School of Molecular and Life Sciences

Determining prey spectra of carnivorous sundews using DNA-metabarcoding

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Master of Research (Environmental Science)

of Curtin University

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Declaration

To the best of my knowledge and belief this thesis contains no material previously published by any other person except where due acknowledgment has been made.

This thesis contains no material which has been accepted for the award of any other degree or diploma in any university.

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Human Ethics (For projects involving human participants/tissue, etc) The research presented and reported in this thesis was conducted in accordance with the National Health and Medical Research Council National Statement on Ethical Conduct in Human Research (2007) – updated March 2014. The proposed research study received human research ethics approval from the Curtin University Human Research Ethics Committee (EC00262), Approval Number #........................
or
Animal Ethics (For projects involving animal use) The research presented and reported in this thesis was conducted in compliance with the National Health and Medical Research Council Australian code for the care and use of animals for scientific purposes 8th edition (2013). The proposed research study received animal ethics approval from the Curtin University Animal Ethics Committee, Approval Number #........................

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Date: 04/03/2020 ………………………..

29

Acknowledgement of Country

We acknowledge that Curtin University works across hundreds of traditional lands and custodial groups in Australia, and with First Nations people around the globe. We wish to pay our deepest respects to their ancestors and members of their communities, past, present, and to their emerging leaders. Our passion and commitment to work with all Australians and peoples from across the world, including our First Nations peoples are at the core of the work we do, reflective of our institutions' values and commitment to our role as leaders in the Reconciliation space in Australia.

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Abstract

Prey spectra (the number and composition of captured prey) represent a crucial ecological aspect for carnivorous plants (CPs), yet remain poorly studied. This study examined prey spectra of five closely-related, scented or unscented, often sympatric species of *Drosera* sect. *Arachnopus* (Droseraceae) from the remote Kimberley Region of Western Australia, investigating the possibility that species or individuals with scented traps would exhibit prey selectivity in capturing more or different prey. A novel DNA-metabarcoding approach was compared with traditional morphology-based methods to identify prey spectra, with *in-situ* macro photography used as a plausibility control for the DNA-metabarcoding (to detect false-positives and contaminations) and to facilitate prey quantity estimations. This approach allowed accurate analysis of CP prey spectra (even of heavily digested prey lacking characteristic morphological features) at a taxonomic resolution and level of completeness unachievable by morphology-based methods. Significant intraspecific prey spectra differences were mostly attributed to different habitats, but multiple comparisons of sympatric odourless *D. cucullata* and scented *D. fragrans* showed possible selective attraction of winged Hymenoptera by the latter. This study provides the first evidence for differential prey selectivity among morphologically similar, sympatric *Drosera* species and further strongly supports the existence of a scent-based prey attraction strategy in *D. fragrans*.

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Attribution statement

	Conception and Design	Acquisition of Data and Method	Data Conditioning and Manipulation	Analysis and Statistical Method	Interpretation and Discussion	Final Approval	Total % contribution
Thilo Krueger	60	80	90	50	70	70	70
I acknowledge that these represent my contribution to the above research output Signed: 03/03/2021							
Andreas Fleischmann	30	10	5	10	20	15	15
I acknowledge that these represent my contribution to the above research output Signed: 04/03/2021							
Adam Cross	10	10	5	40	10	15	15
I acknowledge that these represent my contribution to the above research output Signed: 04/03/2021							

1. Thesis introduction

1.1. Prey spectra of carnivorous plants

Carnivorous Plants (CPs) comprise approximately 860 of the World's known ~300,000 (Christenhusz & Byng, 2016) species of flowering plants and are characterised by adaptations to trap, kill and derive nutritional benefit from animal prey (Fleischmann et al., 2018a). These plants produce modified leaves which function as adhesive traps (*Byblis* Salisb., *Drosera* L., *Drosophyllum* Link, *Philcoxia* P.Taylor & V.C.Souza, *Pinguicula* L., *Roridula* L., *Triphyophyllum* Airy Shaw), pitcher traps (*Brocchinia* Schult. f., *Catopsis* Griseb., *Cephalotus* Labill., *Darlingtonia* Torr., *Heliamphora* Benth., *Nepenthes* L., *Sarracenia* L.), snap traps (*Aldrovanda* L., *Dionaea* Sol. ex J.Ellis), suction traps (*Utricularia* L.) or eel traps (*Genlisea* A.St.-Hil.; Fleischmann et al., 2018a).

While CPs have been a popular subject of research since the early work of Darwin (1875), studies focussing on the number and composition of captured prey (i.e. their prey spectra) remain surprisingly scarce (Darnowski et al., 2018). Most previous studies investigated the pitcher trap genera *Nepenthes* and *Sarracenia* from south-east Asia and North America, respectively, encountering a wide range of arthropod prey frequently dominated by ants (family Formicidae; Ellison & Gotelli, 2009; Chin et al., 2014). Despite Australia representing the World's centre of CP diversity (ca. 250 species; Lowrie, 2014), the prey spectra of only fifteen species from this continent have been previously studied: fourteen species of *Drosera* which mostly captured Diptera or Collembola (Dixon et al., 1980; Verbeek & Boasson, 1993; Krueger et al., 2020), and one aquatic species of *Utricularia* which mostly captured algae (Płachno et al., 2015).

Identifying the prey spectra of CPs is crucial for understanding their ecological requirements, as they typically grow in soils containing very low levels of nitrogen and phosphorous and obtaining these macronutrients by means of arthropod prey capture forms an essential component of the survival strategy of these species (Adamec & Pavlovič, 2018). In addition, Australia is also the country with the highest number of threatened CP species globally (30 species; see Cross et al., 2020). In the face of continued loss and rapid deterioration of suitable habitats across the country (Cross et al., 2020), a better understanding of CP prey spectra could help inform effective strategies for conservation and especially the ecological restoration of remnant habitats.

1.2. Methods for analysing carnivorous plant prey spectra

Prey spectra of CPs have typically been analysed by collecting samples of their trapping leaves in ethanol before identifying captured prey items under a stereo microscope using morphological features (e.g., Zamora 1990; Verbeek & Boasson, 1993; Chin et al., 2014; Bertol et al., 2015; Annis et al., 2018). However, this method is extremely time-intensive and requires considerable knowledge of arthropod taxonomy or help of insect specialists to identify prey items, and identification may still be impossible for heavily digested prey items lacking very characteristic features (Krueger et al., 2020; Hausmann et al., 2020a). It can also be logistically challenging or impossible in extremely remote study sites such as the northern Kimberley Region of Western Australia, which is only accessible by air travel during the CP growing season.

Krueger et al. (2020) tested an alternative approach by capturing macro-photographs of prey items in a systematic pattern, thus allowing rapid and non-invasive *in-situ* collection of prey spectra data even under extreme fieldwork conditions and without harming plants. This method

186 provided highly accurate prey quantity (prey count) data and compositional prey spectra data
187 of roughly comparable resolution to the traditional method of microscopic analysis in a
188 laboratory (Krueger et al., 2020). Although a significant proportion of prey items were deemed
189 unidentifiable and identification below the taxonomic level of order was extremely difficult,
190 often impossible (Krueger et al., 2020), many previous studies using the traditional approach
191 either only collected freshly captured, identifiable prey items (e.g., Thum, 1986; Hagan et al.,
192 2008; Costa et al., 2014; Annis et al., 2018) or reported unrealistically low percentages of
193 unidentifiable prey (e.g., Verbeek & Boasson, 1993). Such unidentifiable prey items usually
194 result from soft-bodied insects (e.g., small Diptera) quickly becoming digested, thus losing their
195 characteristic morphological features allowing identification (Krueger et al., 2020).
196
197 DNA-metabarcoding, described in detail in section 3.1.1., has so far only been used in one study
198 of CP prey spectra (Lekesyte et al., 2018). However, it promises to allow identification even of
199 prey items unidentifiable by morphological-based approaches. In addition, DNA-
200 metabarcoding could yield unprecedented taxonomic resolution of CP prey spectra, even down
201 to species-level (Lekesyte et al., 2018). Prey quantity or biomass estimates, however, are very
202 difficult or impossible with current DNA-metabarcoding methods (Deagle et al., 2013;
203 Lekesyte et al., 2018).
204

205 1.3. *Drosera* section *Arachnopus* – the spider-leg sundews

206 Among Australia's CPs, the *Drosera indica* L. complex (*Drosera* sect. *Arachnopus* Planch.) is
207 of particular interest for prey spectra research. The twelve currently-described species of the
208 group are relatively large representatives of *Drosera*, growing up to 90 cm tall with up to 22
209 cm long trapping leaves that are covered with stalked, mucilage-secreting glands which serve
210 to visually attract and trap small prey animals (functioning as thread-like adhesive-type traps;

211 Fleischmann et al., 2018b; Krueger et al., 2020; T. Krueger, pers. obs.; Figure 1). In contrast to
212 most other *Drosera,* which are perennials, all species of *D.* sect. *Arachnopus* are annuals. These
213 species appear to strongly depend on supplementary nutrition provided by prey capture during
214 their short growth cycle, as is evident from growing experiments (A. Fleischmann, pers. obs.)
215 and judging from prey nutrient acquisition data available for the likewise annual *D.*
216 *glanduligera* Lehm. as opposed to its perennial congeners of similar size from *D.* sect.
217 *Bryastrum* Planch. (Karlsson & Pate, 1992).
218
219 Several species of *D.* sect. *Arachnopus* have evolved highly-specific morphological features
220 which have been hypothesised to function as prey attractants, such as trap scent and eglandular
221 appendages (Fleischmann, 2016; Hartmeyer & Hartmeyer, 2006; Krueger et al., 2020; see
222 section 2.1.2.). In addition, up to five species of *D.* sect. *Arachnopus* can occur in sympatry in
223 Western Australia's Kimberley Region (T. Krueger, pers. obs.). Sympatry is crucial for
224 comparative prey spectra studies as sympatric species (especially those occurring in exactly the
225 same microhabitat) are exposed to the same available prey in the habitat (Krueger et al., 2020).
226 Studies of sympatric populations thus allow for investigation of the role of species-specific
227 morphological features in prey capture.
228
229 Studying seven species of *D.* sect. *Arachnopus* at multiple locations with partially sympatric
230 occurrences in northern Australia using *in-situ* macro photography, Krueger et al. (2020) found
231 that their prey spectra, which mainly consist of flying insects of the order Diptera, is primarily
232 influenced by species-specific trap size differences. Species with larger trapping leaves were
233 found to capture both a larger number of prey items and physically larger prey than sympatric
234 species with smaller trapping leaves (Krueger et al., 2020). In addition, allopatric comparisons
235 of individual species showed that prey composition significantly differs among habitats likely
236 due to differential invertebrate communities present at the sites (Krueger et al., 2020). However,

237 exceptionally high percentages of winged Hymenoptera (bees, sawflies and wasps in a wider
238 sense) were found in the prey spectrum of a species with scented traps, *D. fragrans* Lowrie,
239 compared to sympatric unscented species. This shows that the role of some of the unusual
240 morphological features in *D.* sect. *Arachnopus* on prey spectra requires further investigation
241 (Krueger et al., 2020).

242

243 1.4. Overall research aims

244 This study aimed to characterise the prey spectra of five species in *D.* sect. *Arachnopus* at their
245 natural habitats in Western Australia, to 1) investigate the role of leaf scent in sundew prey
246 attraction that was hypothesised by Fleischmann (2016) and Krueger et al. (2020), 2) detect
247 potential prey specialisation in any of the studied species, 3) test the functionality of a novel
248 approach involving both DNA-metabarcoding and *in-situ* macro photography for studying CP
249 prey spectra, and 4) compare this new method with traditional morphology-based methods for
250 CP prey spectra analysis.

251

252 The first part (Chapter 2) focusses on the potential role of scent-based prey attraction in *D.*
253 *fragrans*, which is compared with a sympatric unscented species (*D. cucullata* Lowrie) at three
254 remote locations in the northern Kimberley Region, using *in-situ* macro photography following
255 the methods established by Krueger et al. (2020). The potential specialisation of *D. fragrans* in
256 capturing winged Hymenoptera (reported by Krueger et al., 2020) or Lepidoptera (reported by
257 Fleischmann, 2016) is investigated.

