran larvae
141 favours the oxidation of polyphenols and inhibits their protein precipitation functions (Salminen
142 and Karonen 2011). In addition, flavonols are often involved in abiotic protection, such as
143 against UV irradiation (Escobar-Bravo et al. 2017; Harborne and Williams 2000).

144 Our aim was to document elevational trends in the concentration, diversity, and composition of
145 *Ficus* alkaloids and polyphenols. We analysed trends in chemical data in the context of
146 caterpillar and leaf-chewing beetle communities. Furthermore, we reported patterns in the
147 elevational species richness of mammalian herbivores because these may represent an
148 important factor driving investment in defence. We expected a general elevational increase in
149 *Ficus* defences as the plants growing at high elevations need to protect their biomass against
150 both biotic and abiotic factors more intensely.

151

152 METHODS AND MATERIALS

153 *Study Sites and Field Sampling*. We carried out a detailed survey at six study sites along an
154 elevational gradient (200, 700, 1200, 1700, 2200, and 2700 m a.s.l.) on Mt. Wilhelm in Papua
155 New Guinea from June 2013 to February 2014 (Figure S1, Table S1). Our study transect has
156 been subject to intensive study and is home to 51% species of New Guinea mainland birds, 27%
157 of PNG butterflies and 15% of PNG frogs (Novotny and Toko 2015). There are 157 *Ficus*
158 species known from New Guinea (Whitfeld and Weiblen 2010), including 73 species
159 documented along the Mt Wilhelm transect. The majority of species surveyed at our study site
160 are widespread in Papua New Guinea and frequently recorded in large scale floristic surveys
161 (Berg and Corner 2005). We focused on nine *Ficus* species common along the gradient: *F.*
162 *arfakensis* King, *F. copiosa* Steud., *F. pungens* Reinw. ex Blume, *F. erythrosperma* Miq., *F.*
163 *hahliana** Diels, *F. hombroniana** Corner, *F. itoana* Diels, Diels, *F. microdictya* and *F. umbrae*
164 Weiblen. The last three species are part of a monophyletic complex, with *F. umbrae* Weiblen

165 being a newly described species recently split from *F. itoana* (Ezedin and Weiblen 2019; Souto-
166 Vilarós et al. 2018). We treated the *F. itoana* species complex as a single species for the purpose
167 of statistical analyses. Species marked with an asterisk may comprise further genetically distinct
168 entities above the population level. Highland individuals of *F. hombroniana* resemble the
169 closely related *F. ihuensis* and populations of *F. hahliana* at 1700 m a.s.l. and above are
170 genetically and morphologically distinct from lowland populations, although they form a
171 monophyletic clade within the current sampling context (Segar et al. 2016).

172 At each elevation, we set up ten 10 x 500 m transects and marked all focal *Ficus* species with a
173 DBH (diameter at breast height) greater than 1 cm that were growing within the transect. We
174 identified each tree and gave it a unique identifier number (Segar et al. 2016). Our selection of
175 individual trees for sampling chemistry was guided largely by the range of sizes used to sample
176 insects (see below), although in both cases we aimed to avoid extremely young individuals (i.e.
177 saplings with a DBH <1.0 cm). We sampled 142 trees for chemical data and recorded DBH
178 data for 132 of these individuals. The mean diameter at breast height (DBH) for each species
179 was as follows (standard error in parentheses): *Ficus afarkensis* 5.0 cm (±0.9), *Ficus copiosa*
180 7.5 cm (±2.2), *Ficus erythrosperma* 6.8 cm (±0.9), *Ficus hahliana* 5.8 cm (±0.8), *Ficus*
181 *hombroniana* 2.5 cm (±0.4), *Ficus itoana* complex 7.8 (±0.9) and *Ficus pungens* 11.6 (±1.6).
182 We collected forty leaf discs from up to six individuals per species per elevation using a cork
183 borer 2.4 cm in diameter (avoiding the midrib) from fully expanded mature leaves. We avoided
184 sampling from plants heavily damaged by herbivores or pathogens. We stored half of the leaf
185 discs in HPLC grade acetone in order to prevent enzymatic degradation and oxidization of the
186 studied metabolites in the field and transferred them to a dark -20ºC freezer on return to the
187 New Guinea Binatang Research Centre. Later, we used these discs for secondary metabolite
188 analysis. We weighed the other half of leaf discs fresh and dry in order to estimate both the
189 percentage of water per leaf disc and the dry weight contained in each tube of acetone.

190 We sampled all *Ficus* individuals for Lepidoptera leaf-chewing larvae (caterpillars) and adult
191 leaf chewing beetles. Trained collectors walked the same ten transects per elevation as
192 described above and systematically (leaf to leaf) searched all accessible (≤3m height) foliage
193 for herbivores on *Ficus* trees. Collection was exhaustive across the accessible foliage such that
194 the number of leaves surveyed varied from tree to tree. We repeated this sampling ten times, in
195 approximately ten-day intervals over a 3.5 month period, for each transect and across all study
196 sites. A total of 300 km across sites was walked across surveys and months. We tested all
197 herbivores for feeding on the plant species from which they were collected in 24-hour no-choice
198 experiments to confirm host associations. Where possible we reared the larvae to adults and
199 photographed both stages. We morphotyped individuals by cross-referencing them to
200 collections at the New Guinea Binatang Research Center. We shipped the adult Lepidoptera to
201 the National Museum of Natural History, Smithsonian Institution for further identification.
202 Legs of representative samples were shipped to Institute of Entomology, Biology Centre, Czech
203 Academy of Sciences. We sampled dry legs from 486 Lepidoptera individuals to obtain COI
204 barcode sequences (Wilson 2012). Following this we either shipped the samples directly for
205 sequencing with standard Sanger protocols at the Biodiversity Institute of Ontario or sent them
206 as extracted and amplified DNA for sequencing at Macrogen Korea. We uploaded the
207 sequences to BOLD and assigned them to Barcoding Index Numbers (BINs) which we used as
208 corroborating evidence, alongside photographs and taxonomic examination by SEM, to further
209 improve our field-based identifications. Our approach allowed us to place the barcoded
210 specimens within a wider sampling context (of 25,000 New Guinean Lepidoptera sequences)
211 and to connect and refine species concepts across tens of years of sampling. We have released
212 data for 408 sequences representing 198 barcode clusters (putative species) on GenBank
213 (accession numbers pending) including the standard fields for the BARCODE data standard
214 and more data, including images and host plants, are available on BOLD

215 (www.boldsystems.org; Ratnasingham and Hebert 2007; Ratnasingham and Hebert 2013), in a
216 dataset accessible using a DOI (dx.doi.org/10.5883/DS-WILFC).

217 We used the leaf area sampled for herbivores to standardize insect abundance across sites and
218 *Ficus* species (Table 1). Specifically, we counted the number of leaves sampled for herbivores
219 on each tree. We then haphazardly sampled one leaf per tree and photographed it. We randomly
220 selected at least ten individuals per *Ficus* species and elevation (if available), measured the leaf
221 area from photographs and used these data to generate mean area of one leaf per *Ficus* species
222 per elevation. The final estimates of the leaf area sampled for herbivores were calculated by
223 multiplying the number of leaves sampled for a given *Ficus* species and elevation by the
224 corresponding mean area per leaf.

225 Non-volant mammals were surveyed at every elevation during the dry season of 2019 (June-
226 September). We sampled every site for ten consecutive nights using between 177 - 266 traps
227 per night. We used the following trap types: rat-type snap traps, medium Sherman box live
228 traps, Elliott box live traps, roofed Tomahawk cage live traps (cat size and squirrel size), and
229 roofed pitfall live traps (provided with hay or moss in higher altitudes). We positioned trapping
230 lines to start at least 50 m from each camp. The terrestrial traps were in 4-6 lines, at ~7 m
231 intervals and placed in diverse habitats (primary and secondary forest, creeks and food gardens).
232 The pitfalls were set 10 m apart along a 50 mm high barrier from a black plastic foil.
233 Additionally, we set a mean of 39 arboreal traps per site in accessible trees between a height of
234 seven to 15 meters at the altitudes of 700, 1700, and 2700 m a.s.l., using a combination of snap
235 traps, Sherman box live traps, and roofed Tomahawk cage live traps. We checked our traps at
236 least twice per 24-hour sampling period (dusk and sunrise). We baited all traps except for the
237 pitfalls before dawn, mostly with a mixture of peanut butter, tinned fish, and rolled oats or with
238 sweet potatoes. Arboreal traps were occasionally baited with banana. We also conducted
239 spotlighting and night walks with local hunters to find and capture mammals. We inspected

240 hunted animals, including older bones and skins, provided by local hunters (a total of 142 bones
241 and 18 skins and other remains). Finally, we conducted opportunistic interviews with local
242 inhabitants and recorded their mammal sightings for each site. The methods, including sampling
243 protocol, were approved by the PNG National Research Institute as a basis for the issue of a
244 Special Exemption Research Visa no. 99902702887. All animals were handled in accordance
245 with ethical guidelines approved by the State of Papua New Guinea.

246 Finally, we measured average temperature and humidity at each elevation as surrogates for
247 climatic changes along the gradient as described in detail in Sam et al. (2019a). Temperature
248 and humidity at each site were recorded every hour by R3120 dataloggers (Comet Systems,
249 Rožnov pod Radhoštěm) placed in the understory (1 m above ground). The temperature and
250 humidity were monitored for 12 months in 2010 and six months in 2013. Only at 700 m and
251 1200 m, where the original dataloggers were stolen, the data represent six months of
252 measurements in 2011 and six months of measurements in 2013. The values obtained were used
253 for calculating mean temperature and humidity at each elevation.

254 *Chemical Analysis.* We stored the leaf discs collected for alkaloid and polyphenol analysis (ca
255 0.5 g of dry leaf tissue in total for each individual) in 40 ml of HLPC grade acetone. In the
256 laboratory, we transferred this first acetone extract into a 50 ml falcon tube. We added 5 ml of
257 ultrapure water and concentrated the solution to water phase under a flow of nitrogen at room
258 temperature. We cut the leaf discs into smaller blades and transferred them into grinding tubes
259 (DT-50, IKA-Werke GmbH & Co. KG, Germany) containing 35 ml acetone/water (80:20, v/v).
260 We extracted the remaining alkaloids and polyphenols from the leaves by grinding them for 30
261 min using tube dispensers at room temperature (Ultra-Turrax Tube Drive, IKA-Werke GmbH
262 & Co. KG, Germany). Then we removed the leaf material and combined the extract with the
263 water phase obtained from the first acetone extraction above. We diluted the combined extract
264 with acetone to a uniform volume of 50 ml. We split this volume of extract, with 10 ml being

265 taken for polyphenol analysis and the remaining 40 ml being freeze-dried and used for alkaloid
266 analysis.

267 For the analysis of alkaloids, we suspended the dried extract in 10 ml of 5 % aq. HCl, vortexed
268 it and transferred it into a 15 ml Falcon tube and centrifuged it (9000 rpm, 10 min) before
269 transferring it to a 10 ml clear vial. Subsequently, we took 8 ml of the sample and adjusted its
270 pH to 10 with 25% NH_3. We extracted the alkaline solution in a 50 ml extraction funnel with
271 an equal volume of $CHCl_3$. We dried the chloroform solution under nitrogen and dissolved it
272 into ethanol, filtered it with a 0.2 µm PTFE filter and analysed it by UPLC-DAD-HESI-
273 Orbitrap-MS in the positive ion mode as described in Volf et al. (2018). The Acquity UPLC
274 systems consisted of a binary solvent manager, a sample manager, a column oven and a diode
275 array detector (Waters Corporation, Milford, MA, USA). We used an Acquity UPLC BEH
276 phenyl column (30 mm × 2.1 mm i.d., 1.7 µm; Waters Corporation). The UPLC system was
277 attached to a Q Exactive Orbitrap mass spectrometer with a heated electrospray ion source
278 (HESI II; Thermo Fisher Scientific GmbH, Bremen, Germany). The flow rate of the eluent was
279 0.650 mL/min and 0.1% HCOOH (A) and acetonitrile (B) were used in the gradient elution.
280 The gradient profile was as follows: 0–0.1 min: 97% A and 3% B (isocratic); 0.1–3.0 min:
281 97%–55% A and 3%–45% B (linear gradient); 3.0–5.0 min: 55%–10 % A and 45%–90% B
282 (linear gradient); 5.0–7.0 min: 10% A and 90% B (isocratic); 7.0–7.1 min: 10%–97% A and
283 90%–3% B (linear gradient); 7.1–7.2 min: 97% A and 3% B (isocratic). The injection volume
284 was 5 µL by full loop injection. The resolution of the mass spectrometer was set to 70 000,
285 automatic gain control (AGC) was 3×10^6, maximum injection time was 200 ms and the scan
286 range was 150–1200 *m/z*. The HESI conditions were as follows: spray voltage +4.0 kV,
287 capillary temperature 380°C, sheath gas (N_2) flow rate 60 units, auxiliary gas (N_2) flow rate 20
288 units and S-lens RF level 60. The mass spectrometer was calibrated with Pierce LTQ Velos ESI
289 Positive Ion Calibration Solution (Thermo Fischer Scientific, Rockford, IL, USA). We

290 processed the data with Thermo Xcalibur Qual Browser and Thermo Xcalibur Quan Browser
291 software packages (Thermo Fischer Scientific). To identify the alkaloids in the samples, we
292 took a portion of each alkaloid extract and pooled them together by plant species. We then
293 identified the alkaloids from each plant species by analysing the pooled samples with UPLC-
294 DAD-HESI-Orbitrap-MS/MS. We identified the compounds mainly by their molecular
295 formulas, which we constructed from the high-resolution mass spectrometric data and then
296 compared them to literature (e.g. Damu et al. 2005; Khan et al. 1993; Lee et al. 2011).
297 Additionally, we used UV spectra and MS^2 data for the compound identification (Baumgartner
298 et al. 1990; Bruneton et al. 1983; Cui et al. 2004; Xiang et al. 2002). We assigned the individual
299 compounds to following structural sub-groups: phenanthroindolizidines, *seco*-
300 phenanthroindolizidines, dehydro-*seco*-phenanthroindolizidines,
301 tetrahydrobenzylisoquinolines, and ficuseptamines. Subsequently, we semi-quantified the
302 alkaloids from the extracts with extracted ion chromatograms (EIC) as area of peak/mg (dry
303 weight) of plant material. To control for the possible fluctuations in the performance of the MS
304 system, we analysed a *Ficus septica* extract periodically and monitored the area of ficuseptine
305 with an EIC. We normalized all initial peak areas of the EICs of the analytes taking into account
306 the possible changes in the ficuseptine peak areas.

307 In the case of polyphenols, we ran two separate sets of assays. First, we quantified
308 concentrations of the main polyphenol sub-groups (in mg/g dry weight) by UPLC-QqQ-MS/MS
309 with the methods of Engström *et al.* (Engström et al. 2014; 2015) as described in e.g. Malisch
310 et al. (2016). The measured polyphenol sub-groups included (1) hydrolysable tannins that we
311 divided into galloyl derivatives and hexahydroxydiphenoyl derivatives (HDDP, ellagitannins),
312 (2) proanthocyanidins that we divided into procyanidin and prodelphinidin subunits, (3)
313 flavonol glycosides that we divided into kaempferol, quercetin and myricetin derivatives, and
314 (4) quinic acid derivatives. Second, from each species we chose all individual polyphenols we

315 were able to characterize on the basis of their UV and MS spectra (e.g. Moilanen et al. 2013).
316 For the quantification of the selected compounds from the negative ion full scan trace of the
317 UPLC-QqQ-MS/MS analyses, we used the *m/z* value of each compound that corresponded to
318 its deprotonated molecule. We quantified these compounds against calibration curves obtained
319 with our own standards (chlorogenic acid, epicatechin, quercetin galactoside, kaempferol
320 glucoside).