258

259 In the second part of this study (Chapter 3), a novel approach for analysing CP prey spectra is
260 presented and evaluated. By using DNA-metabarcoding, this study aimed to characterise the
261 prey spectra of three additional species of *D.* sect. *Arachnopus* at unprecedented taxonomic

262 resolution, while also obtaining prey quantity data via *in-situ* macro photography. *In-situ* macro
263 photographs were used as a control for the DNA-metabarcoding data, helping to detect false
264 positive identifications or contaminations. It was hypothesised that prey spectra obtained by
265 this novel method would confirm earlier results of prey spectra compositions in *D.* sect.
266 *Arachnopus* (Krueger et al., 2020; Chapter 2) at coarse taxonomic levels. Finally, it was
267 attempted to detect significant prey spectra differences among species with different leaf sizes,
268 habitats and scented/unscented plants (Krueger et al., 2020; Chapter 2) via this new method.
269
270

271 2. Scent-based prey selectivity among sympatric species of 272 morphologically similar carnivorous sundews

273 2.1. Introduction

274 2.1.1. Prey specialisation in carnivorous plants

275 Investigating prey specialisation of sympatric CP species could help understand evolutionary
276 drivers and sympatric speciation processes in CPs, as such specialisations may potentially be
277 the consequence of interspecific competition in situations where prey may be a limiting
278 resource (Darnowski et al., 2018) – i.e. comparable to niche shifts regarding food sources in
279 certain animal groups (e.g., the well-studied Darwin finches; Grant & Grant, 2006).
280

281 Prey specialisation has previously been reported for several species of *Nepenthes* (e.g., Chin et
282 al., 2014; Gaume et al., 2016) and in sympatric sundews (*Drosera*) from Europe (Achterberg,
283 1973; Thum, 1986) and south-west Western Australia (Verbeek & Boasson, 1993). For these
284 species, prey spectra were comparatively studied only for morphologically dissimilar species
285 or species occupying different habitats – thereby introducing uncontrolled variables potentially

286 influencing prey spectra. For example, Thum (1986) hypothesised that the differences in prey
287 capture among sympatric *Drosera intermedia* Hayne and *D. rotundifolia* L. are likely the result
288 of their different growth habits (the former species produces erect leaves while the leaves of the
289 latter usually lie flat on the ground) or their different microhabitats (*D. intermedia* prefers wetter
290 areas of the habitat; Thum, 1986). Recent investigation of the prey spectra of seven species
291 from the primarily northern Australian *Drosera* sect. *Arachnopus* confirmed the importance of
292 different habitats and leaf morphologies (especially leaf size) for determining both prey quantity
293 and composition (Krueger et al., 2020). Despite this, Krueger et al. (2020) found several
294 instances of prey spectra dissimilarities even among morphologically very similar, sympatric
295 species, indicating that further research was required to investigate the potential prey selectivity
296 in this group of CPs.

297

298 **2.1.2. The potential role of trap scent in prey attraction**

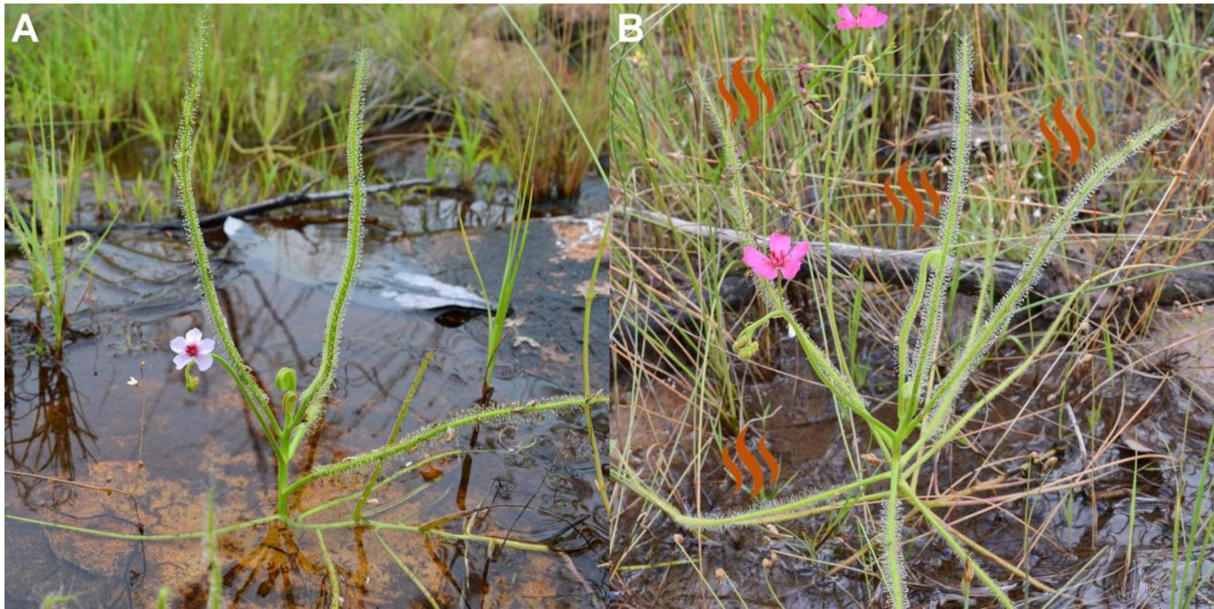
299 One key question arising from the work of Krueger et al. (2020) is the potential role of leaf
300 scent in prey attraction. Although scent-based prey attraction has been demonstrated for other
301 CP genera including *Nepenthes* (Moran, 1996; Di Giusto et al., 2010; Gaume et al., 2016),
302 *Sarracenia* (Jürgens et al., 2009), *Drosophyllum* (Bertol et al., 2015) and *Dionaea* (Kreuzwieser
303 et al., 2014), such studies are conspicuously lacking for *Drosera* (which is the largest genus of
304 CPs, comprising ca. 250 described species; Fleischmann et al., 2018b). Three species of *D.* sect.
305 *Arachnopus* are known to produce sweetly fragrant trapping leaves with a strong, honey-like
306 odour, these being *Drosera fragrans*, *D. finlaysoniana* Wall. ex Arn. and *D. margaritacea*
307 T.Krueger & A.Fleischm. (Fleischmann, 2016; Krueger & Fleischmann, submitted). It has been
308 hypothesised that this leaf scent may be related to prey attraction (Fleischmann, 2016; Krueger
309 et al., 2020), and preliminary observations by Fleischmann (2016) indicated that *D. fragrans*
310 captured greater numbers of Lepidoptera than non-scented, sympatric *D. aquatica* Lowrie
311 (which belongs to the same section). More quantitative analyses of these species growing

sympatrically at one locality in the Northern Territory (Australia) found statistically significant more winged Hymenoptera (comprising wasps in the widest sense, bees and sawflies, but excluding ants) captured by *D. fragrans* (Krueger et al., 2020). Since all members of *D.* sect. *Arachnopus* share a similar erect growth habit and produce morphologically similar, narrowly-linear leaves lined with adhesive carnivorous glands (Lowrie, 2014; Fleischmann et al., 2018a; Krueger et al., 2020), Krueger et al. (2020) subsequently hypothesised that the scented traps of *D. fragrans* may be particularly attractive to this group of insect prey. However, the precise role of trap scent in prey attraction in *D.* sect. *Arachnopus* remains unclear.

2.1.3. Aims and hypotheses

This study aimed to investigate the role of leaf scent in determining the prey spectra of two sympatrically-occurring species from *D.* sect. *Arachnopus*, the odourless *D. cucullata* (Figure 1A) and the scented *D. fragrans* (Figure 1B), at multiple sites in the northern Kimberley region of Western Australia. Both species are morphologically very similar, producing similar-sized leaves with a length of ca. 7-20 cm (Lowrie, 2014; T. Krueger, pers. obs.), thus enabling direct comparison of prey spectra by minimising the strong effect of leaf size on prey capture observed by Krueger et al. (2020). Likewise, the eglandular appendages (which have been hypothesised to play a role in prey attraction; Hartmeyer & Hartmeyer, 2006) are very similar in both species (Lowrie, 2014; Schlauer et al., 2018; T. Krueger, pers. obs.). Therefore, it was hypothesised that *D. fragrans* would capture an increased amount of scent-guided or nectar-seeking insects, such as Hymenoptera (Krueger et al., 2020) or Lepidoptera (Fleischmann, 2016), compared to sympatric *D. cucullata* due to the presence or absence of trap scent (leaf scent) in these two species.

336



337 **Figure 1. Comparison of two species from *Drosera* sect. *Arachnopus* studied for prey**
338 **selectivity. A.** *D. cucullata*. **B.** *D. fragrans*. Brown symbols indicate leaf scent. Photographs
339 by T. Krueger.

340

341 2.2. Material and Methods

342 2.2.1. Study sites

343 Fieldwork was carried out at three locations within the properties of Theda Station (privately
344 owned) in the northern Kimberley Region of Western Australia during April 2019 (Table 1).
345 The three sites are located within five kilometres, Sites 1 and 2 being only approx. 150 metres
346 apart. Sympatric populations were studied where the two species grew side by side within a few
347 cm distance in the same habitat, thereby eliminating the effect of local differences in insect
348 abundance and composition (Krueger et al., 2020).

349

350 **Table 1. Summary of the three study sites in the northern Kimberley region of Western**
351 **Australia.**

Site	Location (coordinates)	Study date	Species studied	Number of plants studied	Number of leaves studied	Number of prey pictures
Site 1	14.7877°S, 126.5308°E	17 April 2019	*D. cucullata*	4	18	1637
			D. fragrans	2	7	522
Site 2	14.7868°S, 126.5316°E	20 April 2019	*D. cucullata*	1	5	999
			D. fragrans	1	3	287
Site 3	14.8271°S, 126.5369°E	21 April 2019	*D. cucullata*	3	13	1541
			D. fragrans	3	10	1081

352

353 **2.2.2. Data collection**

354 Photographic data collection was employed *sensu* Krueger et al. (2020) to study prey quantity
355 and composition of *Drosera* plants *in-vivo* and *in-situ*. Each plant was randomly selected for
356 study and photographed following a systematic pattern, during which all active, mucilage-
357 secreting leaves were examined (old leaves without mucilage were excluded from analysis as
358 very few remaining, undigested prey items on such leaves would likely be identifiable, see
359 Krueger et al., 2020). All photographs were taken using a Panasonic Lumix G81 (Panasonic,
360 Osaka, Japan) with a Panasonic Lumix G Vario 12–60mm f/3.5-5.6 ASPH lens (Panasonic,
361 Osaka, Japan) and Raynox DCR-250 macro adapters (Raynox, Tokyo, Japan). Pictures of all
362 captured prey items (regardless of size or digestive state) were taken from multiple angles,
363 increasing the amount of discernible morphological features needed for assigning it to specific
364 prey groups. All prey count values were analysed per leaf (instead of per-individual) as leaf
365 numbers vary considerably among individuals (Krueger et al., 2020). In contrast to the
366 methodology of Krueger et al.(2020), all prey count values were analysed as per cm of leaf
367 length values for each leaf instead of per leaf values to approximate captured prey per leaf area.
368 This method enables better comparison of prey spectra compositions as it reduces the effect of
369 different overall leaf sizes on prey count values. Although both *Drosera* species produce very

370 similar-sized leaves, it was found that leaf size can vary strongly even within the same
371 individual. For each leaf, this study recorded leaf length, total number of prey items per cm of
372 leaf length, number of prey items assigned to each prey group per cm of leaf length, *Drosera*
373 species and study site, together with the file names of the relevant photographs. Leaf age was
374 also approximated by counting all studied leaves starting with the youngest. In total, 6,067 prey
375 pictures from 56 leaves were analysed (Table 1).

376

377 ### 2.2.3. Data analysis

378 Prey items were identified based on clearly discernible morphological features from the
379 photographs. Prey was classified in informal prey groups (following Krueger et al., 2020),
380 largely representing taxonomic arthropod orders, suborders or superfamilies. Unidentifiable
381 prey items were included in quantitative prey spectra analysis (with '*total captured prey items*
382 *per cm of leaf length*' representing the dependant variable) but excluded from all compositional
383 analysis of prey spectra.

384

385 Total numbers of captured prey per cm of leaf length were compared between both sympatric
386 *Drosera* species at each study site using Mann-Whitney U tests (SPSS Statistics 23, IBM,
387 USA). In addition, the combined data from all three sites were compared between species with
388 the same test. Prey spectra composition was compared between the two *Drosera* species for
389 each site and for the combined site data by analysis of similarity (ANOSIM) in PRIMER 7
390 (Clarke & Gorley, 2015). Leaf samples with no identifiable prey items and prey groups with no
391 observed items were omitted from this analysis. Data was $\log_{(X+1)}$-transformed and Bray-Curtis
392 resemblance matrices were created before ANOSIM analysis. This method quantifies prey
393 spectra dissimilarity with an R-statistic ranging from 0 (identical prey spectra) to 1 (maximal
394 dissimilar prey spectra; Clarke & Gorley, 2015). Similarity percentages (SIMPER) were
395 employed in PRIMER 7 to identify prey groups contributing more than 15% to prey spectra

396 dissimilarity (Krueger et al., 2020; Clarke & Gorley, 2015) and these were subsequently directly
397 compared between sympatric *Drosera* species (at all sites and the combined data) using Mann-
398 Whitney U tests. Total prey, as well as prey groups contributing more than 15% to dissimilarity
399 in SIMPER analysis were further compared between sites for each species using Kruskal-Wallis
400 tests (SPSS Statistics 23, IBM, USA).
401
402 The effect of the independent variables '*species*', '*leaf age*', '*leaf length*' and '*location*' on prey
403 spectra was assessed with linear regression models using backward-stepwise variable selection
404 in SPSS. These linear regression models were constructed to identify the independent variables
405 significantly predicting the total number of captured prey per cm of leaf length and the
406 frequencies of the most common prey groups (those contributing ≥5% of the identifiable prey)
407 per cm of leaf length. Variables featuring a variance inflation factor (VIF) >10 were removed
408 from the models due to collinearity.
409

410 ## 2.3. Results

411 ### 2.3.1. Observed prey spectra of *D. cucullata* and *D. fragrans*

412 A total of 916 prey items were recorded from the 56 leaves sampled, of which 277 (30%) were
413 identifiable to prey group (Table 2). Unidentifiable prey items comprised 70% of the observed
414 prey and were impossible to assign to any specific prey groups due to their heavily digested or
415 degraded state.
416
417 **Table 2. Prey items captured by the two studied *Drosera* species from northern Western**
418 **Australia.** Total count and percentages of each prey group on the identifiable prey (in
419 parenthesis) are given for each species and site. In addition, the percentages of identifiable prey
420 on all counted prey items are indicated. *Setocoris* (Miridae) are sundew mutualists naturally

421 inhabiting the sticky traps and it is thus unclear if they were truly captured as prey (Krueger et
422 al., 2020).

Prey group	*Drosera cucullata*				*Drosera fragrans*				All species
	Site 1	***Site 2***	***Site 3***	***Total***	***Site 1***	***Site 2***	***Site 3***	***Total***	
Araneae	3 (4.2)	0 (0.0)	1 (1.0)	4 (2.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	**4 (1.4)**
Coleoptera	11 (15.5)	1 (11.1)	25 (25.8)	37 (20.9)	7 (15.2)	1 (9.1)	1 (2.3)	9 (9.0)	**46 (16.6)**
Diptera									
Brachycera	3 (4.2)	0 (0.0)	6 (6.2)	9 (5.1)	1 (2.2)	0 (0.0)	13 (30.2)	14 (14.0)	**23 (8.3)**
Large Nematocera	3 (4.2)	0 (0.0)	0 (0.0)	3 (1.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	**3 (1.1)**
Small Nematocera	18 (25.4)	4 (44.4)	24 (24.7)	46 (26.0)	6 (13.0)	1 (9.1)	4 (9.3)	11 (11.0)	**57 (20.6)**
Hemiptera									
Cicadoidea	7 (9.9)	2 (22.2)	14 (14.4)	23 (13.0)	2 (4.3)	1 (9.1)	3 (7.0)	6 (6.0)	**29 10.5)**
Setocoris *****	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.2)	1 (9.1)	0 (0.0)	2 (2.0)	**2 (0.7)**
Other	0 (0.0)	0 (0.0)	2 (2.1)	2 (1.1)	1 (2.2)	0 (0.0)	0 (0.0)	1 (1.0)	**3 (1.1)**
Hymenoptera									
Formicidae	1 (1.4)	0 (0.0)	0 (0.0)	1 (0.6)	2 (4.3)	0 (0.0)	0 (0.0)	2 (2.0)	**3 (1.1)**
Winged Hymenoptera	13 (18.3)	2 (22.2)	13 (13.4)	28 (15.8)	21 (45.7)	7 (63.6)	13 (30.2)	41 (41.0)	**69 (24.9)**
Lepidoptera	8 (11.3)	0 (0.0)	8 (8.2)	16 (9.0)	5 (10.9)	0 (0.0)	6 (14.0)	11 (11.0)	**27 (9.7)**
Odonata	1 (1.4)	0 (0.0)	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	**1 (0.4)**
Orthoptera	0 (0.0)	0 (0.0)	1 (1.0)	1 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	**1 (0.4)**
Thysanoptera	3 (4.2)	0 (0.0)	3 (3.1)	6 (3.4)	0 (0.0)	0 (0.0)	3 (7.0)	3 (3.0)	**9 (3.2)**
Total identifiable	**71 (27.6)**	**9 (17.3)**	**97 (35.1)**	**177 (30.3)**	**46 (30.1)**	**11 (30.4)**	**43 (32.3)**	**100 (30.2)**	**277 (30.2)**
Total prey items	**257**	**52**	**276**	**585**	**153**	**45**	**133**	**331**	**916**
Sample size (leaves)	**n=18**	**n=5**	**n=13**	**n=36**	**n=7**	**n=3**	**n=10**	**n=20**	**n=56**

423

424 Identifiable prey items captured by the two *Drosera* species represented 14 prey groups in nine
425 arthropod orders (Table 2), three of which (Diptera, Hemiptera and Hymenoptera) could be
426 further subdivided. Prey groups comprising more than five percent of all identifiable prey
427 included Winged Hymenoptera (25%), Small Nematocera (21%), Coleoptera (17%),
428 Cicadoidea (10%), Lepidoptera (10%) and Brachycera (8%; Table 2).

429

430 Small Nematocera was the most frequent prey group in the prey spectra of *D. cucullata* at two
431 sites (25% at Site 1; 44% at Site 2), with Coleoptera slightly more abundantly captured (26%)
432 than Small Nematocera (25%) at Site 3 (Table 2). In contrast, Winged Hymenoptera comprised
433 the highest percentage of prey captured by *D. fragrans* at all three sites, ranging from 30–64%
434 of all prey items (Table 2). Coleoptera and Cicadoidea were more commonly captured by *D.*
435 *cucullata* than *D. fragrans* at all three study sites, and no clear pattern in the frequencies of
436 other prey groups was observed (Table 2).

437

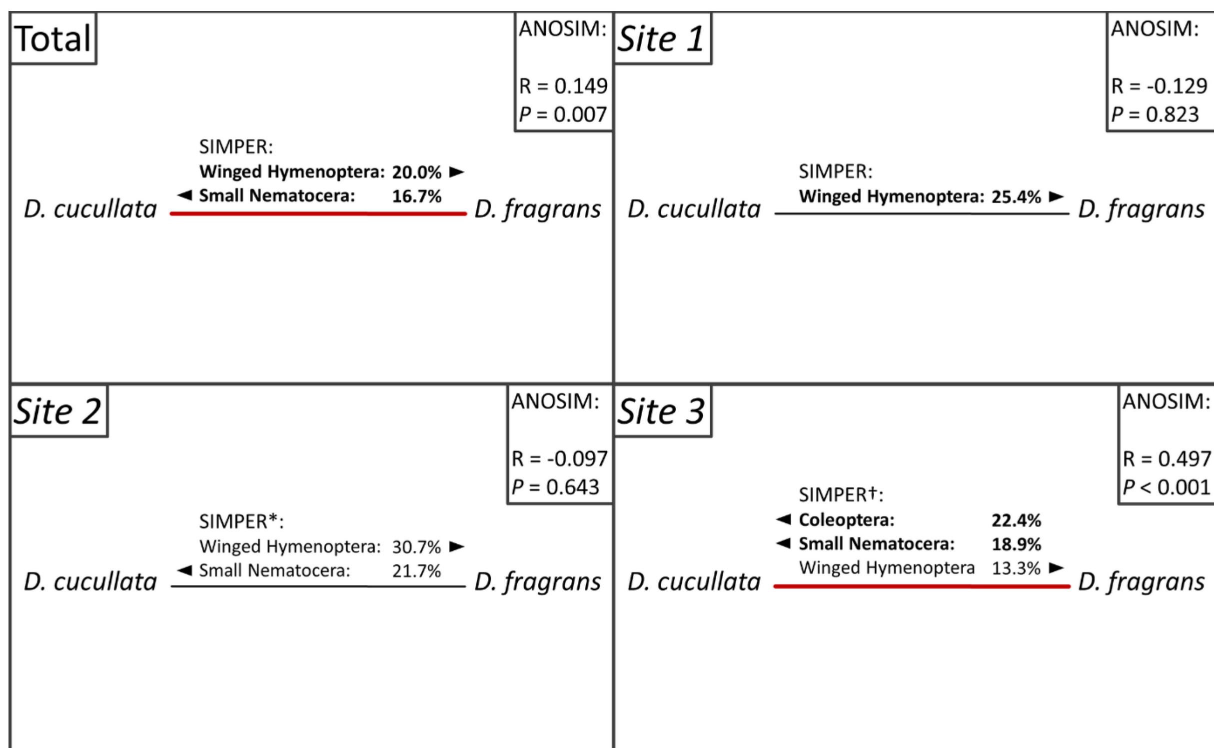
438 ## 2.3.2. Prey spectra comparison of sympatric *D. cucullata* and *D. fragrans*

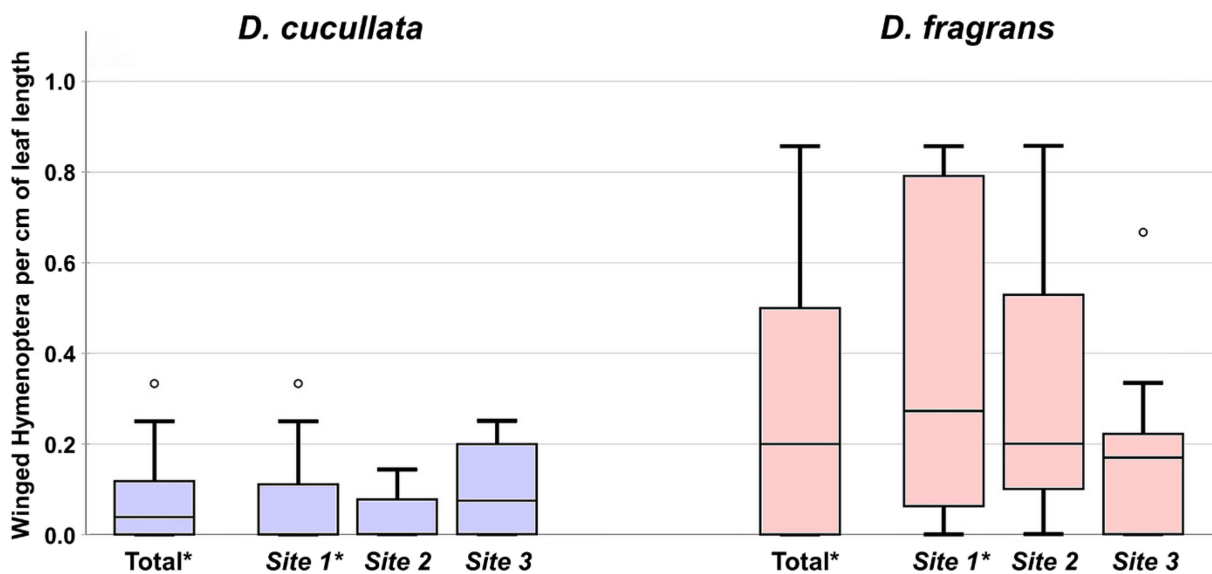
439 Total prey capture per cm of leaf length did not vary among the two species at Sites 1 and 3
440 (and in the combined data for all sites) but was significantly higher in *D. fragrans* at Site 2
441 (Mann-Whitney test, U = 15.00, P = 0.036; Appendix S1).

442

443 Prey composition differed significantly among the two sympatric species at Site 3 and the
444 combined data for all sites, as indicated by ANOSIM (Figure 2). SIMPER analysis indicated
445 that Winged Hymenoptera was the strongest contributor to prey spectra dissimilarity in all
446 comparisons except Site 3, with contributions ranging from 20% (all sites combined) to 31%
447 (Site 2; Figure 2). Direct comparison of the total number of captured Winged Hymenoptera per
448 cm of leaf length showed that *D. fragrans* captured more of this prey group in all comparisons
449 (Figure 3). This difference was found to be significant for Site 1 (Mann-Whitney test, U =
450 95.50, P = 0.047) and for the combined data from all sites (Mann-Whitney test, U = 506.50, P
451 = 0.009; Figure 3).

452

453 

454 **Figure 2. Differences in prey spectra composition among sympatric *Drosera cucullata* and**
455 ***D. fragrans*.** Data are presented for each study site in the northern Kimberley region and for
456 the combined data from all three sites. Significant differences in total prey spectra composition
457 are highlighted by red lines, above which prey groups contributing more than 15% to
458 dissimilarity are listed (prey groups are bold if significant in Mann-Whitney U tests). Black
459 triangles indicate the *Drosera* species having captured more of each listed prey group.
460 **Setocoris* contributed 18% at Site 2 but was excluded from analysis as it is unclear if these
461 sundew mutualists were truly captured as prey. † Winged Hymenoptera contributed less than
462 15% to dissimilarity at Site 3.
463
464 

465 **Figure 3. *Drosera fragrans* captures more Winged Hymenoptera per cm of leaf length than**
466 **sympatric *D. cucullata*.** Data are presented for each study site in the northern Kimberley region
467 and for the combined data from all three sites. Statistical significance ($P < 0.05$) is determined
468 by Mann-Whitney U tests and indicated in the graphic by asterisks.
469
470 Small Nematocera contributed more than 15% to prey spectra dissimilarity in all comparisons
471 except at Site 1 (SIMPER analysis), although it was not strongest contributor to dissimilarity in

472 any comparison (Figure 2). Small Nematocera were captured more frequently by *D. cucullata*
473 in all comparisons, and this difference was found to be significant for Site 3 (Mann-Whitney
474 test, U = 20.00, $P = 0.004$) and for all sites combined (Mann-Whitney test, U = 191.00, $P =$
475 0.003; Appendix S3).
476
477 At Site 3, SIMPER analysis indicated that Coleoptera was the strongest contributor to prey
478 spectra dissimilarity, with a contribution of 22% (Figure 2). However, Site 3 was the only site
479 where this prey group contributed more than 15% to dissimilarity and subsequent direct
480 comparisons further indicated that neither *Drosera* species consistently captured more
481 Coleoptera per cm of leaf length across all sites (Appendix S2). Significant differences
482 regarding capture of Coleoptera were only found at Site 3, where *D. cucullata* captured
483 significantly more Coleoptera per cm of leaf length than *D. fragrans* (Mann-Whitney test, U =
484 12.00, $P < 0.001$; Appendix S2).
485