321 In addition, we ran two activity assays to quantify two major functions of polyphenols in anti-
322 herbivore protection – oxidative activity and protein precipitation capacity. We measured
323 polyphenol oxidative activity following Salminen & Karonen (2011) using gallic acid as the
324 standard. We measured protein precipitation capacity following Hagerman's radial diffusion
325 assay (Hagerman and Butler 1978) using pentagalloylglucose as the standard. Both assays gave
326 activities in mg/g dry weight.

327 Finally, we calculated the Shannon diversity index for alkaloids and polyphenols based on the
328 concentration (in area of peak/mg dry weight and in mg/g dry weight, respectively) of main
329 structural sub-groups listed above to account for structural diversity rather than for the number
330 of compounds in a sample.

331 *Statistical Analysis*. First, we explored overall elevational trends in the concentration and
332 diversity of main alkaloid and polyphenol structural sub-groups, and in the two measured
333 activities. We performed a *Redundancy Analysis* (RDA) with chemical data as the response
334 variables to analyse what percentage of variability in *Ficus* chemical profiles is explained by
335 the elevation. We used elevation as the explanatory variable and *Ficus* species identity as a
336 covariable defining permutation blocks. All chemical and activity data were log-transformed
337 prior to the analyses. We used *Ficus* species from individual elevations as samples. We
338 identified the relative effects of elevation and species identity on alkaloid and polyphenol
339 profiles using 9999 permutations and adjusted the explained variability following Ter Braak

340 and Smilauer (2012). In addition, in the next step we added average temperature and humidity
341 as surrogates for climatic variation along the gradient in the RDA and compared their effects
342 with the effect of elevation by variance partitioning. We conducted all multivariate analyses
343 conducted in CANOCO 5 (Ter Braak and Smilauer 2012).

344 Second, we used compound level data to test for specific elevational trends within focal
345 metabolite sub-groups as individual compounds can exhibit differential responses to elevation.
346 We modelled the overall correlation between the major classes of individual compounds
347 (alkaloids, non-flavonoid polyphenols, flavonoids (flavonols and flavones)) and elevation with
348 a separate linear mixed model for each polyphenol group using the R package 'nlme' (Pinheiro
349 et al. 2019) and a generalised linear mixed model for alkaloids as implemented in the R package
350 'lme4' (Bates et al. 2015). Such an approach is informative when both correlations and
351 opposing trends are expected between explanatory variables. In each model, we used the
352 concentration of each individual compound present in at least 50% of all species and samples
353 as the response variables. For analytical purposes we arranged the data so that the only unique
354 row value was concentration, each individual tree was coded as an observation (repeating 1-
355 142) while species (seven levels), elevation and compound identity were also included to group
356 the rows of concentration values. The fixed explanatory variables were elevation and
357 compound. We used *Ficus* species as the random effect. We also included a constant variance
358 function for the term 'compound' that allowed a different standard deviation for each level (e.g.
359 each compound) along with a general correlation structure between observations from the same
360 individual grouped within species. Finally, we ran mixed models for each individual compound,
361 with the random effect being species. Values in the alkaloid data set were typically high or zero,
362 due to a lack of universal compound presence, as such we converted alkaloid concentration to
363 binary values (presence or absence) and modelled this variable as having a binomial distribution
364 of errors (e.g. we used a generalised linear mixed model with a logit link).

365 Third, we analysed the elevational trends in insect abundance and the number of herbivores
366 shared between the studied *Ficus* species. To assess the elevational trends in leaf-chewer
367 abundance, we analysed the correlation between the elevation and log-transformed insect
368 abundance standardized by leaf area using linear mixed effect models. We used *Ficus* species
369 identity as a random factor. To assess the elevational trends in leaf-chewer specialization, we
370 calculated the dissimilarity of leaf-chewer communities between pairs of studied *Ficus* species
371 at individual elevations using Bray-Curtis abundance-based index and correlated it to elevation.
372 We used quasibinomial generalised linear models with the response variable Bray-Curtis
373 dissimilarity and the explanatory variable elevation, with and without a second order
374 polynomial fit. We chose a quasibinomial error structure because the response variable was
375 bounded by 0 and 1 and the model showed overdispersion. We compared the two models using
376 ANOVA with an F test and selected the more complex model if it explained significantly more
377 of the deviance.
378 To analyse the effects of the studied compounds on the leaf-chewer community structure, we
379 analysed the effects of alkaloids and polyphenols on leaf-chewer communities by hierarchical
380 *Canonical Correspondence Analysis* (CCA). Firstly, we ran an analysis of the effects of total
381 concentrations of alkaloids and polyphenols, their diversities, concentrations of their sub-
382 groups, and the two types of activities. Secondly, we ran an analysis of the effects of individual
383 compounds. We standardized insect data by leaf area, log-transformed them, and down-
384 weighted rare insect species (Ter Braak and Smilauer 2012). We used *Ficus* species trait means
385 at individual elevations as explanatory variables. We used *Ficus* species identity and elevation
386 as covariables and defined the permutation blocks by species identity. We identified the
387 chemical traits with significant effects using 9999 permutations and forward selection. We
388 conducted all multivariate analyses in CANOCO 5 (Ter Braak and Smilauer 2012).

389 We removed singleton herbivore species from all analyses. We also excluded *F. pungens*, which
390 had only a small leaf area sampled for herbivores, and the *F. itoana* complex from 2700m, for
391 which only one singleton herbivore was sampled, from all analyses using the insect data.

392

393 RESULTS

394 In total, we analysed 142 trees for polyphenols and alkaloids. We characterized a total of 29
395 alkaloids belonging to five alkaloid sub-groups and 49 polyphenols belonging to five
396 polyphenol sub-groups (Table S2 and S3). See Appendix 2 for details on their distribution
397 among the studied *Ficus* species.

398 Both polyphenol and alkaloid total and sub-group concentrations, their diversities, and activities
399 changed along the elevational gradient (Figure 1). Diversities of both alkaloids and polyphenols
400 showed an increasing trend along the gradient (Figure S2). There was an increase in alkaloid
401 concentration towards 2200 m while they decreased at 2700 m when not accounting for *Ficus*
402 species identity. This was caused by differential responses of individual alkaloid sub-groups to
403 elevation – phenanthroindolizidines, *seco*-phenanthroindolizidines showed an almost linear
404 increase towards higher elevations while dehydro-*seco*-phenanthroindolizidines and
405 tetrahydrobenzylisokinolines decreased towards higher elevations but more slowly, with a
406 plateau at mid elevations (ca 1700-2200 m a.s.l.). Ficuseptamines were not present at low
407 elevations and were found only in the *F. hahliana* population at 2700 m a.s.l.

408 Importantly, when analysed by the RDA accounting for species identity, most alkaloid
409 structural sub-groups, alkaloid concentration, and their diversity showed significant positive
410 correlation with elevation (Table S4). Elevation explained 7.4% of the adjusted variability in
411 alkaloids (pseudo-F=11.8, $p<0.001$, Figure 1). When combined with average temperature and
412 humidity, all three variables together explained 8.1% of the adjusted variability in alkaloids
413 (pseudo-F=5.0, $p=0.001$). Most of the variation was explained by the covariation between the

414 effects of elevation, average temperature and humidity (5.4% of the explained variability),
415 followed by a significant effect of elevation (1.9% of the explained variability), while the
416 unique effect of average temperature and humidity was not significant (0.8% of the explained
417 variability). The positive correlation in the concentration of several alkaloid groups with
418 elevation was also supported by generalised linear mixed effect models analysing the
419 elevational trends in individual compounds (t_{1826}=9.76 $p<0.001$). Ten out of 13 compounds
420 showed a significant positive trend with elevation (Table S5).

421 The concentration of total phenolics showed a hump-shaped distribution with the maximum at
422 mid elevations. The trend in total phenolics was driven by procyanidins, which were present in
423 the highest concentration. The overall trend in procyanidins was mirrored by the protein
424 precipitation capacity. When analysed by RDA analysis accounting for species identity,
425 polyphenols generally responded to elevation but showed various elevational trends (4.3% of
426 adjusted variability explained, pseudo-F=8.0, $p<0.001$). Polyphenol diversity, quercetins, and
427 quinic acid derivatives showed the strongest positive correlation with elevation whereas
428 prodelphinidins showed the strongest negative correlation with elevation. The response of other
429 polyphenols was much weaker. Galloyl and HHDP derivatives (hydrolysable tannins) were
430 present in very low levels (<0.2 mg/g) in only a few of the samples and did not show any reliable
431 patterns (Table S4). When combined with the average temperature and humidity, all three
432 variables together explained 8.4% of the adjusted variability in polyphenols (pseudo-F=5.1,
433 $p=0.001$). Most of the variation was explained by the unique effects of average temperature and
434 humidity (4.3%), followed by the unique effect of elevation (3.2%), and their covariation
435 (0.9%). The results from linear mixed effect models analysing the elevational trends in
436 individual polyphenol compounds broadly supported the multivariate results outlined above.
437 While flavonoids showed generally a positive correlation with elevation (t=6.086,$_{1262}$,
438 $p<0.001$), non-flavonoid polyphenols did not show a significant trend (t=-1.141,$_{980}$, $p=0.254$;

439 Table S5). Specifically, the concentrations of three out of four flavonoid compounds correlated
440 to elevation showed a positive elevational trend while only epicatechin was negatively
441 correlated ($t=-3.865_{134}$, $p<0.001$). On the contrary, the five non-flavonoid compounds
442 significantly correlated with elevation showing contrasting elevational trends. For example,
443 concentration of PCPC dimer 1 was negatively correlated ($t=-2.364_{134}$, $p<0.001$) while
444 chlorogenic acid was positively correlated ($t=4.272_{134}$, $p<0.001$).

445 We sampled 56 Lepidoptera species (387 individuals) and 148 Coleoptera species (839
446 individuals) during the survey of insect herbivore communities associated with our *Ficus*
447 species (Table S6, Appendix 1). Insect abundance decreased with elevation ($\chi^2(4)=9.5$,
448 $p=0.002$). The dissimilarity in leaf-chewer communities between coexisting pairs of *Ficus*
449 species measured by the Bray-Curtis index showed a hump-shaped distribution with the
450 minimum dissimilarity at mid elevations (Figure 2). The model including a second order
451 polynomial relationship between Bray-Curtis dissimilarity and elevation explained
452 significantly more deviance than the model with a first order relationship ($\Delta DF=1$,
453 $\Delta Deviance=0.487$, $F=4.736$, $p=0.034$). There was a significant curvilinear relationship between
454 elevation and Bray-Curtis dissimilarity ($F_{50,2}=6.671$, $p=0.044$).

455 CCA with forward selection identified ficuseptamines (pseudo-F=2.0, p=0.009) and alkaloid
456 diversity (pseudo-F=1.5, p=0.023) as the chemical traits with significant effects on
457 communities, together explaining 7.9% of the adjusted variability in leaf-chewer composition
458 (p=0.002 for the whole model including both traits). In the analysis of the effect of individual
459 compounds, ficuseptamine (A or B) or pentamethoxy-phenanthroindolizidine (the presence of
460 these compounds was collinear and their effects were identical; pseudo-F=2.1, p=0.002),
461 dihydroxy-dimethoxy-dehydro-seco-phenanthroindolizidine (pseudo-F=1.7, p=0.010),
462 kaempferol glucoside/galactoside (pseudo-F=1.7, p=0.046), hydroxy-trimethoxy-
463 phenanthroindolizidine (pseudo-F=1.5, p=0.042), 5-caffeoylquinic acid (chlorogenic acid,

pseudo-F=1.3, p=0.033), and epicatechin (pseudo-F=1.5, p=0.030) were selected as the variables that best explained herbivore community structure, together explaining 20.4 % of the adjusted variability in leaf-chewer composition ($p<0.001$ for the whole model including all six traits) (Figure 3).

We recorded 21 species of folivorous mammalian herbivores along the gradient (Table S7). Their species richness increased towards higher elevations, with the maximum number of species (15) recorded at 2700 m a.s.l. (Figure 2).

DISCUSSION

We quantified alkaloid and polyphenol-based defences in a community of fig species along a forested elevational gradient in Papua New Guinea. At the community level, we found a hump-shaped trend in the concentration of both alkaloids and phenolics. However, when we accounted for *Ficus* species identity, we found an elevational increase in almost all studied groups of alkaloids that likely serve as potent and phylogenetically restricted anti-herbivore and anti-pathogen defences. The elevational trends in polyphenols were more diverse. We suggest that the elevational trends in individual metabolites and their groups depend on their ecological function.

Elevational increase in plant defences is generally stimulated by unfavourable conditions at higher elevations that cause higher levels of environmental stress and render compensation for lost biomass more costly (Givnish 1999; Salgado et al. 2016). The unfavourable conditions in tropical montane forests involve negative effects of lower temperature and higher rainfall that reduce rates of N mineralization and increase nutrient leaching (Givnish 1999). Here the changes in temperature and humidity explained a larger share of variation in polyphenol composition than the changes in elevation itself. This suggests that these two variables may

488 play important roles in the elevational trends in some groups of polyphenols we studied.
489 Additionally, highland plants are also exposed to higher UV-irradiation. We observed a general
490 correlation between individual flavonoids and elevation while the direct response to elevation
491 was weaker or non-linear in the case of non-flavonoid polyphenols. We did not test the activity
492 of these particular metabolites. But flavonols, such as rutin, or kaempferol derivatives are
493 known for their strong role in anti-UV protection (Harborne and Williams 2000). As they did
494 not show a particularly strong correlation to insect communities, we suggest that their
495 elevational increase in *Ficus* could be most likely attributed the role they play in protecting
496 plants against detrimental environmental effects.

497 We found an elevational increase in almost all sub-groups of phenanthroidolizidine alkaloids.
498 This group of alkaloids represents a specialized defence in *Ficus* species, having a relatively
499 limited distribution among plants and strong effects on insect herbivores (Damu et al. 2005;
500 Han et al. 2013; Konno et al. 2004; Volf et al. 2018). The herbivore communities studied here
501 were most affected by ficuseptamines or pentamethoxy-phenanthroindolizidine, which were
502 unique to *F. hahliana* at the highest elevation. Alkaloid diversity also played a significant role.
503 This highlights the importance of rare or species-specific compounds for structuring insect
504 herbivore communities. Such defences may be especially important in the genus *Ficus*, which
505 harbours many herbivores able to potentially use multiple *Ficus* species as their hosts (Novotny
506 et al. 2010; Volf et al. 2018). Indeed, insect herbivore communities associated with lowland
507 *Ficus* populations are significantly structured by phenanthroidolizidine alkaloid diversity.
508 These alkaloids limit the sharing of certain herbivores between closely related *Ficus* hosts (Volf
509 et al. 2018) and may explain the turnover of specialist caterpillars across populations of the
510 same hosts at different elevations (Novotny et al. 2005). Unlike in the case of polyphenols, their
511 composition was not explained by the unique effects of climatic variables we measured. This

512 is suggestive of their defensive role against insect herbivores in this system, although laboratory
513 experiments with leaf extracts would be needed to confirm this.