486 **2.3.3. Predictors of prey spectra in *D. cucullata* and *D. fragrans***

487 Total number of captured prey per cm of leaf length was significantly predicted by '*leaf age*'
488 (Beta = 0.558, $P < 0.001$) and '*species*' (Beta = 0.419, $P < 0.001$; Table 3). '*Leaf age*'
489 significantly predicted the number of captured Brachycera and Cicadoidea per cm of leaf length,
490 while '*leaf length*' was the only significant predictor for Coleoptera and Small Nematocera
491 (Table 3). The prey group Cicadoidea was also significantly predicted by '*location*', while none
492 of the predictors of Lepidoptera were significant (Table 3). Winged Hymenoptera was the only
493 prey group significantly predicted by '*species*' (Table 3). '*Species*' was the only significant
494 predictor for Winged Hymenoptera, and its beta value was the highest among all predictors for
495 the six prey groups (Beta = 0.505, Table 3).
496

497 **Table 3. Predictors of prey spectra in *D. cucullata* and *D. fragrans* at three sites in**
498 **northern Western Australia.** Summary of regression model statistics and significant
499 predictors for total prey and for the six most captured prey groups (comprising ≥5% of the
500 identifiable prey). *All predictors for Lepidoptera per cm of leaf length were non-significant.
501

			Regression model summary					
Prey group (per cm of leaf length)	Total abundance	n (leaves)	R^2	F	*P*	Significant predictors	Beta	*P*
Brachycera	23	56	0.182	5.916	0.005	Leaf age	-0.334	0.010
Cicadoidea	29	56	0.155	4.860	0.012	Location Leaf age	0.303 0.274	0.020 0.035
Coleoptera	56	56	0.096	5.762	0.020	Leaf length	0.311	0.020
Winged Hymenoptera	69	56	0.254	9.040	< 0.001	Species	0.505	< 0.001
Lepidoptera	27	56	0.025	1.405	0.241	Location*	0.159*	0.241*
Small Nematocera	57	56	0.180	11.861	0.001	Leaf length	0.424	0.001
Total prey	916	56	0.381	16.285	< 0.001	Leaf age Species	0.558 0.419	< 0.001 < 0.001

502

503 2.4. Discussion

504 2.4.1. Evidence for prey selectivity in *D. fragrans*

505 This study provides the first evidence of prey selectivity among sympatric species of
506 morphologically similar sundews. Contrasting all previous studies on *Drosera* with erect
507 leaves, which found Diptera (particularly Nematocera) to contribute the majority to the prey
508 spectrum (Achterberg, 1973; Thum, 1986; Verbeek & Boasson, 1993; Hagan et al., 2008; Costa
509 et al., 2014; Krueger et al., 2020), Winged Hymenoptera were the most commonly observed
510 prey group in *D. fragrans* (contributing 30% to 64% of the identifiable prey, Table 2). In *D.*
511 *cucullata*, however, observed prey spectra were very similar to the above-mentioned studies,

512 with Small Nematocera representing the most common prey group (25–44%) and Winged
513 Hymenoptera comprising a much smaller percentage (13–22%; Table 2). The fact that Winged
514 Hymenoptera was the strongest contributor to prey spectra dissimilarity between the two
515 species in three of the four comparisons (Figure 2), more frequently captured by *D. fragrans* at
516 all sites (significantly so in the combined site comparison and at Site 1; Figure 3) and
517 significantly predicted only by the variable '*species*' in the linear regression model (with a
518 relatively high beta value of 0.505; Table 3) clearly indicates that *D. fragrans* appears to
519 selectively attract this prey group, at least compared to sympatric *D. cucullata*.
520
521 Although selective attraction of Winged Hymenoptera by *D. fragrans* was observed, total prey
522 spectra composition did not differ significantly in the ANOSIM analysis at two of the three
523 sites (Figure 2), indicating considerable prey overlap still exists among both species. Indeed,
524 most other prey groups were captured in similar quantities by both species (Table 2). While
525 direct comparison of captured Coleoptera and Small Nematocera (the only other prey groups
526 contributing more than 15% to prey spectra dissimilarity at any study site; Figure 2) between
527 both species indicated some significant differences (Appendix S2 and S3), regression analysis
528 showed that these differences are unlikely to be the result of differential prey selection among
529 species (none of these prey groups were predicted by '*species*'; Table 3).
530
531 Approximately 70% of all prey items were in a heavily digested or degraded state at the time
532 of examination, which made them impossible to assign to any prey group on the bases of
533 observable morphological characteristics. While this may seem to represent a limitation of this
534 study, the *in-situ* photography-based method enables collection of all necessary data to
535 empirically analyse and compare prey spectra, at least on the relatively coarse taxonomic level
536 required to assess the hypothesis of scent-based prey selectivity. Furthermore, the
537 methodological approach of previous studies on prey spectra in CPs (involving collection of

538 prey items in alcohol for microscopic examination) would have been logistically challenging
539 or impossible due to the extreme inaccessibility of study sites and would not significantly
540 increase the percentage of identifiable prey. Extremely remote areas such as the North
541 Kimberley are only accessible by chartered air travel during the wet season (preserving ethanol
542 is a restricted carriage item on aircraft), which makes field collection and ethanol preservation
543 of large amounts of sampled plants challenging. This extreme remoteness also prevented us
544 from sampling additional populations, especially of *D. fragrans* which was very uncommon at
545 the study sites (resulting in a much lower sample size of n = 20 for this species compared to *D.*
546 *cucullata* (n = 36)).
547

548 2.4.2. Scent-based prey attraction in *D. fragrans*?

549 This study supports prey specialisation among the two investigated *Drosera* species, which are
550 morphologically very similar but differ in the presence/absence of trap scent. While Krueger et
551 al. (2020) also found some instances of significantly different prey spectra between sympatric
552 species of *D.* sect. *Arachnopus*, most differences resulted from species-specific differences in
553 leaf size. However, this factor is unlikely responsible for the observed differences between
554 sympatric *D. cucullata* and *D. fragrans* in this study, as both species share a very similar leaf
555 size of 7-20 cm (Lowrie, 2014; Krueger et al., 2020; T. Krueger, pers. obs.) and prey group
556 frequencies were further analysed as per cm of leaf length values (as opposed to per leaf values
557 in Krueger et al., 2020). Habit, leaf colouration and indumentum of eglandular appendages (all
558 of which have previously been hypothesised to play a role in prey attraction; Hartmeyer &
559 Hartmeyer, 2006; Fleischmann, 2016; Krueger et al., 2020) are likewise identical or extremely
560 similar in both species. Very few significant differences were observed in the comparison of
561 prey spectra between the three study sites (likely due to their close geographic proximity; Table
562 1, Appendix S4), thus excluding another important factor influencing prey spectra (Krueger et
563 al., 2020) and legitimating usage of combined data from all sites in this study. It was therefore

hypothesised that, between the two species studied here, the only significant morphological difference likely to play a role in prey attraction is the strong honey-like leaf scent of *D. fragrans* (*D. cucullata* is odourless; T. Krueger & A. Fleischmann, pers. obs.).

Many species of nectar-feeding Hymenoptera are known to be attracted by floral scent (Howell & Alarcón, 2007; Kehl et al., 2010), and based on the present results regarding prey composition of scented and non-scented species growing in sympatry, it is safe to conclude that scent is the most likely cause of increased capture of Winged Hymenoptera by *D. fragrans* compared to sympatric *D. cucullata*. Winged Hymenoptera were also observed to be captured significantly more frequently by *D. fragrans* compared to sympatric *D. aquatica* by Krueger et al. (2020) and further comprised a higher percentage of identifiable prey in this species than in any of the seven other, non-scented species from *D.* sect. *Arachnopus* investigated by that study. Thus, these results support the existence of a unique olfactory prey attraction strategy in *D. fragrans* and contribute significantly to understanding the ecological needs of the poorly-studied CP species from tropical northern Australia.

3. Determining prey spectra in carnivorous sundews using DNA-metabarcoding

3.1. Introduction

3.1.1. DNA-metabarcoding – a promising tool for prey spectra research in carnivorous plants

With recent advances in technologies such as high throughput DNA-sequencing, DNA-metabarcoding has become a promising tool for analysing environmental samples containing

588 diverse arthropod assemblages (Hebert et al., 2003; Ji et al., 2013; Morinière et al., 2016;
589 Bittleston et al., 2016; Fernandes et al., 2019; Hausmann et al., 2020a), including a single case
590 of prey samples of CPs (Lekesyte et al., 2018). In this approach, all DNA contained in a sample
591 is extracted and amplified using barcode primers targeting the cytochrome c oxidase subunit I
592 (COX1) gene (Hebert et al., 2003; Moriniere et al., 2016). After sequencing, each obtained
593 DNA barcode is subsequently compared with a reference library to obtain taxonomic
594 information (Moriniere et al., 2016). DNA-metabarcoding thus promises to allow for much
595 finer taxonomic resolution and much higher completeness of CP prey spectra. The main
596 disadvantage of this approach is that DNA-metabarcoding usually does not allow for accurate
597 prey quantity estimations (either total count or biomass), as obtained read count data depend on
598 the DNA concentration of prey items which is likely highly variable among different prey taxa,
599 sizes and digestion states (Deagle et al., 2013; Lekesyte et al., 2018). Thus, DNA metabarcoding
600 of CP prey allows for analysis of what is captured (e.g., taxonomic analysis, such as prey
601 composition), but not how much is captured (e.g., prey quantity or biomass).
602
603 In CP ecological research, DNA-metabarcoding has already been used to investigate the
604 eukaryotic and prokaryotic communities found in trap contents of several pitcher plant species
605 of the genera *Sarracenia* and *Nepenthes*, i.e. including both trapped prey and associated infauna
606 living in the pitcher traps (Bittleston et al., 2016; Littlefair et al., 2019; Gilbert et al., 2020).
607 Similarly, this molecular sequencing technique has led to the discovery of symbiotic ciliates in
608 the submerged traps of two aquatic *Utricularia* species (Cheng et al., 2019), and detected
609 microbiota communities on the traps of the Venus flytrap *Dionaea muscipula* J.Ellis ex L.
610 (Sickel et al., 2019). The only published study which was conducted with the specific purpose
611 of analysing prey spectra of CPs using DNA-metabarcoding is Lekesyte et al. (2018), who
612 studied the prey spectra of the sundew *Drosera rotundifolia* growing at two study sites on
613 Lundy Island in the United Kingdom. They successfully identified 20 different prey taxa (16

614 were identified to species-level) on sampled traps and discovered strong differences in prey
615 spectra composition among the two sites, possibly due to differential invertebrate communities
616 present in the two very different habitats (Lekesyte et al., 2018).
617

618 **3.1.2. Aims and hypotheses**

619 A novel approach combining DNA-metabarcoding and *in-situ* macro photography was
620 employed to study the prey spectra (prey composition and quantity) of three Western Australian
621 species of *D.* sect. *Arachnopus*: *D. finlaysoniana*, *D. hartmeyerorum* Schlauer and *D.*
622 *margaritacea*. While the prey spectra of *D. finlaysoniana* and *D. margaritacea* were never
623 studied before, *D. hartmeyerorum* was included in the *in-situ* macro photography study of
624 Krueger et al. (2020).
625

626 DNA-metabarcoding was used for accurate determination of prey composition, and the *in-situ*
627 macro photography (as established by Krueger et al. (2020)) enabled to calculate total prey
628 quantity (this component of CP prey spectra is impossible to determine by DNA-metabarcoding
629 alone, see 3.1.1.). It was hypothesised that *in-situ* macro photography can serve as a control for
630 the DNA-metabarcoding data, as plausibility controls are considered crucial when using DNA-
631 metabarcoding approaches due to the sensitivity of the method and therefore possible false
632 positive identifications by even minuscule DNA contamination, as well as by unresolved DNA
633 barcodes or barcode mismatches (Ji et al., 2013; Creedy et al., 2018). Prey spectra obtained by
634 the present metabarcoding approach were expected to be generally similar to the ones observed
635 by Krueger et al. (2020) for the same or similar species of *D.* sect. *Arachnopus*, at least when
636 compared at a coarse taxonomic level. This study was also expected to confirm significant prey
637 spectra differences between study sites (Lekesyte et al., 2018; Krueger et al., 2020), among
638 species with different trapping leaf sizes (Gibson, 1991; Krueger et al., 2020) and between

639 sympatric scented and unscented plants (as observed by Fleischmann (2016), Krueger et al.
640 (2020) and in Chapter 2).
641