514 The increased alkaloid concentration in high elevation figs may also serve to protect against
515 mammals and pathogens. We observed an elevational increase in species richness of folivorous
516 mammals. Although we cannot present abundance-based data, our findings are in line with the
517 observations of previous studies that report an elevational increase in abundance and diversity
518 of folivorous mammals, such as various possums or cuscuses, in the Austral-Papuan region
519 (Flannery 1995; Tallowin et al. 2017). Several possum species have been shown to be important
520 consumers of *Ficus* leaves (Kanowski et al. 2003). Their dietary preferences are known to be
521 affected by leaf secondary metabolites (Moore et al. 2005). It is thus possible that higher
522 concentration of alkaloids serves as an anti-mammalian defence in highland *Ficus*.
523 Furthermore, several phenanthroindolizidines, such as antofine, show strong anti-fungal
524 activities (Mogg et al. 2008). Fungal pathogens of plants generally decrease in abundance with
525 elevation (Geml et al. 2014). However, the relative costs of compensating for damage by fungal
526 pathogens increases with the elevation too (Brown and Vellend 2014), as with the relative costs
527 of herbivory, possibly making anti-pathogen defences more important. There are very likely
528 several biotic factors driving the elevational increase in *Ficus* alkaloids (and indeed other
529 compound groups). More data on mammalian herbivores, *Ficus* leaf pathogens, and the activity
530 of leaf extracts would be needed to identify their relative contribution to the observed trends.

531 Although we observed an elevational increase in alkaloids and flavonoids this trend was not
532 universal across all the metabolite groups studied. For example, populations of several *Ficus*
533 species from mid elevations were high in procyanidins and showed high protein precipitation
534 capacity. The ability of procyanidins to precipitate proteins is low in alkaline conditions as
535 found in the digestive tract of many caterpillars (Barbehenn et al. 2008; Roslin and Salminen
536 2008; Salminen and Karonen 2011). We did not find any correlation of procyanidins or protein

537 precipitation capacity to the insect community structure, in agreement with studies of lowland
538 fig species (Volf et al. 2018). The mid-elevational populations of *Ficus* also shared the highest
539 number of insect herbivores, suggesting that high procyanidin concentration did not strongly
540 restrict host preferences of the studied insects. On the other hand, procyanidins have been
541 shown to affect feeding preferences and reduce apparent N digestibility in mammalian
542 herbivores, which have low to neutral pH in their digestive system (Foley et al. 1999). The
543 increase in procyanidins towards mid elevations might be an adaptive response to increased
544 pressure from mammalian herbivores (Flannery 1995; Tallowin et al. 2017). However, unlike
545 mammalian species richness and abundance, procyanidins concentration and diversity
546 decreased between middle and high elevations. Procyanidins may thus serve another function
547 in this system, be driven by a combination of several factors, or simply show levels of
548 interspecific variation that are too high for detecting as a simple elevational trend. Relatively
549 low concentrations and high interspecific variation may also explain the limited responses to
550 elevation of other polyphenol groups despite their known biological effects on leaf-chewing
551 insects (Segar et al. 2017; Volf et al. 2018).

552 In agreement with Defossez et al. (2018) and Moreira et al. (2018), we suggest that instead of
553 universal directional trends, plant traits can show contrasting elevational trends depending on
554 their function. Using analyses based on multiple traits and linking them to datasets on
555 herbivores or pathogens is thus necessary to understand elevational trends and interactions in
556 plant defences (Defossez et al. 2018; Escobar-Bravo et al. 2017). Additionally, overall
557 elevational trends in plant defences may be largely dependent on the gradient studied and, in
558 particular, its span (Moreira et al. 2018). Unfavourable conditions can stimulate investment into
559 defensive traits (Givnish 1999; Salgado et al. 2016) but truly adverse conditions can limit
560 investment into secondary metabolites. This effect has been reported from plants exposed to
561 extreme conditions above the tree line (e.g. Pellissier et al. 2014). In turn, the levels of defensive

562 traits may be highest at elevations where conditions are adverse enough to increase the relative
563 costs of compensating for biomass loss, but not adverse enough to hamper secondary metabolite
564 production: resulting in the increase along the forested gradient studied here.

565 Interspecific variability between *Ficus* species can also play an important role in elevational
566 trends. We found some elevational increase in alkaloids and certain polyphenols in most of the
567 species. Exceptions to this rule included *F. copiosa*, which was relatively undefended at all
568 sites. Several previous studies have suggested that closely related species of host-plants often
569 diverge in their defences to avoid sharing insect herbivores (e.g. Becerra 2007; Kursar et al.
570 2009; Volf et al. 2019; Volf et al. 2018). Based on some of our results, it seems that closely
571 related host-plant species may differ in their investment in defences along elevational gradients.
572 As pointed out by Moreira et al. (2018), it would be interesting to analyse whether this can be
573 driven by the costs imposed by herbivores and resulting divergent selection. Indeed,
574 continuously forested gradients provide fascinating systems for studying the biotic and abiotic
575 selective pressures imposed on plants. While generalities are emerging, we suggest that
576 comparative multi-species studies sensitive to variation in herbivore and pathogen diversity are
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578

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596

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776 **Tables**

777 **Table 1** Number of individuals of *Ficus* species sampled for chemical traits and the leaf area
778 of conspecific individuals searched for herbivores (in brackets; m^2) across elevations. Species
779 and elevations with low leaf area sampled for herbivores are marked with NAs and were
780 excluded from the analyses using herbivore data. Species codes used in Figure 3 are given in
781 the brackets following the scientific names.

Species	200m	700m	1200m	1700m	2200m	2700m	Total
F. arfakensis (ARF)	5 (138.08)	5 (64.42)	5 (39.20)	3 (395.41)			17 (637.11)
F. copiosa (COP)	6 (47.41)	5 (165.96)	4 (18.13)	5 (116.67)			20 (348.17)
F. erythrosperma (ERY)		5 (46.63)	4 (114.73)	5 (120.34)			14 (281.7)
F. hahliana (HAH)	5 (148.30)	5 (246.15)	5 (274.08)	5 (96.82)	3 (661.90)	2 (1664.84)	25 (2497.05)
F. hombroniana (HOM)	3 (22.88)	5 (23.63)	5 (4.38)	5 (421.77)	5 (667.71)		23 (1140.37)
F. itoana complex (IXM)	5 (11.94)	4 (147.48)		5 (241.67)	5 (14.96)	5 (NA)	24 (416.05)
F. pungens (PUN)	5 (NA)	5 (NA)	4 (NA)	5 (NA)			19 (NA)
Total	29 (368.61)	34 (694.27)	27 (450.52)	33 (1392.27)	13 (1344.57)	7 (1664.84)	142 (5320.45)

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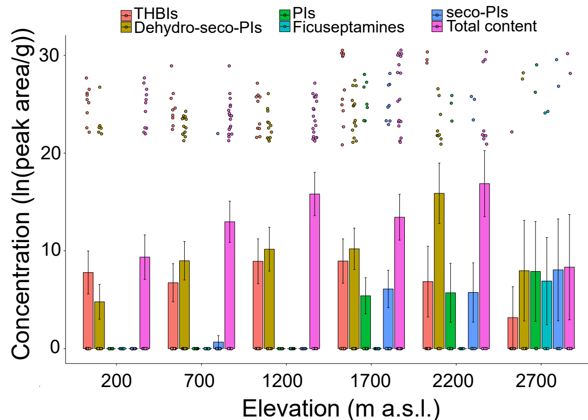
801 **Figure captions**

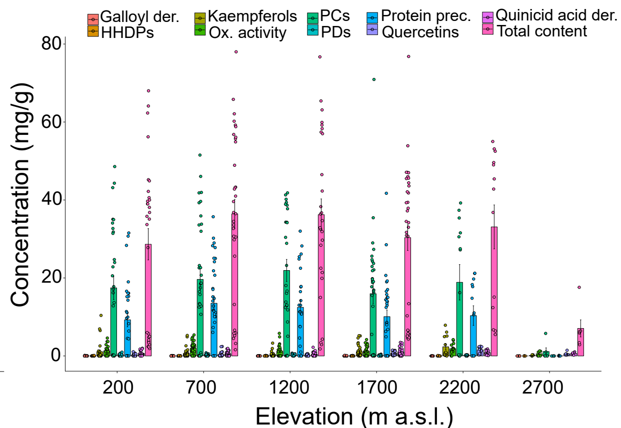
802 **Fig. 1** Elevational trends in individual alkaloid (A) and polyphenol (B). structural sub-groups
803 and effects of elevation on alkaloid (C) and polyphenol (D) composition in the studied *Ficus*.
804 The bars show means ± sd. The concentrations are given per g of dry leaf material. The overall
805 effects of elevation on *Ficus* alkaloids, polyphenols, and their main structural groups were
806 summarized by RDA. Elevation explained 7.4% of the adjusted variability in alkaloids (pseudo-
807 F=11.8, p<0.001,) and 4.3% of the adjusted variability in polyphenols (pseudo-F=8.0, p<0.001).
808 The RDA diagrams show the first two canonical axes. The thick arrow standing for elevation
809 points in the direction of its increase. The thin arrows point in the direction of the increase of
810 the studied chemical traits, while the angle between arrows indicates the correlation between
811 them. The correlation is positive when the angle is sharp and negative when the angle is larger
812 than 90 degrees.

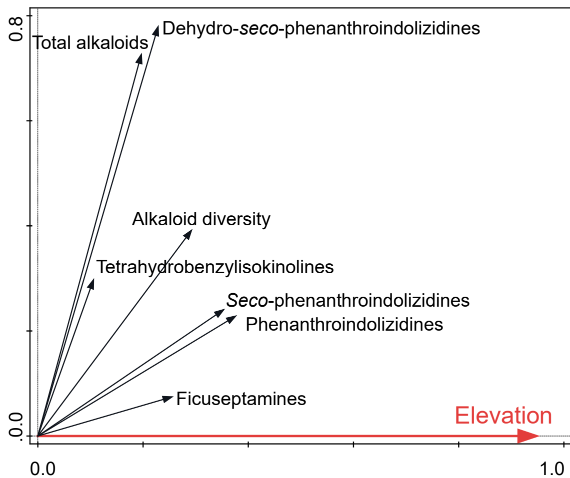
813 **Fig. 2** Elevational trends in insect abundance (A), pairwise insect community dissimilarity
814 between the studied *Ficus* species (B), and species richness of folivorous mammals along the
815 studied gradient (C). The insect abundance decreased with elevation ($\chi^2(4)=9.5$, p=0.0020). The
816 dissimilarity in leaf-chewer communities between coexisting pairs of *Ficus* species measured
817 by the Bray-Curtiss index showed a hump-shaped distribution with the minimum at mid
818 elevations ($F_{50,2}=6.671$, p=0.044). *F. pungens*, which had only a small leaf area sampled for
819 herbivores, and *F. itoana* complex from 2700m, from which only one singleton herbivore was
820 sampled, were removed from the analyses. This left *F. hahliana* as the only *Ficus* species with
821 insect data at 2700m a.s.l. and made bipartite comparisons of community dissimilarity
822 impossible at this elevation. The comparisons of dissimilarity in insect communities thus span
823 only up to 2200 m a.s.l. Mammal species were counted based on records from an active search,
824 identified bone remains, and by questionnaire survey among the local villagers.

825 **Fig. 3** Effects of *Ficus* chemical traits on the associated herbivore communities analysed with
826 CCA. CCA with forward selection identified ficuseptamines (pseudo-F=1.92.0, p=0.009) and
827 alkaloid diversity (pseudo-F=1.65, p=0.023) as the chemical traits with significant effects on
828 communities, together explaining 7.9% of the adjusted variability in leaf-chewer composition
829 (p=0.002 for the whole model including both traits) (A). In the analysis of the effect of
830 individual compounds, ficuseptamine A or B (pseudo-F=2.1, p=0.002), dihydroxy-dimethoxy-
831 dehydro-seco-phenanthroindolizidine (DDDSP, pseudo-F=1.7, p=0.010), kaempferol
832 glucoside/galactosidequercetin glycoside (Kaempferol GL/GA, pseudo-F=1.7, p=0.046),
833 hydroxy-trimethoxy-phenanthroindolizidine (HTP, pseudo-F=1.5, p=0.042), and 5-
834 caffeoylquinic acid (chlorogenic acid, pseudo-F=1.3, p=0.033), and epicatechin (pseudo-F=1.5,
835 p=0.030) were selected as the variables that best explained herbivore community structure,
836 together explaining 20.4% of the adjusted variability in leaf-chewer composition (p<0.001 for
837 the whole model including all four traits) (B). *F. pungens* (all elevations) and *F. itoana* complex
838 (2700m) had low leaf area sampled for herbivores and were excluded from the analysis. The
839 presence of ficuseptamine (A or B) and pentamethoxy-phenanthroindolizidine were collinear
840 and their effects were identical. Pentamethoxy-phenanthroindolizidine is not shown in the
841 figure. Elevations are colour coded. See Table 1 for the species codes. The CCA diagrams show
842 the first two canonical axes and the thick black arrows standing for chemical traits with
843 significant effects on herbivore community structure point in the direction of their increase. The
844 circles represent *Ficus* species and their insect communities from individual elevations. The
845 distance between the circles approximates their insect community dissimilarity as measured by
846 chi-square distances. Perpendicular projections of the circles onto the line overlaying the arrows
847 of chemical traits can be used to approximate the trait values in individual samples.

Figure 1







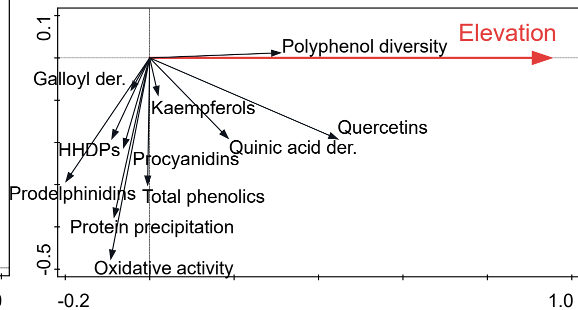
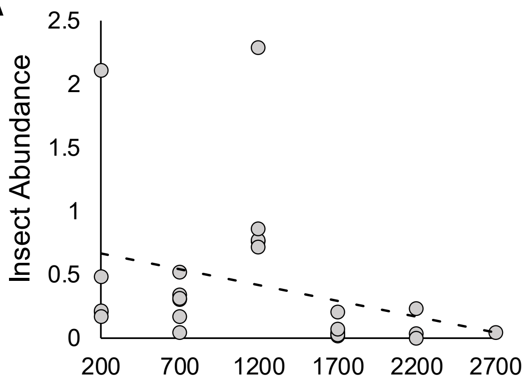
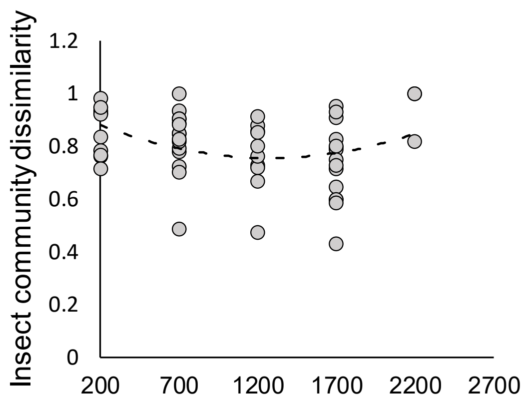


Figure 2





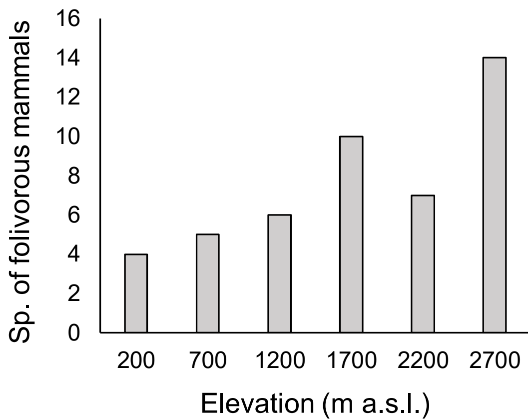


Figure 3

COMPOUND SPECIFIC TRENDS OF CHEMICAL DEFENCES IN *Ficus* ALONG AN ELEVATIONAL GRADIENT REFLECT A COMPLEX SELECTIVE LANDSCAPE

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Appendix 1 Taxonomic notes on Lepidoptera from Mount Wilhelm *Ficus* transect

Taxonomic notes on the lowland fauna can be found in the taxonomic appendices to Craft et al. (2010), Hrcek et al. (2011), and Segar et al. (2017). The highland fauna is more poorly known taxonomically, and many lineages need taxonomic revision.

Choreutidae: Under study by Jadranka Rota, see also Rota et al. (2016)

Erebidae: Taxonomy of diverse and highly sexually dimorphic genus *Ophyx* follows Holloway (1984). We reared three species in the enigmatic genus *Microstola*, which was relatively recently recognized as Arctiinae: Lithosiini, having originally been described in Gelechioidea (Watson et al. 1980). One of these species is a DNA barcode match to *Microstola ammoscia* Lower from Australia, and the other species are probably undescribed.

Euteliidae: *Paectes* sp. AAL8447 is an undescribed species near *Paectes prattii* Bethune-Baker, mentioned by Holloway 1985: 217.

Tortricidae: Taxonomy of the genera *Adoxophyes* and *Homona* follow Hulcr et al. (2007) and Miller et al. (2010). As noted by Hulcr et al. (2007: 551) the complex of highland New Guinea species around *Adoxophyes marmarygodes* needs revision.

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Appendix 2 Details on the distribution of alkaloids and polyphenols among the studied *Ficus* species

Dehydro-*seco*-phenanthroindolizidines were the most abundant group of studied alkaloids and were present in the majority of species (Fig. S3). Other groups of alkaloids showed large interspecific differences in their presence and absence among the studied *Ficus* species. For example, *F. copiosa* and *F. hombroniana* included only dehydro-*seco*-phenanthroindolizidines, while *F. hahliana* included phenanthroindolizidines, *seco*-phenanthroindolizidines, dehydro-*seco*-phenanthroindolizidines, tetrahydrobenzylisokinolines, and (uniquely for this data set) also ficuseptamines (Table S2 and S3).

In the case of polyphenols, proanthocyanidins were the most common sub-group, being present in almost all species except *F. copiosa* and *F. itoana* complex, reaching concentrations of up to ca 70 mg/g and average concentration ca 18 mg/g (Fig. S3). In all species that produced them, proanthocyanidins consisted primarily of procyanidin (PC) units, with less than 3% consisting of prodelphinidin (PD) units. In addition, we detected propelargonidin (PP) units in the individual proanthocyanidin oligomers, but practically only with *F. arfakensis* (Table S2). Other sub-groups of polyphenols showed much lower concentrations, not exceeding 10 mg/g, being absent in several samples and species. This especially applied to hydrolysable tannins (HHDP and galloyl derivatives) that were only detected as trace amounts in *F. hombroniana* and *F. pungens*, and myricetin derivatives that did not appear above our threshold value of 0.01 mg/g in any samples (Table S2). Our analyses also revealed large interspecific differences in the concentration of individual polyphenol compounds. For instance, only the foliage of the *F. itoana* complex contained the simple phenolics 1-3 and their glycosides. We also found a species-specific distribution in the case of flavonoid glycosides, with apigenin diglycoside being found only in *F. copiosa* (Table S3). Some of the individual polyphenols were more widely shared between species, e.g. chlorogenic acid was found in all samples except low elevation *F. hombriana*.

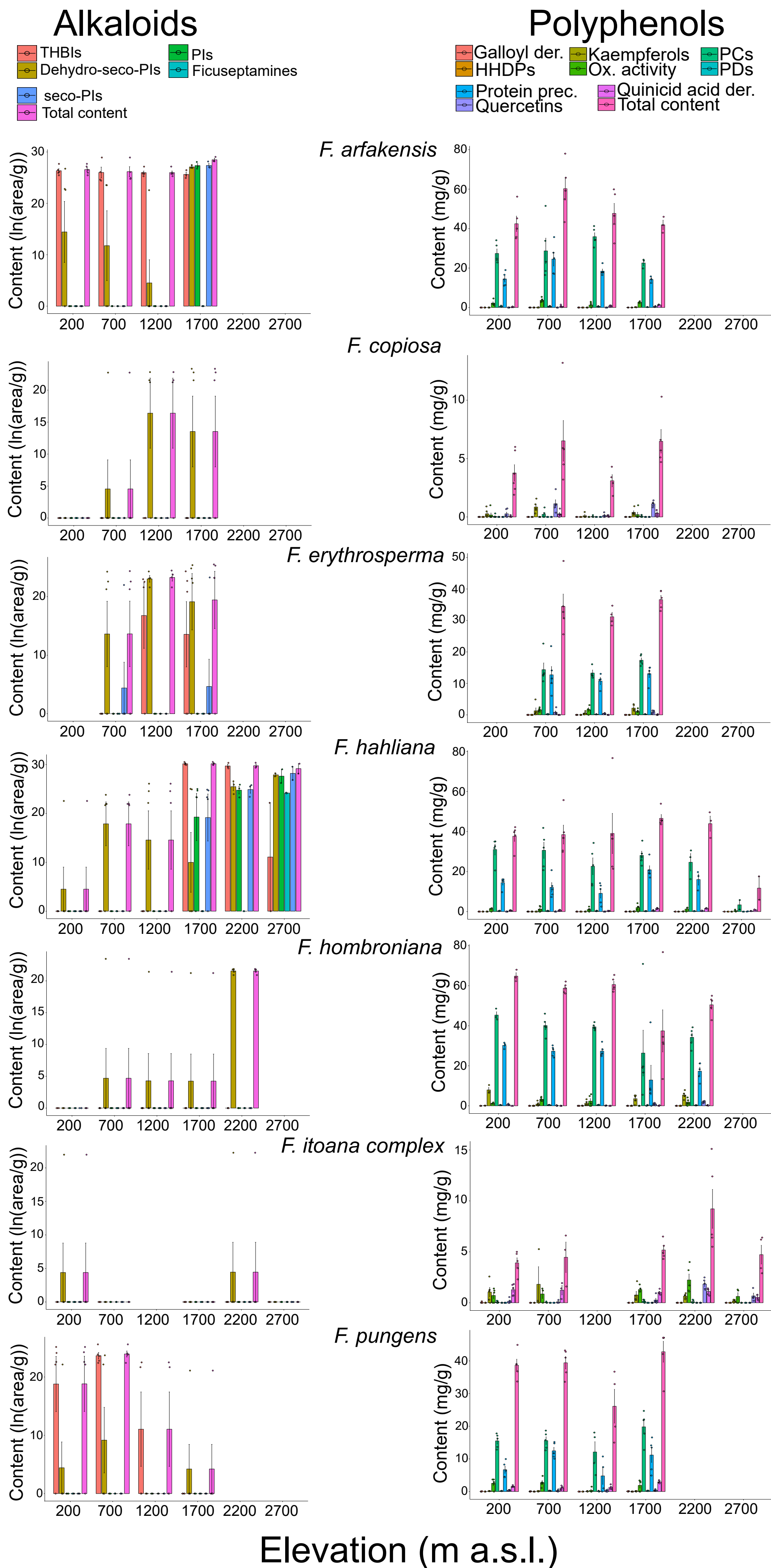

Fig. S3 Species specific trends in alkaloids and polyphenols in *Ficus arfakensis*, *F. copiosa*, *F. erythrosperma*, *F. hahliana*, *F. hombroniana*, *F. itoana* complex, and *F. pungens*. The bars show means ± sd. THBIs - tetrahydrobenzylisoquinolines, PIs - phenanthroindolizidines, HHDPs - hexahydroxydiphenoyl derivatives, PCs - procyanidin subunits of proanthocyanidins, PDs - prodelphinidin subunits of proanthocyanidins.

COMPOUND SPECIFIC TRENDS DRIVE AN ELEVATIONAL INCREASE OF CHEMICAL DEFENCES IN *Ficus*

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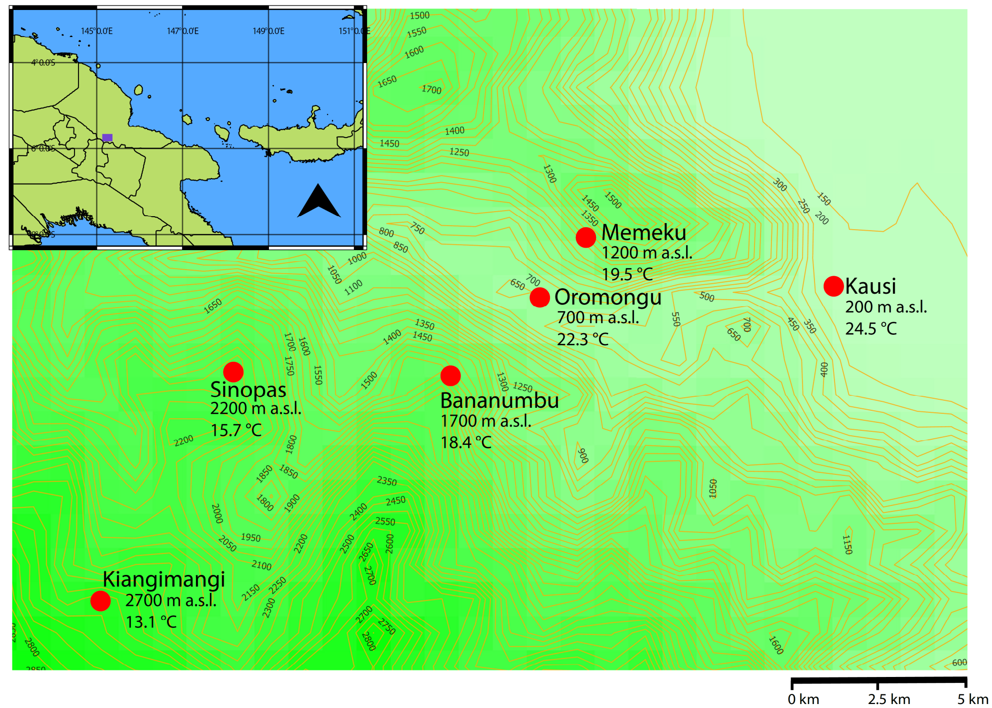

Fig. S1 *Ficus* collection sites along the Mt. Wilhelm gradient in Papua New Guinea, showing their elevation and mean temperature (see Table S1 for details).

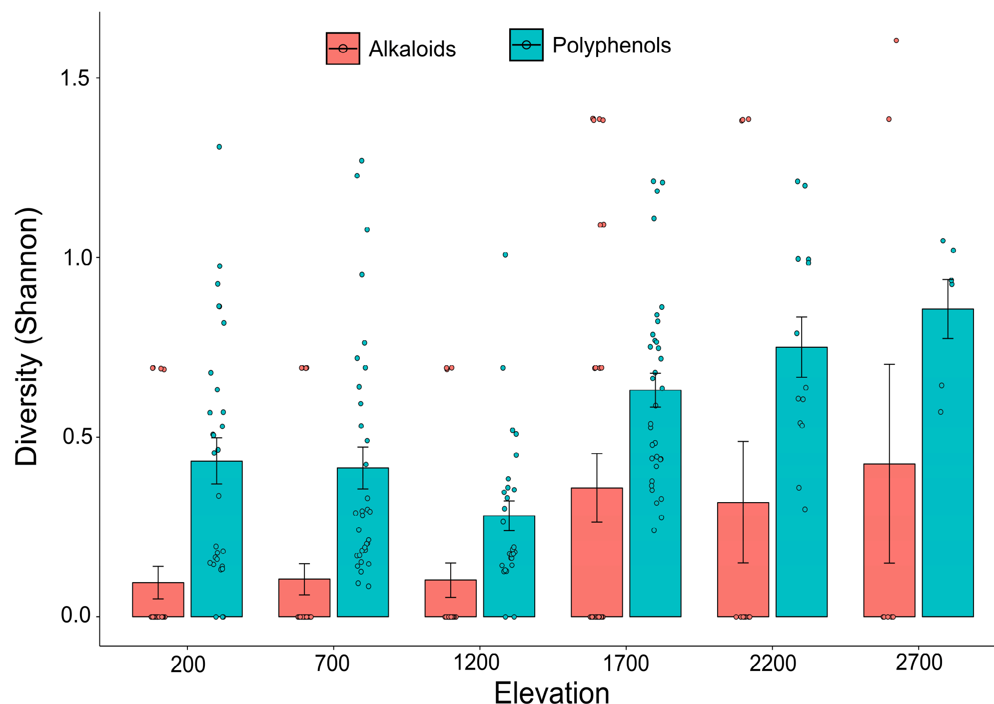

Fig S2 Elevational trends in diversity of alkaloids (red) and polyphenols (blue). The bars show means ± sd.

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Table S1 Names of sampling sites (alternative names in parentheses), their GPS coordinates, distance in a straight line to the site with the lowest elevation (DLE), and sampled *Ficus* species. Temperature and humidity at each site were measured every hour by R3120 dataloggers (Comet Systems, Rožnov pod Radhoštěm) placed in the understory. The temperature and humidity were monitored for 12 months in 2010 and six months in 2013. Only at 700 m and 1200 m, where the original dataloggers were stolen, the data represent six months of measurements in 2011 and six months of measurements in 2013.

Site name	Elevation (m)	Avg. T (°C)	Avg. Hum. (%)	Latitude	Longitude	DLE (km)
Kausi	200	24.5	97.4	05°44’33”S	145°20’01”E	0
Oromongu (Numba)	700	22.3	94.2	05°44’14”S	145°16’12”E	7
Memeku	1,200	19.5	95.8	05°43’18”S	145°16’17”E	7
Bananumbu	1,700	18.4	94.9	05°45’21”S	145°14’11”E	11
Sinopass	2,200	15.7	97.8	05°45’34”S	145°10’49”E	17
Kiangimangi (Bruno Sawmill)	2,700	13.1	99.3	05°48’57”S	145°09’02”E	22

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Table S2 Contents, diversities, subgroups, and activities of phenanthroidolizidine alkaloids (PIs), tetrahydrobenzylisoquinolines, ficuseptamines, and polyphenols. The table shows overall means and standard deviations for each species and individual means and standard deviations for each elevation. Diversities were measured as Shannon diversity indices based on the concentration of main structural polyphenol and alkaloid sub-groups. Alkaloid concentrations are in ln(peak area/mg). Polyphenol concentrations are in mg/g. Compounds found in concentrations lower than 0.1 mg/g are marked with tr (“traces”).