642 # 3.2. Material and Methods

643 ## 3.2.1. Study sites

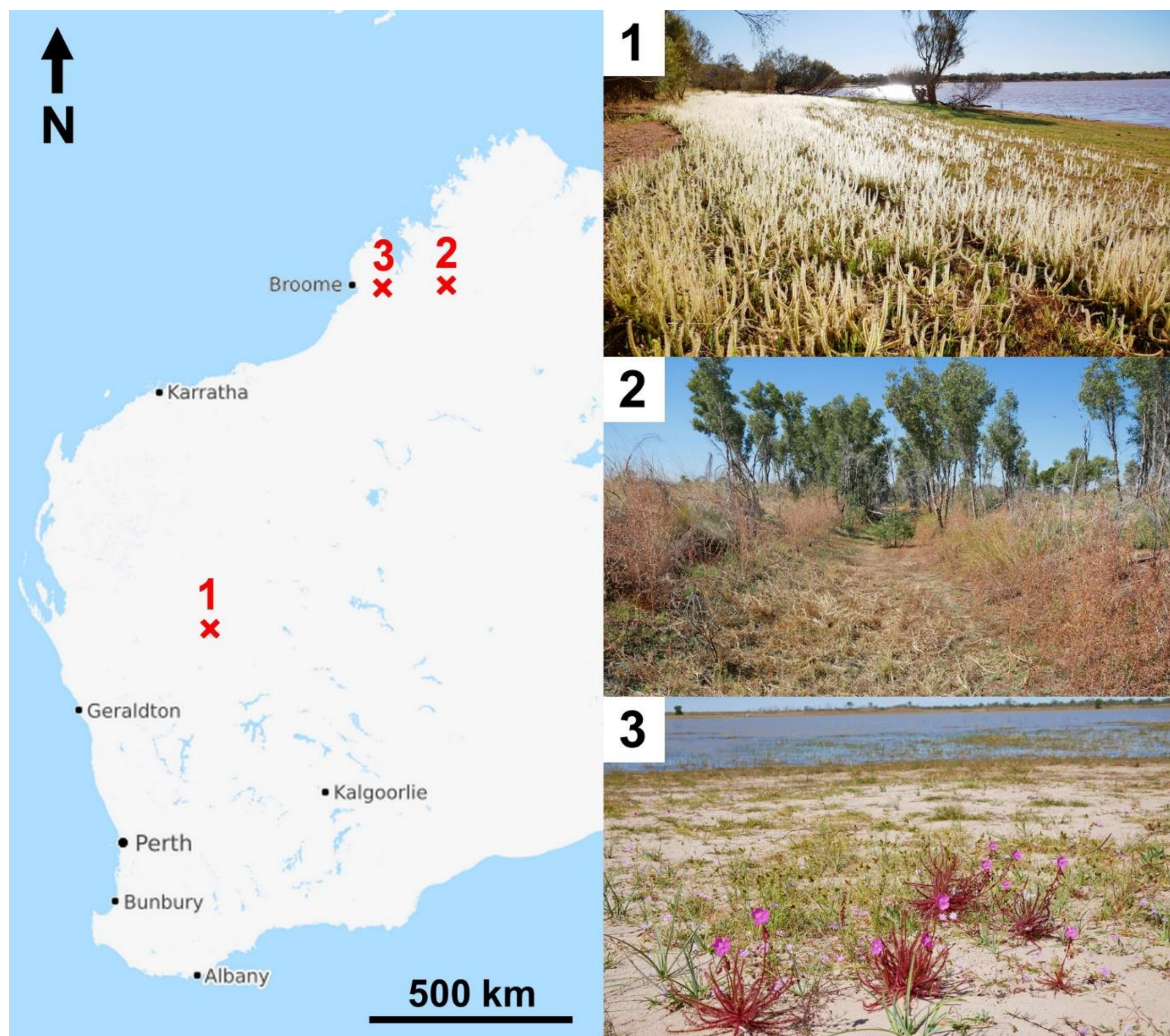
644 Plants were sampled at three road-accessible study sites in Western Australia during July 2020
645 (Table 4). Two of the sites (Sites 2 and 3) are located in the Kimberley Region in the north of
646 the state while Site 1 is located in the Mid-West Region, ca. 1,200 km further south (Figure 4).
647 At each site, the sampled species represented the sole member of *D*. sect. *Arachnopus* present
648 at the time of collection, as no sympatric taxa were found. While large plant populations were
649 present in the freshwater lake margin habitats of Sites 1 and 3 (especially at Site 1 which
650 featured an extremely dense population of *D. finlaysoniana*), only ca. 100 plants of *D.*
651 *margaritacea* were found in a small artificial drainage channel at Site 2 (Figure 4). However,
652 at the latter site scented and unscented individuals of *D. margaritacea* co-occurred, enabling
653 direct comparison of prey spectra among these two biologically different "scent-morphs"
654 (Table 4).
655

656 **Table 4. Summary of the three study sites in Western Australia.**

Site	Location (coordinates)	Sampling date	Species studied	Number of plant individuals sampled	Number of sampled leaves per individual plant	Number of *in-situ* prey pictures
Site 1	27.2584°S, 117.9821°E	13 July 2020	*D. finlaysoniana*	10	5	98
Site 2	17.8439°S, 124.4645°E	18 July 2020	*D. margaritacea*	5 (scented) 5 (unscented)	5	154

Site 3	17.7703°S, 122.8838°E	19 July 2020	*D. hartmeyerorum*	10	5	195

657



658

659 **Figure 4. Locations and habitats of the three study sites in Western Australia.** For each
660 site a picture of the habitat is provided. Site 1 featured a large and very dense population of
661 *Drosera finlaysoniana* adjacent to a freshwater lake near Cue. Site 2 is located in a narrow
662 artificial drainage channel which was completely dry at the time of study and only ca. 100 plants
663 of *D. margaritacea* were found to sparsely populate this habitat. Site 3 featured a large but
664 scattered population of *D. hartmeyerorum* (red plants visible in foreground) growing around an
665 extensive wetland system. Photographs by T. Krueger, map © OpenStreetMap.com
666 contributors.

667

668 3.2.2. Leaf sampling

669 Ten plants from each population were randomly selected for study and five leaves per plant
670 were randomly removed with forceps (under scientific collection license FT61000038-2). Each
671 sample thus constituted of five leaves belonging to the same plant individual. Only fully
672 developed, mucilage-secreting (i.e. "active") leaves were collected as the heavily digested prey
673 items found on old leaves would complicate 1) quantitative analysis (e.g., by counting
674 fragmented prey items multiple times), 2) qualitative prey analysis by DNA-metabarcoding as
675 senescent leaves with old, more decayed prey items would not only increase amounts of
676 degraded prey DNA (thus hampering DNA amplification and sequencing) but also fungal and
677 bacterial contaminations, as well as chances of higher prey loss of prey items by rain, wind and
678 kleptoparasites and 3) using pictures as a control for DNA-metabarcoding due to reduced
679 amounts of identifiable prey items (see Krueger et al., 2020). Three species of *D.* sect.
680 *Arachnopus* were studied, resulting in a total sample size of n = 30 samples (consisting of 150
681 collected leaves and 447 collected *in-situ* macro-photographs; Table 4).

682

683 3.2.3. *In-situ* macro photography

684 The detached sampled leaves were carefully placed on paper sheets (with the adaxial, tentacle-
685 bearing, sticky, prey-containing side facing upwards so that no prey items could get stuck and
686 lost to the sheets) with a scale (ruler) and both leaf length and leaf scent were recorded. To
687 determine total prey counts for each leaf, the method employed by Krueger et al. (2020) was
688 used. Multiple macro-photographs of each collected leaf were taken using a Panasonic Lumix
689 G81 (Panasonic, Osaka, Japan) with a Panasonic Lumix G Macro 30mm f/2.8 ASPH lens
690 (Panasonic, Osaka, Japan) and total prey was counted for each sample based on these images.
691 In contrast to Krueger et al. (2020) and Chapter 2, prey count values were analysed on a per-

692 individual basis to further reduce the probability of pseudoreplication. Thus, the prey count
693 value per sample was defined as the total number of observed prey items on five randomly
694 selected leaves of a single individual. Finally, the strong effect of leaf size on prey counts
695 (Krueger et al., 2020) was mitigated by calculating prey count values as per cm of leaf length
696 (because even within a single individual of *D*. sect. *Arachnopus*, leaf size can be highly variable;
697 T. Krueger, pers. obs.). All three studied *Drosera* species have a narrowly linear-lanceolate leaf
698 shape and prey counts per cm of leaf length thus closely approximate prey counts per leaf area.
699 Leaf length of the five collected leaves was averaged for each sample.
700

701 **3.2.4. Sample preparation, lysis and DNA extraction**

702 After all leaves were measured and photographed, the five leaves belonging to each individual
703 plant were placed in 15 ml sterile sample tubes containing 96% denatured ethanol (Recochem
704 Inc., Lytton, Australia) and temporarily stored at ~5 °C for DNA conservation and
705 metabarcoding. The ethanol supernatant of all 30 samples was carefully removed immediately
706 before shipment to the Botanische Staatssammlung Munich (Germany) for further processing
707 (export permit WT2020-001235). There, 96% denatured ethanol (Sigma-Aldrich Chemie
708 GmbH, Taufkirchen, Germany) was re-added to the samples. Prey items were separated from
709 the leaves in order to reduce the amount of plant tissue per sample relative to the amount of
710 insect tissue (*Drosera* leaf tissue is rich in polyphenols and polysaccharides which are known
711 to infer DNA extraction and amplification; Fleischmann & Heubl, 2009); for this, prey items
712 still attached to the leaves were carefully detached from the leaves using forceps under a
713 stereomicroscope, and prey items were transferred into 2 µl lysis cups that were filled with 96%
714 denatured ethanol. Therefore, most of the leaf tissue (except for the tentacles) was removed
715 before lysis.
716

717 For better lysis and DNA extraction from the insect tissue, all samples were subsequently
718 homogenised by adding steel beads (1 mm diameter, 100Cr6 steel) and placing each sample in
719 a FastPrep-96 homogeniser (MP Biomedicals, Irvine, USA; M. Hübner, pers. comm.). Lysis
720 was conducted using the Pall protocol “Glass Fiber Plate DNA Extraction” (Pall Corporation,
721 Port Washington, USA; Ivanova et al., 2006; M. Hübner, pers. comm.). 200 µl of insect lysis
722 buffer with proteinase K in a 1:20 mixture ratio was added to the solution (M. Hübner, pers.
723 comm.). Polyvinylpyrrolidone (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany) was
724 added (until 2% concentration in the solution) to block inhibiting substances such as
725 polyphenols (M. Hübner, pers. comm.). Samples were incubated overnight at a temperature of
726 56 °C and lysis products were frozen before extraction (M. Hübner, pers. comm.). Extraction
727 was conducted using multi-well filter plates from Pall (Pall Corporation, Port Washington,
728 USA) following the Pall protocol “Glass Fiber Plate DNA Extraction” (Ivanova et al., 2006;
729 M. Hübner, pers. comm.).
730

731 ### 3.2.5. DNA amplification and metabarcoding

732 DNA amplification and DNA-metabarcoding was conducted at the AIM Lab (AIM - Advanced
733 Identification Methods GmbH, Leipzig, Germany), with methods following Morinière et al.
734 (2016), Hardulak et al. (2020), Hausmann et al. (2020a) and Hausmann et al. (2020b).
735

736 From each sample, 5 µl of extracted total DNA was used for multiplex PCR, along with Plant
737 MyTAQ (Bioline, Luckenwalde, Germany) and High Throughput Sequencing (HTS) adapted
738 mini-barcode primers for the cytochrome c oxidase subunit I (COX1) gene of the mitochondrial
739 DNA compartment (mtDNA) (primers and amplification following Morinière et al., 2016).
740 Amplification success and fragment lengths were verified by gel electrophoresis. Amplified
741 DNA was cleaned up and resuspended in 50 µl purified water for each sample before
742 proceeding. Illumina Nextera XT (Illumina Inc., San Diego, USA) indices were ligated to the

743 samples in a second PCR reaction applying the same annealing temperature as for the first PCR
744 reaction but with only seven cycles, and ligation success was confirmed by gel electrophoresis.
745 DNA concentrations were measured using a Qubit fluorometer (Life Technologies, Carlsbad,
746 USA), and adjusted to 40 µl pools containing equimolar concentrations of 100 ng DNA
747 template each. Pools were purified using MagSi-NGSprep Plus (Steinbrenner Laborsysteme
748 GmbH, Wiesenbach, Germany) beads. A final elution volume of 20 µl was used. High
749 Throughput Sequencing (HTS) was performed on an Illumina MiSeq (Illumina Inc., San Diego,
750 USA) using v3 chemistry (2×300 basepairs, 600 cycles, maximum of 25 million paired-end
751 reads).
752

753 **3.2.6. Barcode sequence analysis, processing and OTU identification**

754 Following the methods established by Morinière et al. (2016), Hardulak et al. (2020),
755 Hausmann et al. (2020a) and Hausmann et al. (2020b), FASTQ files were combined and
756 sequence processing was performed with the VSEARCH v2.4.3 suite (Rognes et al., 2016) and
757 cutadapt v1.14 (Martin, 2011). Due to not all of the sequenced samples yielding reverse reads
758 of high enough quality to enable paired-end merging, only forward reads were utilised. Forward
759 primers were removed with cutadapt. Quality filtering was done with the fastq_filter program
760 of VSEARCH (fastq_maxee 2, minimum length of 100 bp). Sequences were dereplicated with
761 derep_fulllength, first at the sample level, and then concatenated into one fasta file, which was
762 then dereplicated. Chimeric sequences were filtered out from the large fasta file using
763 uchime_denovo. Remaining sequences were clustered into Operational Taxonomic Units
764 (OTUs) at 97% identity with cluster_size, and an OTU table was created with usearch_global.
765 To reduce false positives, a cleaning step was employed which excluded read counts in the OTU
766 table of less than 0.01% of the total. OTUs were blasted against a custom database downloaded
767 from GENEBANK (a local copy of the NCBI nucleotide database downloaded from
768 ftp://ftp.ncbi.nlm.nih.gov/blast/db/), including taxonomy and BIN (Barcode Index Number)

769 information, by using Geneious (v.10.2.5; Biomatters, Auckland, New Zealand) and following
770 the methods described in Morinière et al. (2016). The resulting csv file which included the OTU
771 ID, BOLD Process ID, BIN, Hit-%-ID value (percentage of overlap similarity (identical base
772 pairs) of an OTU query sequence with its closest counterpart in the database), length of the top
773 BLAST hit sequence, phylum, class, order, family, genus, and species information for each
774 detected OTU was exported from Geneious and combined with the OTU table generated by the
775 bioinformatic pipeline (Appendix S5).
776

777 **3.2.7. Sample pooling, data exclusion and plausibility control**

778 OTUs were first pooled to arthropod families, as prey spectra analysis was not conducted below
779 this taxonomic level and only 303 of the 741 retrieved OTUs could be identified to genus or
780 below by DNA-metabarcoding (Appendix S5). This also resulted in the exclusion of 87 OTUs
781 above the taxonomic level of organismic order. In addition, microorganisms (such as the
782 ubiquitous arthropod intracellular bacteria of the genus *Wolbachia*), marine taxa, fungi and
783 other obvious contaminants (such as *Homo sapiens* which likely contaminated some of the
784 samples during the leaf removal and photographing procedure) were excluded from analysis.
785 The rather ubiquitous phytophagous mealybugs and mites of Pseudococcidae, Trombidiformes
786 and Mesostigmata were not considered to have been captured as prey, but rather parasitised the
787 collected plant tissues, and were thus also excluded. The *in-situ* macro-photographs obtained
788 during sampling were used as a plausibility control of the prey spectra data generated by DNA-
789 metabarcoding. Each taxon in each sample was carefully attempted to be matched with one or
790 several of the prey items visible in the pictures. This pictorial plausibility control process was
791 conducted conservatively, as taxa were only excluded from further analysis if they consisted of
792 physically large prey animals (such as, for example, wasps, beetles or moths) which would have
793 been clearly visible in the pictures if they were truly present. Families mostly consisting of
794 small prey animals were generally impossible to confirm or exclude by pictorial plausibility

795 control as small unrecognisable "crumbs" of prey material were present on most leaves (see
796 Krueger et al. (2020) for a discussion of this problem). Data on prey spectra composition was
797 therefore compiled and analysed as presence/absence only, because DNA-metabarcoding does
798 not allow for accurate estimations of prey quantity (Deagle et al., 2013; Morinière et al., 2016;
799 Lekesyte et al., 2018). Finally, the number of samples in which each prey taxon was present
800 was counted for each *Drosera* species, as well as combined for all three species.
801

802 3.2.8. Statistical analysis

803 Prey spectra composition was compared between scented and non-scented individuals of *D.*
804 *margaritacea* as well as among all three species (including all pairwise comparisons) by using
805 analysis of similarity (ANOSIM) in PRIMER 7 (Clarke & Gorley, 2015). After creating Bray-
806 Curtis resemblance matrices, prey spectra dissimilarity was quantified using the ANOSIM R-
807 statistic which ranges from 0 (100% similarity) to 1 (0% similarity; Clarke & Gorley, 2015).
808 No data transformations were required, as DNA-metabarcoding data was treated as
809 presence/absence only. Subsequently, similarity percentages (SIMPER) were calculated in
810 PRIMER 7 to identify prey groups contributing most to dissimilarity (more than 15% for
811 arthropod orders and the five taxa contributing most to dissimilarity for arthropod families;
812 Krueger et al., 2020).
813

814 Total numbers of captured prey per cm of leaf length (as determined by analysis of *in-situ* prey
815 pictures) were compared between all three species using Kruskal-Wallis tests with Dunn-
816 Bonferroni post-hoc pairwise comparisons (SPSS Statistics 23, IBM, Armonk, USA). Mann-
817 Whitney U tests were employed to detect differences in total numbers of captured prey per cm
818 of leaf length between scented and non-scented individuals of *D. margaritacea* (SPSS Statistics
819 23, IBM, Armonk, USA).
820

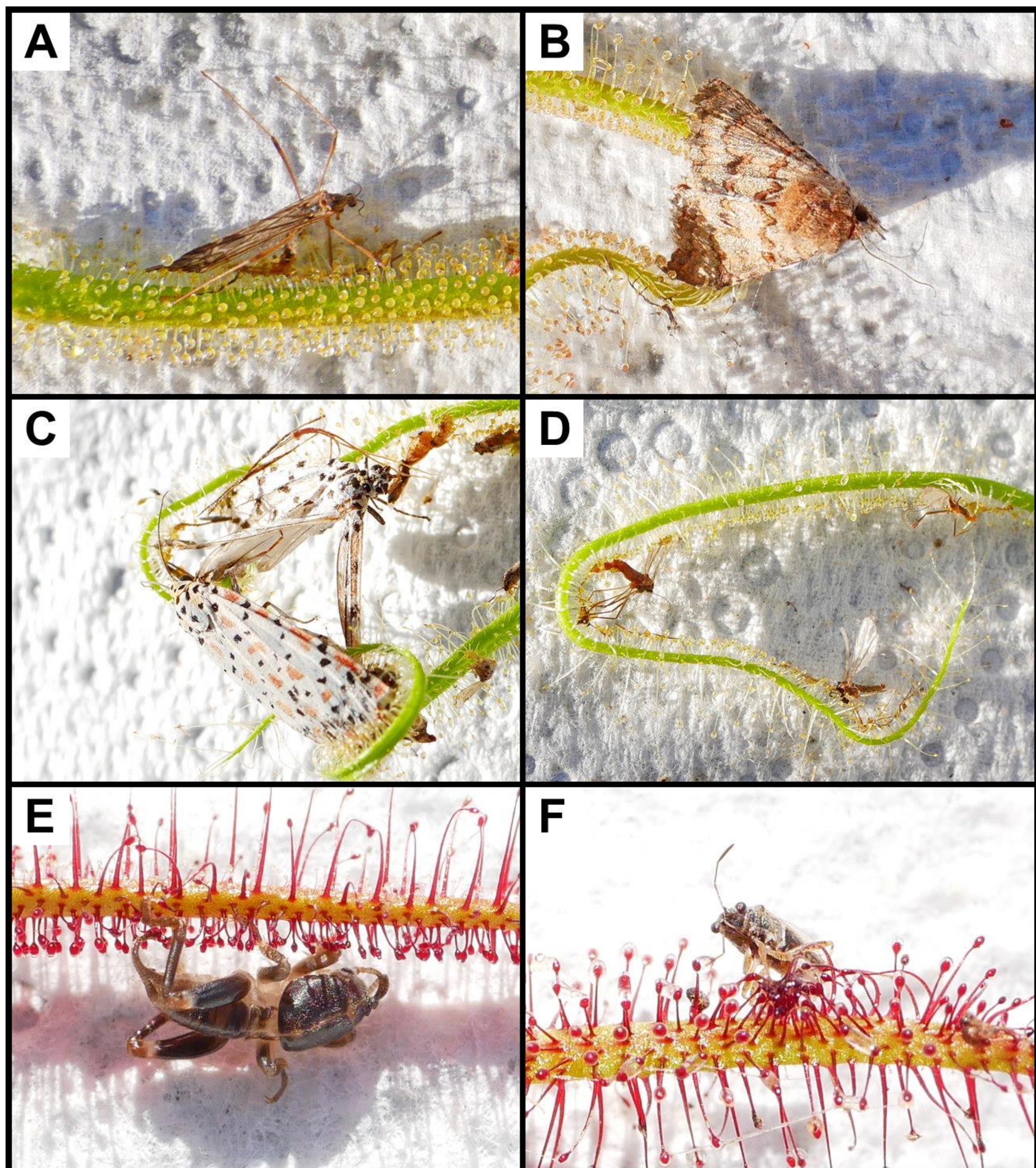
3.3. Results

3.3.1. Prey spectra detected by DNA-metabarcoding

DNA-metabarcoding confirmed 92 arthropod families belonging to 12 orders caught as prey across all 30 *Drosera* samples (Table 5, Figure 5). Samples from 25 arthropod families were excluded by pictorial plausibility control, most of them detected in *D. hartmeyerorum* samples 1, 4 and 9 (Appendix S6).

Curculionidae was the family most commonly excluded by pictorial plausibility control as these characteristic weevil beetles were clearly not present as prey in nine of the thirteen samples where they were detected by DNA-metabarcoding (in the remaining four samples they were either confirmed by the pictorial plausibility control or not excluded with certainty; see Appendix S6).

834



835 **Figure 5. Examples of captured arthropod prey detected and correctly identified by DNA-**
836 **metabarcoding in three Western Australian species of *Drosera* sect. *Arachnopus*.** The
837 lowest taxonomic level determined by DNA-metabarcoding and the corresponding family,
838 order and BOLD Barcode Index Number (BIN) is indicated. **A.** *Symplecta* sp. (Limoniidae,
839 Diptera, BOLD:AAF8963) captured by *D. finlaysoniana* (Sample 5). **B.** *Praxis marmarinopa*
840 (Erebidae, Lepidoptera, BOLD:AAC9474) captured by *D. finlaysoniana* (Sample 9). **C.** 2
841 individuals of *Utetheisa* sp. (Erebidae, Lepidoptera, BOLD:AAA4528) captured by *D.*

842 *margaritacea* (Sample 2). **D.** Cecidomyiidae (Diptera, BOLD:ACK2565) captured by *D.*
843 *margaritacea* (Sample 9). **E.** Early instar nymph of *Gryllotalpa pluvialis* (Gryllotalpidae,
844 Orthoptera, BOLD:AAF7358) captured by *D. hartmeyerorum* (Sample 1). **F.** *Nysius plebeius*
845 (Lygaeidae, Hemiptera, BOLD:AAI3382) captured by *D. hartmeyerorum* (Sample 7). All
846 pictures by T. Krueger.

847

848 Ten of the twelve detected arthropod orders were insects, with only Araneae (spiders,
849 Arachnida, present in 30% of total samples) and Entomobryomorpha (springtails, Collembola,
850 present in 10% of total samples) not belonging to this class (Figure 6). These two orders were
851 also the only orders exclusively consisting of non-flying prey. Although some of the captured
852 insect families such as Formicidae (ants, Hemiptera, present in 17% of samples) and larvae of
853 Gryllotalpidae (mole crickets, Orthoptera, larvae present in 3% of samples; Figure 5E) include
854 non-flying prey taxa, in a majority of samples only flying adult prey insects were detected.

855

856 The prey orders Diptera and Hemiptera were confirmed to be present in all 30 samples (100%),
857 while Hymenoptera (87%), Lepidoptera (77%) and Thysanoptera (57%) were detected in more
858 than half of samples (Figure 6). The most commonly (≥50%) detected prey families were
859 "Other Hemiptera" (i.e. hemipterans which could not be assigned by DNA-metabarcoding to
860 any family; present in 97% of samples), Hemiptera–Cicadellidae (83%), "Other Diptera"
861 (73%), Diptera–Cecidomyiidae (70%) and Hemiptera–Lygaeidae (70%; Table 5).

862

863 **Table 5. Arthropod taxa captured by the three studied species of *Drosera* sect. *Arachnopus***
864 **from Western Australia.** Total numbers and percentages of samples where each prey group
865 was detected by DNA-metabarcoding are indicated. Arthropod orders are presented in bold.