	Total alkaloids	Alkaloid diversity	PIs	Seco-PIs	Dehydro-seco-PIs	Tetrahydrobenzyl-isoquinolines	Ficuseptamines	Total phenolics	Polyphenol diversity	Oxidative activity	Protein precipitation	Galloyl der.	HHDPs	Procyanidins	Prodelphinidins	Quinic acid der.	Kaempferols	Quercetins
F.arfakensis	26.6±1.4	0.5±0.5	4.8±10.8	4.83±10.76	13.1±12.8	26.03±1.11	-	48.77±12.14	0.22±0.09	2.47±1.41	18.34±6.12	-	-	29.33±9.05	0.54±0.2	0.7±0.48	0.03±0.07	0.08±0.21
200m	26.6±0.9	0.4±0.4	-	-	14.4±13.8	26.35±0.84	-	42.46±8.67	0.17±0.02	2.26±1.33	14.48±3.39	-	-	27.41±5.01	0.68±0.18	0.31±0.15	-	-
700m	26.2±1.9	0.4±0.4	-	-	11.8±13.6	26.01±2.08	-	60.24±12.87	0.19±0.01	3.7±1.08	24.57±7.81	-	-	28.71±14.4	0.57±0.16	0.59±0.55	0.02±0.05	-
1200m	26.0±0.7	0.1±0.3	-	-	4.5±10.1	25.97±0.7	-	47.72±11.23	0.18±0.01	1.3±1.3	18.46±2.32	-	-	35.92±4.39	0.58±0.09	0.8±0.29	-	-
1700m	28.5±0.5	1.4±0.0	27.4±0.7	27.38±0.66	27.1±0.3	25.64±0.77	-	41.9±4.05	0.4±0.07	2.7±0.5	14.21±1.67	-	-	22.61±2.42	0.23±0.01	1.37±0.2	-	0.5±0.28
F. copiosa	7.8±10.9	-	-	-	7.8±10.9	-	-	5±2.76	0.72±0.47	0.1±0.31	-	-	-	0.1±0.2	0	0.18±0.22	0.41±0.43	0.67±0.62
200m	-	-	-	-	-	-	-	3.77±1.72	0.49±0.57	0.17±0.41	-	-	-	0.05±0.12	0	0.05±0.08	0.27±0.36	0.27±0.34
700m	4.6±10.2	-	-	-	4.6±10.2	-	-	6.52±3.89	1.04±0.23	-	-	-	-	0.23±0.36	0	0.26±0.27	0.85±0.49	1.13±0.75
1200m	16.4±11.0	-	-	-	16.4±11.0	-	-	3.1±1.04	0.42±0.51	-	-	-	-	0.04±0.08	0	0.08±0.09	0.11±0.21	0.13±0.19
1700m	13.5±12.4	-	-	-	13.5±12.4	-	-	6.48±2.25	0.89±0.26	0.2±0.45	-	-	-	0.07±0.09	0	0.32±0.27	0.39±0.3	1.12±0.23
F.erythrosperma	18.5±10.1	0.4±0.4	-	3.2±8.2	18.3±10.0	9.64±11.58	-	34.3±5.73	0.45±0.17	1.43±0.85	12.29±3.89	-	-	15.14±3.42	0.2±0.05	0.02±0.07	1.39±1.44	0.76±0.62
700m	13.6±12.5	0.1±0.3	-	4.4±9.8	13.6±12.5	-	-	34.48±8.72	0.42±0.22	1.62±0.56	12.73±5.87	-	-	14.38±4.73	0.21±0.08	0	1.3±2.03	0.84±0.92
1200m	23.3±1.2	0.5±0.3	-	-	23.0±1.1	16.78±11.2	-	31.18±2.76	0.35±0.08	1.63±1.27	10.76±2.3	-	-	13.3±1.9	0.18±0.03	0	0.56±0.47	0.44±0.13
1700m	19.4±10.9	0.6±0.3	-	4.7±10.4	19.1±10.7	13.57±12.45	-	36.62±2.92	0.56±0.14	1.08±0.75	13.08±2.7	-	-	17.37±1.63	0.2±0.02	0.05±0.11	2.15±1.01	0.93±0.5

	Total alkaloids	Alkaloid diversity	PIs	Seco-PIs	Dehydro-seco-PIs	Benzil-isokinolines	Ficuseptines	Total phenolics	Polyphenol diversity	Oxidative activity	Protein precipitation	Galloyl der.	HHDPs	Procyanidins	Prodelphinidins	Quinic acid der.	Kaempferols	Quercetins
F.hahliana	19.3±12.7	0.5±0.7	9.03±12.34	9.1±12.4	14.7±12.3	10.48±14.35	1.93±6.69	38.58±13.93	0.27±0.2	1.3±1.14	13.25±6.88	-	-.02	25.7±9.9	0.29±0.12	0.88±0.55	0.03±0.06	0.18±0.24
200m	4.5±10.1	-	-	-	4.5±10.1	-	-	37.68±5.72	0.15±0.02	1.26±0.71	14.48±2.71	-	-	31.01±6.01	0.37±0.07	0.53±0.13	-	-
700m	17.9±10.0	-	-	-	17.9±10.0	-	-	38.5±10.5	0.2±0.0	1.3±1.3	12.1±5.3	-	-	30.6±8.9	0.3±0.1	0.6±0.2	-	tr
1200m	14.6±13.4	-	-	-	14.6±13.4	-	-	39.1±22.4	0.2±0.0	1.0±1.0	9.1±5.2	-	-	22.7±9.3	0.3±0.1	0.3±0.1	-	-
1700m	30.2±0.2	1.0±0.6	19.26±10.79	19.2±10.7	10.0±13.7	30.2±0.2	-	46.6±4.2	0.4±0.1	2.0±1.6	20.9±4.5	-	tr	28.1±4.7	0.2±0.0	1.6±0.3	0.1±0.1	0.5±0.2
2200m	29.8±0.5	1.4±0.0	24.76±1.34	25.0±1.3	25.5±1.4	29.7±0.6	-	43.9±6.5	0.4±0.1	1.3±1.1	16.0±4.8	-	-	24.7±7.5	0.3±0.0	1.6±0.2	0.11±0.1	0.4±0.2
2700m	29.2±1.4	1.0±0.2	27.62±1.98	28.0±1.9	27.9±0.4	11.1±15.7	24.2±0.1	11.8±8.3	0.8±0.3	0.5±0.7	0.9±0.1	-	-	3.4±3.4	-	0.9±0.2	-	0.3±0.1
F. hombroniana	7.7±10.6	-	-	-	7.7±10.	-	-	53.5±14.3	0.4±0.3	1.8±1.9	22.4±10.1	-	tr	36.4±13.0	0.3±0.1	0.1±0.1	3.5±2.9	0.8±0.8
200m	-	-	-	-	-	-	-	64.8±2.9	0.5±0.1	1.3±0.5	30.2±1.7	-	0.1±0.0	45.4±2.9	0.4±0.0	-	8.0±2.3	0.7±0.3
700m	4.7±10.5	-	-	-	4.7±10.5	-	-	58.8±2.5	0.2±0.1	3.3±0.9	27.3±2.6	-	tr	40.1±4.5	0.4±0.0	-	0.9±1.1	0.1±0.1
1200m	4.3±9.6	-	-	-	4.3±9.6	-	-	60.6±3.5	0.2±0.1	2.4±2.8	27.3±2.9	-	0.1±0.1	39.5±1.6	0.5±0.1	-	1.3±1.3	0.1±0.1
1700m	4.3±9.5	-	-	-	4.3±9.5	-	-	37.5±23.5	0.6±0.3	-	12.9±16.5	-	tr	26.4±25.5	0.2±0.2	0.1±0.1	3.6±2.1	1.0±0.6
2200m	21.6±0.4	-	-	-	21.6±0.4	-	-	50.5±4.8	0.6±0.1	1.8±1.7	17.3±4.4	-	tr	34.2±4.7	0.3±0.0	0.2±0.1	5.4±1.8	2.0±0.5
***F.itoana* complex**	1.8±6.2	-	-	-	1.8±6.2	-	-	7.7±10.4	0.8±0.3	1.2±1.0	1.0±4.7	tr	-	1.4±6.4	tr	1.0±0.5	0.8±1.2	0.6±0.8
200m	4.4±9.8	-	-	-	4.4±9.8	-	-	3.9±1.1	0.8±0.3	0.7±0.7	-	tr	-	0.1±0.1	-	1.3±0.6	1.1±1.0	0.2±0.2
700m	-	-	-	-	-	-	-	16.7±24.5	0.4±0.3	1.1±0.8	5.7±11.4	-	-	7.7±15.4	0.1±0.2	0.9±0.9	1.4±2.6	0.1±0.1
1700m	-	-	-	-	-	-	-	5.2±0.9	1.0±0.2	1.3±0.3	-	-	-	0.2±0.2	-	1.0±0.2	0.8±0.8	0.3±0.4
2200m	4.5±10.0	-	-	-	4.5±10.0	-	-	9.2±4.3	1.1±0.1	2.2±1.3	-	-	-	0.1±0.1	-	1.1±0.3	0.7±0.3	1.8±0.5
2700m	-	-	-	-	-	-	-	4.7±1.8	0.9±0.2	0.6±0.7	-	-	-	-	-	0.5±0.3	0.2±0.1	0.7±0.5
F. pungens	14.7±11.6	0.1±0.3	-	-	4.7±9.3	13.5±11.9	-	37.3±8.6	0.5±0.1	2.0±1.6	9.0±4.8	tr	-	16.0±4.6	0.2±0.1	1.8±0.9	0.1±0.1	0.3±0.2
200m	18.8±10.6	0.1±0.3	-	-	4.4±9.9	18.8±10.6	-	38.7±4.1	0.5±0.6	2.5±1.5	6.7±2.4	tr	-	15.5±2.2	0.2±0.02	1.6±0.2	0.2±0.0	0.4±0.1
700m	24.0±1.1	0.3±0.4	-	-	9.2±12.6	23.7±1.2	-	39.4±4.2	0.4±0.1	2.8±1.3	12.5±2.3	tr	-	15.6±2.6	0.2±0.0	1.1±0.5	0.1±0.1	0.3±0.3
1200m	11.1±12.8	-	-	-	-	11.1±12.8	-	26.1±10.3	0.4±0.1	0.4±0.8	4.8±5.2	-	-	12.1±6.3	0.1±0.1	1.3±0.7	0.1±0.1	0.2±0.3
1700m	4.2±9.4	-	-	-	4.2±9.4	-	-	42.8±7.1	0.6±0.1	1.9±1.7	11.2±5.0	0.1±0.1	-	19.8±4.7	0.2±0.1	3.0±0.4	0.1±0.1	0.4±0.3

COMPOUND SPECIFIC TRENDS OF CHEMICAL DEFENCES IN *Ficus* ALONG AN ELEVATIONAL GRADIENT REFLECT A COMPLEX SELECTIVE LANDSCAPE

Journal of Chemical Ecology

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Table S3 Concentrations of individual alkaloids (ln(peak area/mg DW)) and polyphenols (mg/g DW) found in the studied species at different elevations. *Ficus* species are coded with three letter codes – *F. arfakensis* (ARF), *F. copiosa* (COP), *F. erythrosperma* (ERY), *F. hahliana* (HAH), *F. hombroniana* (HOM), *F. itoana* complex (IXM), and *F. pungens* (PUN). Compounds found in concentrations lower than 0.1 mg/g are marked with tr ("traces").