Prey group	All 3 Drosera species	Present in % of samples	*D. margaritacea*	Present in % of samples	*D. finlaysoniana*	Present in % of samples	*D. hartmeyerorum*	Present in % of samples

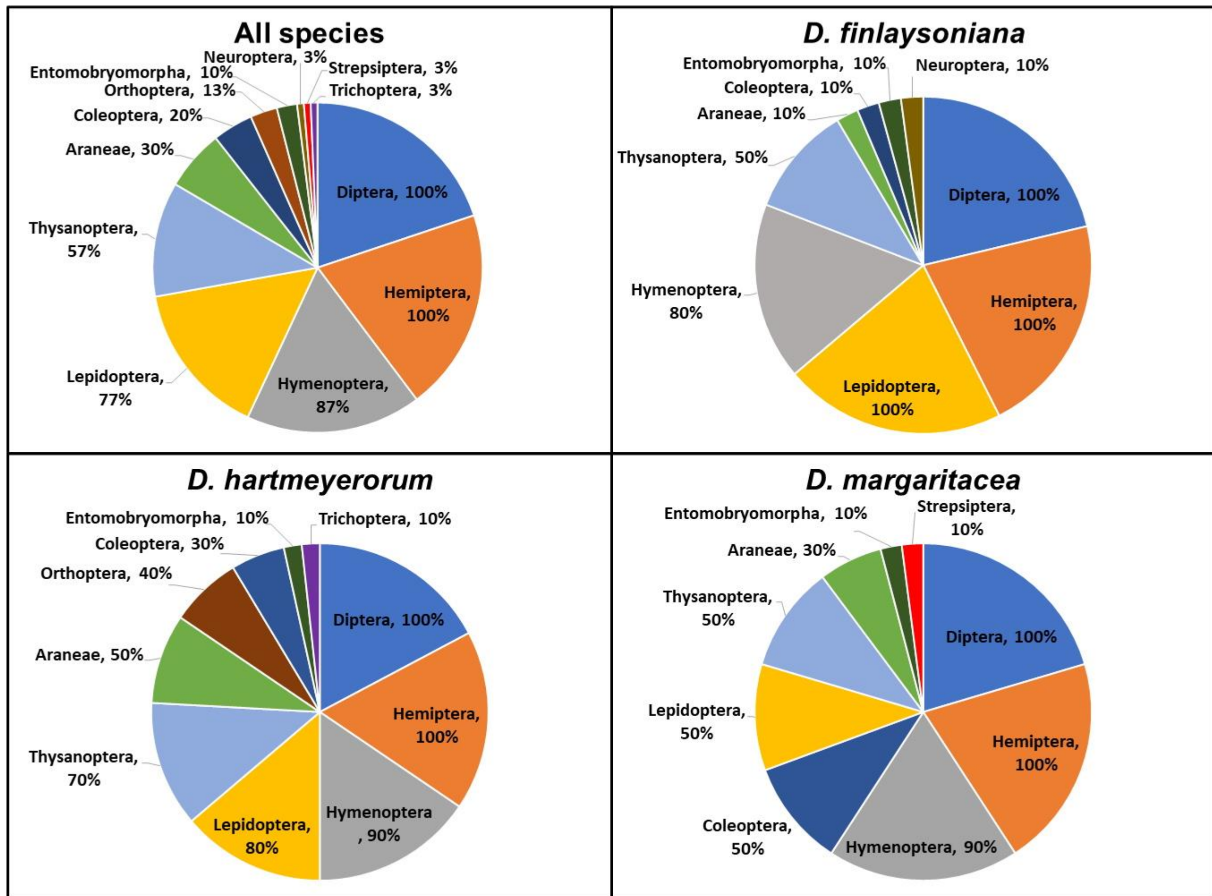
Araneae (Arachnida)	**9**	**30%**	**3**	**30%**	**1**	**10%**	**5**	**50%**
Linyphiidae	1	3%	0	0%	0	0%	1	10%
Lycosidae	1	3%	0	0%	0	0%	1	10%
Oxyopidae	1	3%	0	0%	1	10%	0	0%
Pisauridae	1	3%	0	0%	0	0%	1	10%
Other Araneae	5	17%	3	30%	0	0%	2	20%
Coleoptera	**6**	**20%**	**5**	**50%**	**1**	**10%**	**3**	**30%**
Brentidae	1	3%	1	10%	0	0%	0	0%
Cantharidae	1	3%	0	0%	1	10%	0	0%
Chrysomelidae	2	7%	0	0%	0	0%	2	20%
Coccinellidae	2	7%	1	10%	0	0%	1	10%
Curculionidae	4	13%	4	40%	0	0%	0	0%
Other Coleoptera	1	3%	0	0%	0	0%	1	10%
Diptera	**30**	**100%**	**10**	**100%**	**10**	**100%**	**10**	**100%**
Agromyzidae	2	7%	0	0%	1	10%	1	10%
Anthomyiidae	5	17%	0	0%	1	10%	4	40%
Bibionidae	1	3%	0	0%	1	10%	0	0%
Calliphoridae	6	20%	0	0%	6	60%	0	0%
Canacidae	2	7%	0	0%	2	20%	0	0%
Cecidomyiidae	21	70%	9	90%	6	60%	6	60%
Ceratopogonidae	5	17%	0	0%	1	10%	4	40%
Chironomidae	10	33%	0	0%	8	80%	2	20%
Chloropidae	3	10%	0	0%	2	20%	1	10%
Culicidae	1	3%	1	10%	0	0%	0	0%
Dolichopodidae	2	7%	0	0%	2	20%	0	0%
Drosophilidae	5	17%	0	0%	5	50%	0	0%
Ephydridae	4	13%	3	30%	0	0%	1	10%
Fanniidae	1	3%	1	10%	0	0%	0	0%
Heleomyzidae	1	3%	0	0%	0	0%	1	10%
Limoniidae	6	20%	1	10%	5	50%	0	0%
Muscidae	14	47%	0	0%	7	70%	7	70%
Mycetophilidae	3	10%	0	0%	1	10%	2	20%
Phoridae	5	17%	2	20%	1	10%	2	20%
Pipunculidae	2	7%	1	10%	0	0%	1	10%
Psychodidae	3	10%	1	10%	2	20%	0	0%
Sarcophagidae	10	33%	1	10%	7	70%	2	20%
Scathophagidae	1	3%	0	0%	1	10%	0	0%
Sciaridae	6	20%	1	10%	2	20%	3	30%
Sphaeroceridae	3	10%	0	0%	2	20%	1	10%
Stratiomyidae	1	3%	0	0%	1	10%	0	0%
Syrphidae	8	27%	1	10%	7	70%	0	0%
Tachinidae	4	13%	0	0%	2	20%	2	20%
Tephritidae	2	7%	0	0%	2	20%	0	0%
Tipulidae	6	20%	0	0%	0	0%	6	60%
Other Diptera	22	73%	3	30%	10	100%	9	90%
Entomobryomorpha (Collembola)	**3**	**10%**	**1**	**10%**	**1**	**10%**	**1**	**10%**
Hemiptera	**30**	**100%**	**10**	**100%**	**10**	**100%**	**10**	**100%**
Aleyrodidae	10	33%	9	90%	0	0%	1	10%
Aphididae	5	17%	5	50%	0	0%	0	0%
Cicadellidae	25	83%	10	100%	5	50%	10	100%
Delphacidae	5	17%	4	40%	0	0%	1	10%
Issidae	1	3%	0	0%	0	0%	1	10%
Liviidae	2	7%	0	0%	0	0%	2	20%
Lygaeidae	21	70%	10	100%	9	90%	2	20%
Miridae	6	20%	0	0%	3	30%	3	30%
Monophlebidae	1	3%	1	10%	0	0%	0	0%
Psyllidae	2	7%	0	0%	0	0%	2	20%
Triozidae	1	3%	0	0%	0	0%	1	10%
Other Hemiptera	29	97%	9	90%	10	100%	10	100%
Hymenoptera	**26**	**87%**	**9**	**90%**	**8**	**80%**	**9**	**90%**
Bethylidae	1	3%	0	0%	1	10%	0	0%
Braconidae	1	3%	0	0%	0	0%	1	10%

Dryinidae	2	7%	0	0%	1	10%	1	10%
Eucharitidae	2	7%	2	20%	0	0%	0	0%
Eulophidae	1	3%	1	10%	0	0%	0	0%
Formicidae	5	17%	2	20%	1	10%	2	20%
Ichneumonidae	12	40%	0	0%	5	50%	7	70%
Mymaridae	5	17%	3	30%	1	10%	1	10%
Platygastridae	6	20%	5	50%	0	0%	1	10%
Pompilidae	2	7%	1	10%	0	0%	1	10%
Torymidae	9	30%	7	70%	2	20%	0	0%
Trichogrammatidae	4	13%	1	10%	0	0%	3	30%
Other Hymenoptera	14	47%	8	80%	3	30%	3	30%
Lepidoptera	**23**	**77%**	**5**	**50%**	**10**	**100%**	**8**	**80%**
Cosmopterigidae	1	3%	0	0%	0	0%	1	10%
Crambidae	10	33%	2	20%	3	30%	5	50%
Erebidae	4	13%	1	10%	2	20%	1	10%
Gelechiidae	4	13%	1	10%	2	20%	1	10%
Geometridae	3	10%	0	0%	1	10%	2	20%
Gracillariidae	1	3%	1	10%	0	0%	0	0%
Lycaenidae	4	13%	0	0%	4	40%	0	0%
Noctuidae	3	10%	0	0%	2	20%	1	10%
Oecophoridae	1	3%	1	10%	0	0%	0	0%
Pterophoridae	7	23%	2	20%	0	0%	5	50%
Pyralidae	1	3%	0	0%	1	10%	0	0%
Scythrididae	3	10%	2	20%	1	10%	0	0%
Tineidae	1	3%	0	0%	0	0%	1	10%
Tortricidae	2	7%	0	0%	2	20%	0	0%
Other Lepidoptera	4	13%	0	0%	1	10%	3	30%
Neuroptera	**1**	**3%**	**0**	**0%**	**1**	**10%**	**0**	**0%**
Coniopterygidae	1	3%	0	0%	1	10%	0	0%
Orthoptera	**4**	**13%**	**0**	**0%**	**0**	**0%**	**4**	**40%**
Acrididae	2	7%	0	0%	0	0%	2	20%
Gryllidae	1	3%	0	0%	0	0%	1	10%
Gryllotalpidae	1	3%	0	0%	0	0%	1	10%
Other Orthoptera	2	7%	0	0%	0	0%	2	20%
Strepsiptera	**1**	**3%**	**1**	**10%**	**0**	**0%**	**0**	**0%**
Corioxenidae	1	3%	1	10%	0	0%	0	0%
Thysanoptera	**17**	**57%**	**5**	**50%**	**5**	**50%**	**7**	**70%**
Phlaeothripidae	10	33%	2	20%	5	50%	3	30%
Thripidae	10	33%	5	50%	0	0%	5	50%
Trichoptera	**1**	**3%**	**0**	**0%**	**0**	**0%**	**1**	**10%**
Sample size	**n=30**		**n=10**		**n=10**		**n=10**	

866

867

868



869 **Figure 6. Arthropod orders comprising the prey spectra of three species from *Drosera***
870 **sect. *Arachnopus* as detected by DNA-metabarcoding.** The percentage numbers denote the
871 proportion of *Drosera* samples in which each arthropod order was detected.

872

873 The orders Diptera and Hemiptera were the only orders found in 100% of samples of each
874 studied *Drosera* species (Figure 6). Hymenoptera (present in 80-90% of samples), Lepidoptera
875 (50-100%) and Thysanoptera (50-70%) were also among the five most commonly detected
876 arthropod prey orders in each of the three *Drosera* species (Figure 6). Coleoptera (50% in *D.*
877 *margaritacea*) and Araneae (50% in *D. hartmeyerorum*) were the only other prey orders present
878 in more than 50% of samples of one of the three sampled sundew species (Figure 6). Prey
879 families/taxa detected in more than 50% of samples in each of the three species were "Other
880 Hemiptera" (present in 90-100% of samples), Diptera–Cecidomyiidae (60-90%; Figure 5D)
881 and Hemiptera–Cicadellidae (50-90%; Table 5).

882

883 ANOSIM indicated that differences in the prey spectra between the three species were highly
884 significant at prey family-level (R = 0.784, *P* < 0.001) but non-significant at the level of order
885 (R = 0.079, *P* = 0.063). Additionally, all three species-pairwise comparisons at prey family-
886 level were significant, with the highest R value observed in the comparison between *D.*
887 *margaritacea* and *D. finlaysoniana* (R = 0.918, *P* < 0.001; Table 6). The only significant
888 pairwise comparison at prey order-level was *D. margaritacea* – *D. finlaysoniana* (R = 0.134,
889 *P* = 0.046; Table 6). SIMPER analysis indicated that no single prey family contributed more
890 than 5% to prey spectra dissimilarity in any of the three pairwise comparisons (Table 6).
891 Aleyrodidae (Hemiptera) contributed most to dissimilarity in both pairwise comparisons
892 involving *D. margaritacea* (this prey family was detected in much more samples of this
893 species), while Lygaeidae had the highest contribution in the SIMPER comparison of *D.*
894 *finlaysoniana* and *D. hartmeyerorum* (where it was more commonly detected in the latter
895 species; Table 6). However, the individual contributions to dissimilarity of most prey families
896 were generally very similar within the pairwise species comparisons (Table 6). When analysed
897 at order level, Lepidoptera contributed most to prey dissimilarity in both the *D. margaritacea*
898 – *D. finlaysoniana* and *D. margaritacea* – *D. hartmeyerorum* comparisons (in both cases
899 detected much less commonly in the *D. margaritacea* samples) but did not contribute more than
900 15% to dissimilarity in the *D. finlaysoniana* – *D. hartmeyerorum* comparison (Table 6). Other
901 prey orders contributing more than 15% to dissimilarity in the pairwise comparisons were
902 Thysanoptera and Coleoptera (both in the *D. margaritacea* – *D. finlaysoniana* and *D.*
903 *margaritacea* – *D. hartmeyerorum* comparisons) and Araneae and Orthoptera in the
904 comparison between *D. finlaysoniana* and *D. hartmeyerorum* (Table 6). SIMPER analysis
905 further indicated that all pairwise comparisons among species showed higher average
906 dissimilarity than samples of the same species.