Akaloids	ARF 200	ARF 700	ARF 1200	ARF 1700	COP 200	COP 700	COP 1200	COP 1700	ERY 700	ERY 1200	ERY 1700	HAH 200	HAH 700	HAH 1200	HAH 1700	HAH 2200	HAH 2700	HOM 200	HOM 700	HOM 1200	HOM 1700	HOM 2200	IXM 200	IXM 700	IXM 1700	IXM 2200	IXM 2700	PUN 200	PUN 700	PUN 1200	PUN 1700
Trimethoxy-phenantroindolizidine (363 Da)	-	-	-	26.7± 0.9	-	-	-	-	-	-	-	-	-	-	13.8± 12.6	-	26.7± 2.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydroxy-trimethoxy-phenantroindolizidine (379 Da)	-	-	-	26.5± 0.4	-	-	-	-	-	-	-	-	-	-	18.0± 10.1	24.1± 1.1	26.7± 1.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tetramethoxy-phenantroindolizidine (393 Da)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	18.4± 10.3	23.6± 1.2	12.3± 17.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dihydroxy-dimethoxy-phenantroindolizidine (365 Da)	-	-	-	23.3± 0.3	-	-	-	-	-	-	-	-	-	-	-	15.2± 13.1	24.9± 1.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydroxy-tetramethoxy-phenantroindolizidine (409 Da)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	9.1± 12.5	8.0± 13.9	12.1± 17.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pentamethoxy-phenantroindolizidine (423 Da)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	23.2± 0.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dihydroxy-trimethoxy-phenantroindolizidine (395 Da)	-	-	-	23.5± 0.2	-	-	-	-	-	-	-	-	-	-	-	15.4± 13.3	25.1± 1.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Trihydroxy-dimethoxy-phenantroindolizidine (381 Da)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydroxy-methoxy-*seco*-phenantroindolizidine (321 Da)	-	-	-	25.2± 0.3	-	-	-	-	-	-	-	-	-	-	4.4± 9.9	-	12.9± 18.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dimethoxy-*seco*-phenantroindolizidine (335 Da)	-	-	-	22.9± 0.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydroxy-dimethoxy-*seco*-phenantroindolizidine (351 Da)	-	-	-	24.8± 0.5	-	-	-	-	4.4± 9.8	-	4.7± 10.4	-	-	-	4.5± 10.1	7.8± 13.5	26.1± 1.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Trimethoxy-*seco*-phenantroindolizidine (365 Da)	-	-	-	26.4± 1.1	-	-	-	-	-	-	-	-	-	-	9.4± 12.8	-	27.4± 2.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydroxy-trimethoxy-*seco*-phenantroindolizidine (381 Da)	-	-	-	26.3± 0.5	-	-	-	-	-	-	-	-	-	-	13.7± 12.5	24.2± 1.2	27.3± 1.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tetramethoxy-*seco*-phenantroindolizidine (395 Da)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	18.5± 10.4	24.1± 1.4	12.2± 17.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydroxy-methoxy-dehydro-*seco*-phenantroindolizidine (318 Da)	-	-	-	24.4± 0.7	-	-	-	9.1± 12.4	-	-	-	-	-	-	8.4± 11.5	7.0± 12.1	22.4± 2.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dimethoxy-dehydro-*seco*-phenantroindolizidine (332 Da)	14.4± 13.3	11.8± 13.6	4.5± 10.1	23.7± 0.8	-	4.6± 10.2	16.4± 11.0	8.7± 12.0	13.6± 12.5	23.0± 1.1	14.7± 13.4	4.5± 10.1	17.9± 10.0	14.6± 13.4	9.0± 12.3	25.4± 1.3	23.1± 0.2	-	4.7± 10.5	4.3± 9.6	4.3± 9.5	21.6± 0.4	4.4± 9.8	-	-	4.5± 10.0	-	4.4± 9.9	9.2± 12.6	-	4.2± 9.4
Hydroxy-dimethoxy-dehydro-*seco*-phenantroindolizidine (348 Da)	4.8± 10.7	-	-	23.7± 0.2	-	-	-	-	-	-	-	-	-	-	4.5± 10.1	-	26.0± 0.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Trimethoxy-dehydro-*seco*-phenantroindolizidine (362 Da)	-	-	-	26.7± 0.5	-	-	-	-	-	-	4.5± 10.0	-	-	-	9.7± 13.2	-	27.3± 0.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydroxy-trimethoxy-dehydro-*seco*-phenantroindolizidine (378 Da)	-	-	-	24.9± 0.3	-	-	-	-	-	-	-	-	-	-	4.5± 10.1	15.9± 13.8	26.3± 0.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Tetramethoxy-dehydro-*seco*-phenantroindolizidine (392 Da)	-	-	-	23.2± 0.7	-	-	-	-	-	-	-	-	-	-	4.9± 10.9	8.0± 13.8	24.0± 0.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dihydroxy-dimethoxy-dehydro-*seco*-phenantroindolizidine (364 Da)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	7.6± 13.2	24.8± 0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Alkaloids	**ARF 200**	**ARF 700**	**ARF 1200**	**ARF 1700**	**COP 200**	**COP 700**	**COP 1200**	**COP 1700**	**ERY 700**	**ERY 1200**	**ERY 1700**	**HAH 200**	**HAH 700**	**HAH 1200**	**HAH 1700**	**HAH 2200**	**HAH 2700**	**HOM 200**	**HOM 700**	**HOM 1200**	**HOM 1700**	**HOM 2200**	**MIC 200**	**MIC 700**	**MIC 1700**	**MIC 2200**	**MIC 2700**	**PUN 200**	**PUN 700**	**PUN 1200**	**PUN 1700**
Hydroxy-dimethoxy-N-methyl-tetrahydrobenzylisoquinoline (313 Da)	-	5.9± 11.7	-	25.4± 0.7	-	-	-	-	-	-	-	-	-	-	4.4± 9.9	14.8± 12.8	10.9± 15.4	-	-	-	-	-	-	-	-	-	-	18.7± 10.5	23.0± 1.3	11.1± 12.8	-
Dihydroxy-methoxy-N-methyl-tetrahydrobenzylisoquinoline (329 Da)	26.0± 1.0	19.3± 13.1	19.7± 11.1	16.2± 14.1	-	-	-	-	-	-	-	-	-	-	24.8± 0.5	26.3± 1.6	-	-	-	-	-	-	-	-	-	-	-	13.3± 12.1	18.6± 10.5	-	-
Hydroxy-trimethoxy-N-methyl-tetrahydrobenzylisoquinoline (343 Da)	24.9± 0.7	24.8± 1.8	24.5± 0.6	22.9± 0.3	-	-	-	-	-	-	-	-	-	-	25.4± 0.2	25.8± 0.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Dihydroxy-trimethoxy-N-methyl-tetrahydrobenzylisoquinoline (359 Da)	23.6± 0.2	24.0± 1.4	24.6± 0.7	-	-	-	-	-	-	-	4.7± 10.4	-	-	-	28.9± 0.7	28.7± 0.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Hydroxy-tetramethoxy-N-methyl-tetrahydrobenzylisoquinoline (373 Da)	-	17.2± 11.5	19.0± 10.6	-	-	-	-	-	-	16.8± 11.2	13.5± 12.4	-	-	-	29.7± 0.2	29.1± 0.4	10.5± 14.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pentamethoxy-N-methyl-tetrahydrobenzylisoquinoline (387 Da)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	26.0± 0.4	25.3± 0.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Trihydroxy-tetramethoxy-tetrahydrobenzylisoquinoline (405 Da)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	24.4± 0.2	23.5± 0.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ficuseptamine A or B (265 Da)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	24.2± 0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Polyphenols	**ARF 200**	**ARF 700**	**ARF 1200**	**ARF 1700**	**COP 200**	**COP 700**	**COP 1200**	**COP 1700**	**ERY 700**	**ERY 1200**	**ERY 1700**	**HAH 200**	**HAH 700**	**HAH 1200**	**HAH 1700**	**HAH 2200**	**HAH 2700**	**HOM 200**	**HOM 700**	**HOM 1200**	**HOM 1700**	**HOM 2200**	**MIC 200**	**MIC 700**	**MIC 1700**	**MIC 2200**	**MIC 2700**	**PUN 200**	**PUN 700**	**PUN 1200**	**PUN 1700**
3-Caffeoylquinic acid (354 Da)	-	0.2± 0.3	0.8± 0.5	1.1± 0.5	-	-	-	-	-	-	-	0.2± 0.1	tr	tr	3.2± 1.3	2.0± 1.0	tr	-	-	-	-	-	2.4± 1.4	1.7± 1.9	0.3± 0.1	0.3± 0.2	0.2± 0.1	tr	tr	tr	tr
5-Caffeoylquinic acid (chlorogenic acid)	0.5± 0.3	1.3± 1.7	1.2± 0.5	3.4± 0.6	0.2± 0.2	0.6± 0.8	0.2± 0.1	0.8± 0.7	-	-	0.2± 0.2	0.8± 0.3	1.1± 0.7	0.5± 0.2	2.7± 0.9	2.7± 0.8	2.1± 0.3	-	-	-	0.3± 0.3	0.6± 0.3	2.1± 1.0	1.5± 1.6	2.0± 0.3	3.0± 1.2	1.2± 0.8	3.2± 0.5	2.5± 1.8	2.5± 1.7	8.7± 2.7
4-Caffeoylquinic acid (354 Da)	-	tr	0.2± 0.1	0.2± 0.0	-	-	-	-	-	-	-	tr	tr	-	0.5± 0.1	0.3± 0.1	tr	-	-	-	-	-	1.1± 0.5	1.0± 1.0	0.3± 0.1	0.3± 0.1	tr	tr	tr	tr	0.2± 0.1
Catechin (290 Da)	1.5± 1.2	4.3± 1.3	4.4± 2.5	0.9± 0.2	0.3± 0.2	0.7± 0.6	tr	0.2± 0.2	1.9± 0.8	2.0± 0.6	3.7± 0.6	2.5± 1.2	2.7± 1.1	2.6± 0.8	0.4± 0.2	0.4± 0.0	tr	2.2± 0.2	3.0± 0.4	0.7± 0.3	2.0± 0.7	3.4± 0.3	tr	0.8± 1.6	tr	tr	tr	2.0± 0.3	2.4± 0.4	2.4± 0.6	1.7± 0.5
Epicatechin (290 Da)	4.7± 0.2	4.6± 2.5	2.2± 2.9	3.8± 0.2	-	-	-	-	1.6± 0.6	1.1± 0.2	0.2± 0.1	3.1± 0.8	3.5± 0.4	3.5± 0.5	3.2± 0.4	3.0± 0.3	1.1± 0.1	3.3± 0.5	2.8± 0.6	5.0± 0.2	1.5± 2.2	0.7± 0.3	-	0.6± 1.1	-	-	-	0.3± 0.1	0.3± 0.2	0.6± 0.3	0.2± 0.1
PCPC dimer 1 (578 Da)	tr	0.3± 0.1	0.3± 0.1	0.4± 0.1	-	-	-	-	1.1± 0.5	0.7± 0.1	1.2± 0.1	0.7± 0.1	0.6± 0.2	0.6± 0.2	0.2± 0.1	0.2± 0.0	-	2.5± 0.3	2.1± 0.5	0.5± 0.2	0.8± 0.3	1.8± 0.2	-	0.4± 0.9	-	-	-	1.2± 0.2	1.1± 0.1	1.1± 0.6	1.5± 0.4
PCPC dimer 2 (578 Da)	1.0± 0.3	2.0± 0.5	2.5± 1.0	0.9± 0.4	-	tr	-	-	0.7± 0.2	0.5± 0.2	0.9± 0.1	1.9± 0.5	2.0± 0.4	1.6± 0.5	0.4± 0.2	0.3± 0.0	-	3.1± 0.7	2.9± 0.3	1.7± 0.3	1.8± 0.8	3.0± 0.3	-	0.5± 1.1	-	-	-	0.9± 0.2	1.0± 0.2	0.7± 0.4	0.8± 0.3
PCPC dimer 3 (578 Da)	0.3± 0.1	0.3± 0.2	0.2± 0.3	5.8± 1.0	-	-	-	-	0.3± 0.2	0.2± 0.1	tr	0.3± 0.2	0.3± 0.0	0.3± 0.1	4.6± 1.5	4.4± 0.5	0.4± 0.1	2.1± 0.3	2.0± 1.5	7.8± 1.2	3.1± 6.7	0.2± 0.1	-	0.2± 0.4	-	-	-	0.2± 0.0	tr	tr	0.2± 0.1
Apigenin diglycoside 4 (578 Da)	-	-	-	0.7± 0.1	-	-	-	tr	-	-	-	-	-	-	-	0.8± 0.7	1.1± 0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PCPCPC trimer 1 (866 Da)	-	-	tr	tr	-	-	-	-	0.2± 0.1	tr	0.3± 0.0	tr	tr	tr	tr	-	-	1.4± 0.2	1.1± 0.3	0.3± 0.1	0.3± 0.2	1.0± 0.1	-	0.2± 0.4	-	-	-	0.2± 0.1	0.2± 0.1	tr	0.3± 0.1
PCPCPC trimer 2 (866 Da)	tr	0.3± 0.3	0.6± 0.4	0.3± 0.2	-	-	-	-	0.4± 0.2	0.2± 0.1	0.5± 0.0	0.4± 0.2	0.4± 0.2	0.3± 0.2	0.2± 0.1	0.2± 0.0	-	1.0± 0.1	0.9± 0.2	0.4± 0.1	0.6± 0.3	1.3± 0.2	-	0.2± 0.3	-	-	-	0.5± 0.1	0.4± 0.2	0.4± 0.2	0.7± 0.2
PCPP dimer 1 (562 Da)	0.5± 0.1	0.8± 0.5	0.2± 0.3	-	-	-	-	-	-	-	-	tr	tr	-	-	-	-	tr	tr	-	-	-	-	-	-	-	-	tr	-	tr	tr
PCPP dimer 2 (562 Da)	1.9± 0.3	3.3± 2.2	0.8± 0.9	-	-	0.1± 0.2	-	-	-	-	-	tr	tr	tr	-	-	-	-	tr	-	-	-	-	-	-	-	-	-	-	-	-
PCPPPP trimer 1 (834 Da)	tr	0.2± 0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PCPPPP trimer 2 (834 Da)	0.5± 0.1	1.1± 0.8	0.2± 0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PCPPPP trimer 3 (834 Da)	tr	0.4± 0.3	tr	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PCPC A-type dimer (576 Da)	-	-	tr	-	-	-	-	-	0.3± 0.2	0.2± 0.1	0.3± 0.1	tr	tr	0.2± 0.1	-	tr	tr	tr	0.2± 0.1	-	tr	tr	-	tr	-	-	-	tr	tr	tr	-
Simple phenolic 1 (206 Da)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.6± 0.8	4.2± 2.0	4.9± 3.0	-	-	-	-
Simple phenolic 2 (204 Da)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.6± 0.2	0.9± 0.3	0.6± 0.3	-	-	-	-
Simple phenolic 3 (236 Da)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2.1± 1.0	2.3± 1.3	0.6± 0.6	-	-	-	-
Phenolic glycoside 1 (366 Da)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.6± 0.2	0.3± 0.2	0.7± 0.3	-	-	-	-
Phenolic glycoside 2 (396 Da)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.3± 0.1	0.2± 0.1	0.2± 0.1	-	-	-	-
Apigenin glycoside 1 (432 Da)	0.2± 0.2	0.8± 0.6	0.4± 0.3	1.2± 1.0	-	tr	-	-	-	-	-	tr	tr	-	1.3± 0.3	0.6± 0.7	tr	-	-	-	-	-	-	-	-	-	-	1.6± 0.6	1.2± 1.2	1.2± 1.4	0.7± 0.3

Polyphenols	ARF 200	ARF 700	ARF 1200	ARF 1700	COP 200	COP 700	COP 1200	COP 1700	ERY 700	ERY 1200	ERY 1700	HAH 200	HAH 700	HAH 1200	HAH 1700	HAH 2200	HAH 2700	HOM 200	HOM 700	HOM 1200	HOM 1700	HOM 2200	MIC 200	MIC 700	MIC 1700	MIC 2200	MIC 2700	PUN 200	PUN 700	PUN 1200	PUN 1700
Apigenin glycoside 2 (432 Da)	1.0±0.6	2.6±1.0	2.9±1.4	-	-	tr	-	-	-	-	-	0.3±0.3	0.4±0.3	0.2±0.1	1.5±0.5	1.5±0.8	-	-	-	-	-	-	-	-	-	-	-	0.3±0.1	0.2±0.3	0.2±0.2	tr
Kaempferol C-glycoside 1 (448 Da)	0.7±0.2	1.2±0.6	0.5±0.2	1.6±1.7	-	0.2±0.2	-	0.2±0.2	-	-	-	tr	tr	-	1.6±1.0	0.6±0.4	0.3±0.1	-	-	-	-	-	-	-	-	-	-	6.2±1.1	3.8±2.8	1.4±1.9	5.9±1.7
Kaempferol C-glycoside 2 (448 Da)	1.0±0.2	1.5±0.6	0.9±0.2	tr	tr	0.3±0.3	tr	0.4±0.4	-	-	-	tr	0.2±0.1	tr	1.9±0.8	1.2±0.4	-	-	-	-	-	-	-	-	-	-	-	4.0±0.6	2.3±1.7	0.5±0.7	2.2±0.5
Kaempferol glycoside 1 (448 Da)	-	-	-	-	-	tr	-	tr	-	-	-	-	-	-	-	-	-	-	-	-	0.2±0.3	0.4±0.2	-	tr	tr	tr	-	-	-	-	-
Quercetin glycoside (464 Da)	-	-	-	tr	-	0.3±0.2	-	0.4±0.2	-	-	0.2±0.1	-	-	-	0.2±0.1	0.2±0.0	tr	tr	tr	-	0.2±0.2	0.6±0.3	-	-	tr	0.6±0.1	0.2±0.1	tr	tr	tr	0.2±0.1
Kaempferol glycoside 2 (490 Da)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.5±0.1	0.4±0.3	tr	0.8±0.2
Apigenin diglycoside 1 (534 Da)	-	-	-	-	0.3±0.2	0.6±0.2	0.4±0.3	0.9±0.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Apigenin diglycoside 2 (564 Da)	-	tr	tr	-	1.0±0.9	2.2±0.4	0.8±0.5	2.0±0.4	-	-	-	tr	tr	-	-	-	-	-	-	-	-	-	-	-	-	-	-	tr	tr	tr	-
Apigenin diglycoside 3 (564 Da)	-	-	-	-	0.3±0.3	0.8±0.3	0.2±0.2	0.9±0.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	tr	tr	-	-
Kaempferol diglycoside 1 (580 Da)	-	-	-	-	-	tr	-	0.3±0.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	tr	tr	-	tr
Kaempferol diglycoside 2 (580 Da)	-	-	-	-	tr	0.5±0.3	tr	0.9±0.9	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	tr	tr	-	tr
Kaempferol diglycoside 3 (592 Da)	-	-	-	-	-	-	-	-	tr	-	tr	-	-	-	-	-	-	-	-	tr	1.5±2.0	2.2±3.1	-	-	-	-	-	-	-	-	-
Apigenin diglycoside 5 (594 Da)	-	tr	0.2±0.1	-	0.9±1.1	0.9±0.5	0.2±0.3	0.8±0.6	-	-	-	tr	tr	-	tr	tr	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Kaempferol diglycoside 4 (594 Da)	-	-	-	0.4±0.3	-	-	-	-	-	-	-	-	-	-	-	0.5±0.5	1.3±0.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Apigenin diglycoside 6 (594 Da)	1.0±0.3	1.5±0.4	1.4±0.5	0.5±0.8	-	tr	-	-	-	-	-	1.4±0.5	1.3±0.5	0.5±0.3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Apigenin diglycoside 7 (594 Da)	-	-	-	5.5±0.6	tr	-	-	0.1±0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2±0.1	tr	-	-	-	-
Kaempferol diglycoside 5 (594 Da)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3.3±1.1	0.8±1.5	0.1±0.2	-	-	-	-	-	-	-	-	-	-	-
Kaempferol diglycoside 6 (594 Da)	-	-	-	-	tr	0.2±0.1	-	tr	-	-	-	-	-	-	-	-	-	-	-	-	0.8±1.4	1.6±1.2	0.3±0.5	0.8±1.6	0.2±0.3	tr	-	tr	tr	-	tr
Kaempferol diglycoside 7 (594 Da)	-	-	-	-	-	-	-	-	1.4±2.0	0.8±0.6	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Quercetin diglycoside 1 (608 Da)	-	-	-	-	-	0.1±0.2	tr	-	tr	-	tr	-	-	-	-	-	-	0.3±0.2	-	-	0.4±0.5	0.6±0.8	-	-	-	-	-	-	-	-	-
Flavonol diglycoside 1 (610 Da)	-	-	-	-	0.2±0.2	0.3±0.2	tr	0.4±0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Flavonol diglycoside 2 (610 Da)	-	-	-	1.3±0.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Quercetin diglycoside 2 (610 Da)	-	-	-	0.5±0.5	0.3±0.4	1.1±0.9	tr	1.1±0.6	-	-	-	-	-	-	0.3±0.2	0.2±0.1	0.2±0.1	-	-	-	0.6±0.6	2.5±1.3	0.2±0.3	tr	0.3±0.4	2.0±0.7	0.7±0.8	0.2±0.1	0.2±0.3	0.3±0.4	0.4±0.3
Quercetin diglycoside 3 (610 Da)	-	-	-	-	-	-	-	-	0.4±0.4	0.3±0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Flavonol triglycoside 2 (754 Da)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2±0.1	-	-	tr	-	-	-	-	-	-	-	-	-	-
Flavonol triglycoside 1 (738 Da)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.3±0.6	-	-	-	-	-	-	-	-	-	-	-	-	-

COMPOUND SPECIFIC TRENDS OF CHEMICAL DEFENCES IN *Ficus* ALONG AN ELEVATIONAL GRADIENT REFLECT A COMPLEX SELECTIVE LANDSCAPE

Journal of Chemical Ecology

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Table S4 Regression coefficients from RDA analysis for contents, diversities, subgroups, and activities of alkaloids and polyphenols. Identity of *Ficus* species was used as a grouping covariable.

Alkaloids	**R^2**
Seco-phenanthroindolizidines	0.3532
Phenanthroindolizidines	0.3759
Alkaloid diversity	0.2920
Tetrahydrobenzylisoquinolines	0.1058
Dehydro-*seco*-phenanthroindolizidines	0.2286
Total alkaloids	0.1967
Ficuseptamines	0.2550
Phenolics	**R^2**
Quercetins	0.4450
Polyphenol diversity	0.3093
Quinic acids	0.1855
Kaempferols	0.0199
Galloyls	-0.0437
Oxidative activity	-0.0920
Total phenolics	-0.0049
Protein precipitation	-0.0843
Procyanidins	-0.0616
HHDPs	-0.0893
Prodelphinidins	-0.1980

Table S5 The results of linear mixed model analyses including individual values as the response variable and elevation and compound identity as the fixed effect variables. Correlation between different compounds is included as a random effect (see main text for more details).

Fixed Effect (Alkaloids)	Estimate	Std.Error	DF	z-value	p-value
Elevation	0.0015	0.0002	1826	9.767	0.000
Dimethoxy-dehydro-*seco*-phenantroindolizidine (332 Da)	0.0007	0.0003	134	2.425	0.015
Dihydroxy-methoxy-N-methyl-tetrahydrobenzylisoquinoline (329 Da)	0.0002	0.0004	134	0.368	0.713
Hydroxy-trimethoxy-N-methyl-tetrahydrobenzylisoquinoline (343 Da)	0.0020	0.0008	134	2.408	0.016
Dihydroxy-trimethoxy-N-methyl-tetrahydrobenzylisoquinoline (359 Da)	0.0007	0.0005	134	1.444	0.149
Hydroxy-tetramethoxy-N-methyl-tetrahydrobenzylisoquinoline (373 Da)	0.0020	0.0006	134	3.374	0.001
Hydroxy-dimethoxy-N-methyl-tetrahydrobenzylisoquinoline (313 Da)	0.0005	0.0005	134	1.056	0.291
Hydroxy-trimethoxy-phenantroindolizidine (379 Da)	0.0135	0.0063	134	2.138	0.033
Hydroxy-trimethoxy-*seco*-phenantroindolizidine (381 Da)	0.0128	0.0061	134	2.104	0.035
Hydroxy-dimethoxy-*seco*-phenantroindolizidine (351 Da)	0.0031	0.0010	134	3.027	0.002
Trimethoxy-phenantroindolizidine (363 Da)	0.0034	0.0012	134	2.867	0.004
Trimethoxy-dehydro-*seco*-phenantroindolizidine (362 Da)	0.0036	0.0012	134	2.978	0.003
Pentamethoxy-N-methyl-tetrahydrobenzylisoquinoline (387 Da)	0.0020	0.0008	134	2.422	0.015
Hydroxy-trimethoxy-dehydro-*seco*-phenantroindolizidine (378 Da)	0.0065	0.0023	134	2.863	0.004

Fixed Effect (Non-Flavonoids)	Estimate	Std.Error	DF	t-value	p-value
Elevation	-0.00001	0.0000	1121	-1.141	0.254
5-Caffeoylquinic acid (chlorogenic acid)	0.00072	0.00017	134	4.272	0.000
PCPC dimer 1 (578 Da)	-0.00012	0.00005	134	-2.364	0.020
PCPC dimer 2 (578 Da)	-0.00025	0.00007	134	-3.427	0.001
PCPC dimer 3 (578 Da)	0.00041	0.00025	134	1.683	0.095
PCPCPC trimer 2 (866 Da)	0.00001	0.00003	134	0.211	0.833
PCPP dimer 2 (562 Da)	-0.00014	0.00007	134	-1.976	0.050
4-Caffeoylquinic acid (354 Da)	-0.00008	0.00003	134	-2.516	0.013
3-Caffeoylquinic acid (354 Da)	-0.00002	0.00010	134	-0.209	0.835

Fixed Effect (Flavonoids)	Estimate	Std.Error	DF	t-value	p-value
Elevation	0.00006	0.00001	1262	6.086	0.000
Catechin (290 Da)	-0.00022	0.00014	134	-1.544	0.125
Epicatechin (290 Da)	-0.00051	0.00014	134	-3.685	0.000
Kaempferol C-glycoside 1 (448 Da)	0.00006	0.00013	134	0.456	0.649
Kaempferol C-glycoside 2 (448 Da)	-0.00005	0.00009	134	-0.587	0.558
Quercetin diglycoside 2 (610 Da)	0.00043	0.00007	134	5.886	0.000
Apigenin glycoside 1 (432 Da)	0.00005	0.00006	134	0.936	0.351
Kaempferol diglycoside 3 (592 Da)	0.00021	0.00009	134	2.301	0.023
Quercetin glycoside (464 Da)	0.00014	0.00002	134	7.775	0.000

COMPOUND SPECIFIC TRENDS OF CHEMICAL DEFENCES IN *Ficus* ALONG AN ELEVATIONAL GRADIENT REFLECT A COMPLEX SELECTIVE LANDSCAPE

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Table S6 Insect data for Lepidoptera (LEP) and Coleoptera (COL) used in the analyses including the identifications and number of individuals found on the studied *Ficus* hosts at each elevation. Species codes are working names for each species that incorporate genus and species names along with BOLD BINs where available (several Lepidoptera species lacked adequate sequence data for BIN assignment.) Where assignation to species is not possible based on barcode data, we revert to field morphospecies codes used to assign specimens to provisional species. Species (morphospecies) codes are consistent across projects conducted at the New Guinea Binatang Rese arch Centre. In some cases morphospecies codes proved to consist of multiple BINs, or BINs represent multiple morphospecies codes, we provide details of such instances for future reference". Host codes: ARF - *Ficus arfakensis*, COP - *F. copiosa*, ERY - *F. erythrosperma*, HAH - *F. hahliana*, HOM - *F. hombroniana*, IXM - *F. itoana* complex.

Species	Order	Family	Species code	BIN	ARF 200	ARF 700	ARF 1200	ARF 1700	COP 200	COP 700	COP 1200	COP 1700	ERY 700	ERY 1200	ERY 1700	HAH 200	HAH 700	HAH 1200	HAH 1700	HAH 2200	HAH 2700	HOM 200	HOM 700	HOM 1200	HOM 1700	HOM 2200	IXM 200	IXM 700	IXM 1700	IXM 2200	IXM 2700
Glyphodes margaritaria	LEP	Crambidae	Glyphodes margaritaria	BOLD:AAA6663	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Talanga exquisitalis	LEP	Crambidae	Talanga exquisitalis	BOLD:AAD8828	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	2	0	0	0	0	1	4	0	0	2	0	0
Talanga polyzonalis	LEP	Crambidae	Talanga polyzonalis	BOLD:AAB2801	0	0	0	0	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Argina astrea	LEP	Erebidae	Argina astrea	BOLD:AAB3700	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Asota eusemioides	LEP	Erebidae	Asota eusemioides	BOLD:AAB2494	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Asota heliconia	LEP	Erebidae	Asota heliconia	BOLD:ABY6186	0	0	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
Asota plana	LEP	Erebidae	Asota plana	BOLD:AAA5335	0	0	0	26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Euproctis (s.l.) sp.	LEP	Erebidae	LYMA146		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Euproctis (s.l.) ADY2407	LEP	Erebidae	Euproctis sp. ADY2407	BOLD:ADY2407	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0
Microstola ammoscia species complex	LEP	Erebidae	ARCT057		0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Nudaria sp. ACM4366	LEP	Erebidae	Nudaria sp. ACM4366	BOLD:ACM4366	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Ophyx bilinea	LEP	Erebidae	Ophyx bilinea	BOLD:ADV2286	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
Ophyx crinipes	LEP	Erebidae	Ophyx crinipes	BOLD:AAC2400	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ophyx sp. ADW4520	LEP	Erebidae	Ophyx sp. ADW4520	BOLD:ADW4520	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ophyx sp. ADX2851	LEP	Erebidae	Ophyx sp. ADX2851	BOLD:ADX2851	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Ophyx sp.	LEP	Erebidae	NOCT 288		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Ophyx sp. 10899	LEP	Erebidae	Ophyx sp. AAQ2186	BOLD:AAQ2186	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0
Ophyx sp. AAL8318	LEP	Erebidae	Ophyx sp. AAL8318	BOLD:AAL8318	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
Ophyx sp. ACD4038	LEP	Erebidae	Ophyx sp. ACD4038	BOLD:ACD4038	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0
Ophyx sp. ADB6016	LEP	Erebidae	Ophyx sp. ADB6016	BOLD:ADB6016	0	0	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
Piratisca minax	LEP	Erebidae	Piratisca minax	BOLD:ADC1232	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Teia sp.	LEP	Erebidae	Teia sp. LYMA089		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
Alcis papuensis	LEP	Geometridae	Alcis papuensis ACB9977	BOLD:ACB9977	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Geometridae sp.	LEP	Geometridae	GEOM465		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
Gracillariidae sp.	LEP	Gracillariidae	GRAC024	BOLD:ADX2454	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0
Brenthia JR38	LEP	Choreutidae	Brenthia sp. AAB9832	BOLD:AAB9832	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	49	0	0	0	0	0	1	0	0	0	0	0
Brenthia JR62 AAC1201	LEP	Choreutidae	Brenthia sp. AAC1201	BOLD:AAC1201	0	0	4	0	0	0	0	0	0	11	0	4	12	32	0	0	0	0	1	0	0	0	0	0	0	0	0
Brenthia JR62 ADA7419	LEP	Choreutidae	Brenthia sp. ADA7419	BOLD:ADA7419	1	0	0	0	0	0	0	0	0	7	0	28	0	13	0	0	0	0	0	0	0	0	0	0	0	0	0
Brenthia n. sp.	LEP	Choreutidae	Brenthia n. sp. CHOR001		0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brenthia sp. ADT5777	LEP	Choreutidae	Brenthia sp. ADT5777	BOLD:ADT5777	4	0	2	0	49	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Brenthia sp. ADV7326	LEP	Choreutidae	Brenthia sp. ADV7326	BOLD:ADV7326	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0
Choreutidae sp.	LEP	Choreutidae	CHOR008		0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Choreutidae sp.	LEP	Choreutidae	CHOR030		0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Choreutidae sp.	LEP	Choreutidae	CHOR033		0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Choreutidae sp.	LEP	Choreutidae	CHOR036		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	2	0	0	0	0	0
Choreutis sp.	LEP	Choreutidae	CHOR035	BOLD:ADY4579	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0

Species	Order	Family	Species code	BIN	ARF 200	ARF 700	ARF 1200	ARF 1700	COP 200	COP 700	COP 1200	COP 1700	ERY 700	ERY 1200	ERY 1700	HAH 200	HAH 700	HAH 1200	HAH 1700	HAH 2200	HAH 2700	HOM 200	HOM 700	HOM 1200	HOM 1700	HOM 2200	IXM 200	IXM 700	IXM 1700	IXM 2200	IXM 2700
Choreutis cf. anthorma	LEP	Choreutidae	Choreutis cf. anthorma TORT005	BOLD:AAA3330	1	0	0	0	0	0	0	0	0	0	0	3	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Choreutis cf. niphocrypta	LEP	Choreutidae	Choreutis sp. ADV6794	BOLD:ADV6794	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Choreutis niphocrypta	LEP	Choreutidae	Choreutis niphocrypta	BOLD:AAC1274	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	4	0	0	0	0	0
Moca congrualis	LEP	Immidae	Moca congrualis	BOLD:AAC3049	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
Lactura sp. AAI2177	LEP	Lacturidae	Lactura sp. AAI2177	BOLD:AAI2177	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Lacturidae sp. ADX4389	LEP	Lacturidae	Lacturidae ADX4389	BOLD:ADX4389	0	0	0	0	0	0	0	0	4	12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Adoxophyes nr. marmarygodes	LEP	Tortricidae	Adoxophyes sp. AAP7433	BOLD:AAP7433	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Adoxophyes sp. AAP5694	LEP	Tortricidae	TORT283	BOLD:AAP5694	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
Adoxophyes sp. ACL3493	LEP	Tortricidae	Adoxophyes sp. ACL3493	BOLD:ACL3493	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Homona mermerodes	LEP	Tortricidae	Homona mermerodes	BOLD:AAA3890	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Isotenes sp. AAD4971	LEP	Tortricidae	Isotenes sp. AAD4971	BOLD:AAD4971	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Olethreutinae sp.	LEP	Tortricidae	Tortricidae sp. ADX6828	BOLD:ADX6828	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Scopariinae sp. ADX1782	LEP	Tortricidae	Scopariinae sp. ADX1782	BOLD:ADX1782	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	1
Tortricidae sp.	LEP	Tortricidae	TORT423		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Tortricidae sp. ACU4096	LEP	Tortricidae	Tortricidae sp. ACU4096	BOLD:ACU4096	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Tortricidae sp. ADT2318	LEP	Tortricidae	Tortricidae sp. ADT2318	BOLD:ADT2318	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Tortricidae sp. ADX6286	LEP	Tortricidae	Tortricidae sp. ADX6286	BOLD:ADX6286	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Tortricidae sp. AAF9348	LEP	Tortricidae	Tortricidae sp. AAF9348	BOLD:AAF9348	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Epipleminae sp.	LEP	Uraniidae	URAN032	BOLD:ADY5878	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
Lepidoptera sp. ADS7051	LEP		Lepidoptera sp. ADS7051	BOLD:ADS7051	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
Attelabidae sp.	COL	Attelabidae			0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Brentidae sp.	COL	Brentidae	BREN030		0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	5	0	0	0	0	0	0	0	0	0	0
Cerambycidae sp.	COL	Cerambycidae	CERA166		0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cerambycidae sp.	COL	Cerambycidae	CERA255		0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Cerambycidae sp.	COL	Cerambycidae	CERA265		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Cerambycidae sp.	COL	Cerambycidae	CERA268		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Cerambycidae sp.	COL	Cerambycidae	CERA279		0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Cerambycidae sp.	COL	Cerambycidae	CERA301		0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glenea heptagoda	COL	Cerambycidae	Glenea heptagoda		0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Symphyletes sp. nr. defloratus	COL	Cerambycidae	Symphyletes sp. nr. defloratus		0	0	0	0	0	2	0	0	2	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tmesisternus dissimilis	COL	Cerambycidae	Tmesisternus dissimilis		0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tmesisternus marmoratus	COL	Cerambycidae	Tmesisternus marmoratus		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
Tmesisternus sp. nr. longicollis	COL	Cerambycidae	Tmesisternus sp. nr. longicollis		0	0	0	0	0	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tmesisternus sp.	COL	Cerambycidae	Tmesisternus sp. CERA101		0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Coccinellidae sp.	COL	Coccinellidae	COCC001		0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Coccinellidae sp.	COL	Coccinellidae	COCC002		0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Coccinellidae sp.	COL	Coccinellidae	COCC003		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0
Alcidodes elegans	COL	Curculionidae	Alcidodes elegans		1	0	0	0	0	2	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Apirocalus ebrius	COL	Curculionidae	Apirocalus ebrius		7	0	0	1	6	2	1	0	0	0	0	8	1	1	0	0	4	3	1	0	0	0	1	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC001		0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC302		0	2	1	3	2	3	2	0	1	9	0	5	0	18	0	0	0	0	0	2	0	0	0	2	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC303		3	0	0	0	3	0	0	0	0	0	0	1	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC304		0	0	0	0	4	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC305		0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC306		0	2	1	0	0	4	1	0	3	1	0	1	6	4	0	0	0	0	1	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC309		0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC311		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
Curculionidae sp.	COL	Curculionidae	CURC312		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

Species	Order	Family	Species code	BIN	ARF 200	ARF 700	ARF 1200	ARF 1700	COP 200	COP 700	COP 1200	COP 1700	ERY 700	ERY 1200	ERY 1700	HAH 200	HAH 700	HAH 1200	HAH 1700	HAH 2200	HAH 2700	HOM 200	HOM 700	HOM 1200	HOM 1700	HOM 2200	IXM 200	IXM 700	IXM 1700	IXM 2200	IXM 2700
Curculionidae sp.	COL	Curculionidae	CURC313		0	4	0	0	0	1	0	0	0	0	0	0	1	0	0	2	1	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC316		0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC010		1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC317		0	0	0	0	0	4	0	0	1	5	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC318		1	3	0	0	0	14	0	0	1	2	0	0	9	1	0	0	0	0	0	1	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC323		0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC325		0	0	1	0	0	1	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC328		0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC332		0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC333		0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC334		0	0	0	0	0	1	0	0	0	2	0	0	0	2	0	0	0	0	0	1	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC337		0	0	1	0	0	0	1	0	0	1	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC338		0	0	0	0	0	0	1	0	0	4	0	0	0	3	0	0	0	0	0	1	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC012		0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC340		0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC341		0	0	0	0	0	0	0	0	0	0	0	0	1	3	0	0	0	0	0	1	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC342		0	0	0	0	0	0	0	0	0	1	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC343		0	0	0	0	0	0	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC347		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	8	0	0	0	2	5	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC349		0	0	0	4	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2	0	0	0	1	0	0
Curculionidae sp.	COL	Curculionidae	CURC353		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Curculionidae sp.	COL	Curculionidae	CURC355		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC358		0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC363		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	1	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC026		0	2	0	11	1	0	0	0	0	0	0	0	1	0	1	0	0	0	0	0	1	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC370		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	0	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC372		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC377		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC379		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC380		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC381		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Curculionidae sp.	COL	Curculionidae	CURC386		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC388		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC390		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC392		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC067		0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC393		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC398		0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC399		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC402		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC412		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC415		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC421		0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC423		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC441		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	2	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC442		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0
Curculionidae sp.	COL	Curculionidae	CURC267		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
Curculionidae sp.	COL	Curculionidae	CURC443		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0

Species	Order	Family	Species code	BIN	ARF 200	ARF 700	ARF 1200	ARF 1700	COP 200	COP 700	COP 1200	COP 1700	ERY 700	ERY 1200	ERY 1700	HAH 200	HAH 700	HAH 1200	HAH 1700	HAH 2200	HAH 2700	HOM 200	HOM 700	HOM 1200	HOM 1700	HOM 2200	IXM 200	IXM 700	IXM 1700	IXM 2200	IXM 2700
Curculionidae sp.	COL	Curculionidae	CURC274		0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	1	0	0
Curculionidae sp.	COL	Curculionidae	CURC283		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
Curculionidae sp.	COL	Curculionidae	CURC288		0	0	0	0	0	0	1	0	0	2	0	0	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0
Lobotrachelus sp.	COL	Curculionidae	Lobotrachelus? sp. CURC045		0	0	0	1	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Oribius sp.	COL	Curculionidae	Oribius sp. CURC033		1	0	0	3	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trigonopterus sp.	COL	Curculionidae	Trigonopterus sp. CURC027		0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Atysa sp.	COL	Chrysomelidae	Atysa sp. CHRY004		2	1	6	3	0	2	2	1	0	0	1	2	5	8	0	37	7	0	0	0	1	2	0	0	1	0	0
Aulacophora sp. nr. pallidifasciata	COL	Chrysomelidae	Aulacophora sp. nr. pallidifasciata		0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aulacophora sp.	COL	Chrysomelidae	Aulacophora sp. CHRY041		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
Deretrichia sp.	COL	Chrysomelidae	Deretrichia sp. CHRY054		0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Chrysomelidae sp.	COL	Chrysomelidae	CHRY141		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
Chrysomelidae sp.	COL	Chrysomelidae	CHRY228		0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
Chrysomelidae sp.	COL	Chrysomelidae	CHRY233		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
Chrysomelidae sp.	COL	Chrysomelidae	CHRY234		0	0	0	0	4	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chrysomelidae sp.	COL	Chrysomelidae	CHRY242		0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chrysomelidae sp.	COL	Chrysomelidae	CHRY249		0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chrysomelidae sp.	COL	Chrysomelidae	CHRY257		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
Chrysomelidae sp.	COL	Chrysomelidae	CHRY266		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
Chrysomelidae sp.	COL	Chrysomelidae	CHRY278		0	0	1	0	0	0	0	0	0	0	0	0	1	3	0	0	0	0	0	0	0	0	0	0	0	0	0
Chrysomelidae sp.	COL	Chrysomelidae	CHRY281		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
Chrysomelidae sp.	COL	Chrysomelidae	CHRY284		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0	0
Chrysomelidae sp.	COL	Chrysomelidae	CHRY144		0	0	0	0	0	5	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Chrysomelidae sp.	COL	Chrysomelidae	CHRY286		0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chrysomelidae sp.	COL	Chrysomelidae	CHRY288		0	1	9	5	0	0	1	1	0	29	2	0	1	57	1	1	1	0	0	3	1	0	0	0	4	0	0
Chrysomelidae sp.	COL	Chrysomelidae	CHRY290		0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Chrysomelidae sp.	COL	Chrysomelidae	CHRY296		0	0	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
Chrysomelidae sp.	COL	Chrysomelidae	CHRY305		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0
Chrysomelidae sp.	COL	Chrysomelidae	CHRY306		0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	4	0	0	0	0	0	0	0	0	0	0	0
Chrysomelidae sp.	COL	Chrysomelidae	CHRY310		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
Chrysomelidae sp.	COL	Chrysomelidae	CHRY313		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
Chrysomelidae sp.	COL	Chrysomelidae	CHRY314		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
Chrysomelidae sp.	COL	Chrysomelidae	CHRY322		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Chrysomelidae sp.	COL	Chrysomelidae	CHRY221		0	0	0	2	0	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chrysomelidae sp.	COL	Chrysomelidae	CHRY324		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Chrysomelidae sp.	COL	Chrysomelidae	CHRY326		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Chrysomelidae sp.	COL	Chrysomelidae	CHRY335		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0	0	0	0	0	0	0	0
Chrysomelidae sp.	COL	Chrysomelidae	CHRY338		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
Chrysomelidae sp.	COL	Chrysomelidae	CHRY342		0	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chrysomelidae sp.	COL	Chrysomelidae	CHRY344		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
Chrysomelidae sp.	COL	Chrysomelidae	CHRY351		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0
Chrysomelidae sp.	COL	Chrysomelidae	CHRY378		0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Chrysomelidae sp.	COL	Chrysomelidae	CHRY379		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
Chrysomelidae sp.	COL	Chrysomelidae	CHRY283		0	2	1	1	0	7	0	0	0	0	0	0	3	1	0	0	0	0	1	0	0	0	0	0	2	0	0
Chrysomelidae sp.	COL	Chrysomelidae	CHRY115		0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0
Chrysomelidae sp.	COL	Chrysomelidae	CHRY039		0	1	0	0	0	15	2	0	0	0	0	1	8	1	0	0	0	0	0	0	0	0	0	5	0	0	0
Chrysomelidae sp.	COL	Chrysomelidae	CHRY087		0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Chrysomelidae sp.	COL	Chrysomelidae	CHRY188		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
Chrysomelidae sp.	COL	Chrysomelidae	CHRY211		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

Species	Order	Family	Species code	BIN	ARF 200	ARF 700	ARF 1200	ARF 1700	COP 200	COP 700	COP 1200	COP 1700	ERY 700	ERY 1200	ERY 1700	HAH 200	HAH 700	HAH 1200	HAH 1700	HAH 2200	HAH 2700	HOM 200	HOM 700	HOM 1200	HOM 1700	HOM 2200	IXM 200	IXM 700	IXM 1700	IXM 2200	IXM 2700
Lomirana sp. nr. sulcipennis	COL	Chrysomelidae	Lomirana sp. nr. sulcipennis		0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Monolepta sp.	COL	Chrysomelidae	Monolepta sp. CHRY088		2	0	0	0	0	0	0	0	1	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Prasyptera sp. nr. ornata	COL	Chrysomelidae	Prasyptera sp. nr. ornata		0	0	0	0	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhyparida picticollis	COL	Chrysomelidae	Rhyparida picticollis		0	0	0	0	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rhyparidella sewana	COL	Chrysomelidae	Rhyparidella sewana gr.		0	0	0	1	0	3	0	0	1	0	1	0	2	0	0	0	0	0	0	0	1	0	0	0	0	0	0
Sastra sp.	COL	Chrysomelidae	Sastra sp. CHRY033		0	1	0	0	0	2	0	0	0	0	0	4	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sastra sp.	COL	Chrysomelidae	Sastra sp. CHRY131		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
Stethotes nigritula	COL	Chrysomelidae	Stethotes nigritula		0	1	0	1	0	5	1	0	0	3	0	0	2	11	0	0	2	0	0	0	0	0	0	0	0	0	0
Lycidae sp.	COL	Lycidae	LYCI001		0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Lycidae sp.	COL	Lycidae	LYCI003		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
Lycidae sp.	COL	Lycidae	LYCI004		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Lycidae sp.	COL	Lycidae	LYCI007		0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lycidae sp.	COL	Lycidae	LYCI012		2	1	0	1	0	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Lycidae sp.	COL	Lycidae	LYCI024		0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lycidae sp.	COL	Lycidae	LYCI026		0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Lycidae sp.	COL	Lycidae	LYCI027		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
Lycidae sp.	COL	Lycidae	LYCI033		1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Scarabaeidae sp.	COL	Scarabaeidae	SCAR009		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0
Holostrongylium gravidum	COL	Tenebrionidae	Holostrongylium gravidum tuperipenne		2	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
Tenebrionidae sp.	COL	Tenebrionidae	LAGR009		0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tenebrionidae sp.	COL	Tenebrionidae	LAGR011		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
Tenebrionidae sp.	COL	Tenebrionidae	TENE016		0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Tenebrionidae sp.	COL	Tenebrionidae	TENE018		0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
Tenebrionidae sp.	COL	Tenebrionidae	TENE027		0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0

COMPOUND SPECIFIC TRENDS OF CHEMICAL DEFENCES IN *Ficus* ALONG AN ELEVATIONAL GRADIENT REFLECT A COMPLEX SELECTIVE LANDSCAPE

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Table S7. Folivorous mammal species detected at the studied elevations. Mammal species were counted based on records from an active search, trapping, identified bone remains, and by questionnaire survey among the local villagers.

				Elevation (m a.s.l.)					
				200	**700**	**1200**	**1700**	**2200**	**2700**
Species	**Common name**	**Family**	**Order**						
Dendrolagus dorianus	Doria's Tree-kangaroo	Macropodidae	Diprotodontia						present
Dendrolagus goodfellowi	Goodfellow's Tree-kangaroo	Macropodidae	Diprotodontia			present	present	present	present
Dorcopsulus vanheurni	Small Forest Wallaby	Macropodidae	Diprotodontia		present	present	present		present
Thylogale browni	New Guinea Pademelon	Macropodidae	Diprotodontia	present	present			present	
Phalanger carmelitae	Mountain Cuscus	Phalangeridae	Diprotodontia				present		present
Phalanger gymnotis	Ground Cuscus	Phalangeridae	Diprotodontia		present	present	present	present	
Phalanger orientalis	Northern Common Cuscus	Phalangeridae	Diprotodontia	present	present				
Phalanger sericeus	Silky Cuscus	Phalangeridae	Diprotodontia						present
Spilocuscus maculatus	Common Spotted Cuscus	Phalangeridae	Diprotodontia	present	present				
Pseudochirops corinnae	Plush-coated Ring-tailed Possum	Pseudocheiridae	Diprotodontia				present	present	present
Pseudochirops cupreus	Coppery Ring-tailed Possum	Pseudocheiridae	Diprotodontia				present	present	present
Pseudochirulus larvatus	Masked Ring-tailed Possum	Pseudocheiridae	Diprotodontia			present	present	present	present
Chiruromys sp. A	Tree Mouse sp.	Muridae	Rodentia			present			
Hyomys goliath	Eastern White-eared Giant Rat	Muridae	Rodentia				present		present
Mallomys aroaensis	De Vis's Woolly Rat	Muridae	Rodentia						present
Mallomys rothschildi	Rothschild's Woollly Rat	Muridae	Rodentia				present	present	present
Pogonomys loriae	Loria´s Tree Mouse	Muridae	Rodentia				present		
Pogonomys macrourus	Chestnut Tree Mouse	Muridae	Rodentia			present			

				Elevation (m a.s.l.)					
				200	**700**	**1200**	**1700**	**2200**	**2700**
Species	**Common name**	**Family**	**Order**						
Pogonomys sp. A	Tree Mouse sp.	Muridae	Rodentia	present					present
Rattus "niobe"	Eastern New Guinea Mountain Rat	Muridae	Rodentia						present
Unknown sp. B	Unknown	Muridae	Rodentia						present