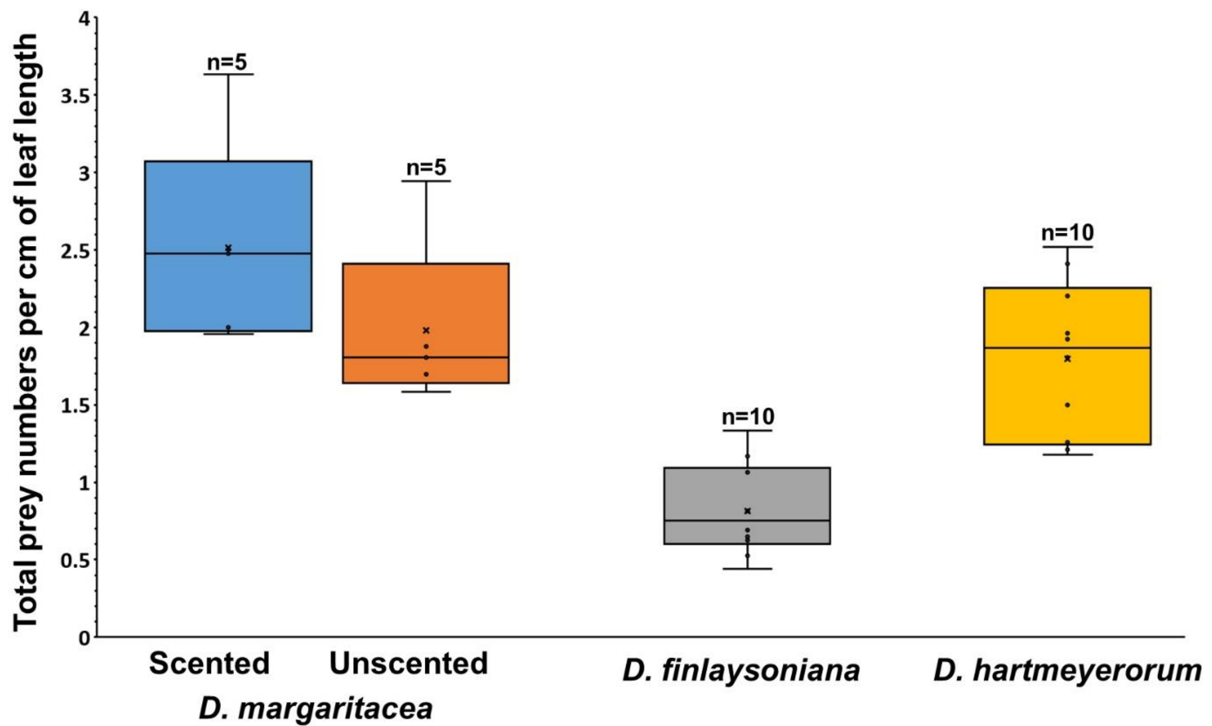
907

Table 6. **DNA-metabarcoding detection of family- and order-level prey spectra differences among three species from *D.* sect. *Arachnopus* in Western Australia.** Prey compositions are compared by Analysis of Similarity (ANOSIM) and Similarity Percentages (SIMPER) for all pairwise comparisons of studied species. *D. f.* = *D. finlaysoniana*, *D. h.* = *D. hartmeyerorum*, *D. m.* = *D. margaritacea*.

	Pairwise *Drosera* species comparison	**ANOSIM R**	***P***	**5 prey families contributing most to dissimilarity in SIMPER analysis (contribution in %; species in which prey family was more commonly detected)**
Family-level	*D. margaritacea* – *D. finlaysoniana*	0.918	< 0.001	Aleyrodidae (4.46; *D. m.*) Chironomidae (4.01; *D. f.*) Other Diptera (3.62; *D. f.*) Muscidae (3.50; *D. f.*) Syrphidae (3.25; *D. f.*)
	D. margaritacea – *D. hartmeyerorum*	0.749	< 0.001	Aleyrodidae (4.15; *D. m.*) Lygaeidae (4.11; *D. m.*) Torymidae (3.59; *D. m.*) Muscidae (3.51; *D. h.*) Other Diptera (3.47; *D. h.*)
	D. finlaysoniana – *D. hartmeyerorum*	0.642	< 0.001	Lygaeidae (3.59; *D. f.*) Chironomidae (3.47; *D. f.*) Syrphidae (3.47; *D. f.*) Calliphoridae (3.12; *D. f.*) Sarcophagidae (3.08; *D. f.*)
Order-level	*D. margaritacea* – *D. finlaysoniana*	0.134	0.046	Lepidoptera (20.38; *D. f.*) Thysanoptera (20.12; N/A) Coleoptera (19.62; *D. m.*)
	D. margaritacea – *D. hartmeyerorum*	0.033	0.264	Lepidoptera (17.54; *D. h.*) Thysanoptera (17.49; *D. h.*) Coleoptera (17.16; *D. m.*) Araneae (16.57; *D. h.*)
	D. finlaysoniana – *D. hartmeyerorum*	0.046	0.196	Thysanoptera (20.51; *D. h.*) Araneae (18.38; *D. h.*) Orthoptera (15.15; *D. h.*)

3.3.2. Observed total numbers of captured prey

916 Total prey capture per cm of leaf length, as observed by counting prey items in the *in-situ*
917 macro-photographs, did differ significantly among all three studied *Drosera* species (Kruskal-
918 Wallis test, H = 19.19, $P < 0.001$) and in the two pairwise comparisons *D. margaritacea* – *D.*
919 *finlaysoniana* ($P < 0.001$) and *D. finlaysoniana* – *D. hartmeyerorum* ($P = 0.004$). Prey numbers
920 did not differ in the comparison *D. margaritacea* – *D. hartmeyerorum* ($P = 0.966$). Among the
921 three species, *D. margaritacea* featured the highest average number (2.25) of prey items per cm
922 of leaf length (Figure 7). The average measured leaf length of this species was 7.1 cm
923 (Appendix S7). For *D. hartmeyerorum*, the average number of prey items per cm of leaf length
924 was 1.80, with an average leaf length in this species of 5.3 cm (Figure 7; Appendix S7). Despite
925 having by far the largest leaves (average leaf length of 10.4 cm; Appendix S7), *D. finlaysoniana*
926 had the lowest observed number of prey items per cm of leaf length among the three species
927 (0.81; Figure 7).



928

929 **Figure 7. Total prey numbers of per cm of leaf length in three species from *Drosera* sect.**
930 ***Arachnopus* in Western Australia.** Scented and unscented individuals of *D. margaritacea* are
931 separated.

932

933 ### 3.3.3. Prey spectra comparison of scented and unscented *D.*
934 ***margaritacea***

935 Scented leaves were recorded for *D. margaritacea* and *D. finlaysoniana* but not in *D.*
936 *hartmeyerorum*. While all studied individuals of *D. finlaysoniana* were only slightly scented
937 (to the human nose), ca. 20% of the *D. margaritacea* population (which consisted of ca. 100
938 individuals) produced a notably stronger honey-like scent. The other 80% were completely
939 odourless. Therefore, of each "scent-morph" of *D. margaritacea*, five leaves were collected to
940 allow for prey spectra comparison between them.

941

942 Prey composition did not differ significantly between the two "scent-morphs" when compared
943 at the taxonomic level of family (R = 0.094, $P = 0.262$) but differences were nearly significant
944 at order-level (R = 0.370, $P = 0.056$; Table 7). SIMPER analysis indicated that among the five
945 prey families contributing most to prey spectra dissimilarity, only Curculionidae was detected
946 in more samples of scented individuals compared to unscented ones (Table 7). Of the prey
947 orders contributing most to prey spectra dissimilarity, Lepidoptera and Coleoptera were found
948 in more samples of the scented individuals while Thysanoptera and Araneae were more
949 commonly present in samples of unscented individuals (Table 7).

950

951 Scented plants captured an average of 2.52 prey items per cm of leaf length, while the average
952 for unscented ones was 1.98 (Figure 7). However, a Mann-Whitney test indicated that this
953 difference was not significant (U = 4.00, $P = 0.095$).

954

955 **Table 7. DNA-metabarcoding detection of family- and order-level prey spectra differences**
956 **among scented and unscented individuals of *D. margaritacea* from the Kimberley region**

957 **of Western Australia.** Prey compositions are compared among scent-morphs by Analysis of
958 Similarity (ANOSIM) and Similarity Percentages (SIMPER).

	ANOSIM R	***P***	**Prey taxa contributing most to dissimilarity in SIMPER analysis (contribution in %; scent-morph in which prey taxon was more commonly detected)**
Family-level	0.094	0.262	Thripidae (5.53; unscented) Other Araneae (4.52; unscented) Curculionidae (4.47; scented) Platygastridae (4.17; unscented) Aphididae (4.16; unscented)
Order-level	0.370	0.056	Thysanoptera (22.31; unscented Lepidoptera (21.88; scented) Araneae (18.89; unscented) Coleoptera (17.00; scented)

959

960

961 3.4. Discussion

962 3.4.1. A combined DNA-metabarcoding/*in-situ* macro photography 963 approach to accurately analyse carnivorous plant prey spectra

964 Results indicate that DNA-metabarcoding allows for accurate analysis of prey spectra
965 composition in CPs at a taxonomic resolution and level of completeness unachievable by
966 traditional morphology-based approaches (as performed, for example, by Zamora 1990;
967 Verbeek & Boasson, 1993; Chin et al., 2014; Bertol et al., 2015; Annis et al., 2018). Even in
968 remote northern Western Australia, where many (if not most) arthropod species have not yet
969 been accessioned into the BOLD barcode reference library, this method identified over 90% of
970 obtained OTUs; most of them at family level, but 41% to genus-level, and 17% even down to
971 species rank (Appendix S5). Lekesyte et al. (2018) were able to identify 80% of the analysed
972 prey items found on *D. rotundifolia* from Lundy Island (UK) to species-level. However, their
973 sampling was performed in northern Europe, whose entomofauna is comparatively well studied

974 taxonomically and largely barcoded (often repeatedly on country level) and thus more broadly
975 represented in the BOLD libraries (Gaytán et al., 2020). New insect barcodes are regularly
976 added to the BOLD library through large-scale initiatives such as the International Barcode of
977 Life Project (iBOL; https://ibol.org/) and its Australian node Australian Barcode of Life
978 Network (ABOLN), hence accuracy of future DNA-metabarcoding research performed in
979 Australia can thus be expected to increase to similar levels in the coming years.
980
981 *In-situ* macro photography was found to provide a valuable plausibility control tool for the prey
982 taxa identified by DNA-metabarcoding data. While many of the smaller prey taxa detected by
983 DNA-metabarcoding were impossible to identify from *in-situ* macro photographs due to their
984 tendency to quickly degenerate into small, shapeless "crumbs" (see Krueger et al., 2020), this
985 control method considerably reduced the amount of prey taxa detected which were not actually
986 present as prey in the *Drosera* samples. This is most commonly a consequence of procedural
987 errors resulting in cross-contamination among samples (see Lekesyte et al., 2018), but may also
988 occur if prey was captured by the sundew before it subsequently escaped from the trap (Gibson,
989 1991; Cross & Bateman, 2018), or was kleptoparasitised (by larger animals). In both cases, a
990 DNA imprint on the *Drosera* leaves (as excretions, detached scales, hairs, or frequently as
991 autotomised body parts; Cross & Bateman, 2018) could be detected by DNA-metabarcoding.
992 Additionally, some barcoding-detected taxa may not constitute prey if they were associated
993 with another captured prey taxon (either as part of its diet, or as a parasite). The latter may
994 explain some barcode hits for taxa not immediately apparent from the *in-situ* macro
995 photographs, as they are (endo)parasites of captured prey taxa. This was likely the case in the
996 detected Strepsiptera which are frequently found as larvae and adults in Hymenopteran and
997 Orthopteran hosts (Kathitithambi et al., 2003). However, insect endoparasites and other non-
998 obvious prey taxa were by default not excluded by the very conservative approach of pictorial
999 plausibility control. Additionally, in the case of endoparasites, these organisms would also

1000 contribute to plant nutrition as "bycatch" after being digested together with their host, despite
1001 not having been actively attracted to the carnivorous traps. Finally, the control method tested in
1002 this study showed that even heavily digested prey items in the samples had sufficient amounts
1003 of intact (mitochondrial) DNA present to be detected by DNA-metabarcoding, as no instance
1004 was found of any prey item being visible in the macro photographs but not present in the
1005 barcoding data.

1006

1007 ### 3.4.2. Prey spectra composition of the studied *Drosera* species

1008 The analysed prey spectra of the three studied species from *D.* sect. *Arachnopus* most
1009 commonly contained flying insects (especially of the orders Diptera and Hemiptera, both
1010 present in 100% of the samples; Figure 6), thus confirming earlier *in-situ* macro photography-
1011 based studies of closely-related *D.* sect. *Arachnopus* species by Krueger et al. (2020) and
1012 Chapter 2. All members of *D.* sect. *Arachnopus* are characterised by a large, erect growth habit
1013 and thread-like aerial leaves which usually do not contact the ground (Lowrie, 2014), thereby
1014 excluding most ground-inhabiting prey taxa. This result is also similar to other prey spectra
1015 studies of erect-leaved *Drosera* from different geographic areas, where flying insects
1016 (particularly Diptera) unanimously comprised almost all of the observed prey (Thum, 1986;
1017 Verbeek & Boasson, 1993; Costa et al., 2014). Furthermore, this study confirmed the result of
1018 Krueger et al. (2020) and Chapter 2 that Hemiptera – and within this order especially
1019 Cicadellidae – are exceptionally common in the prey spectra of *D.* sect. *Arachnopus* compared
1020 to any other, previously studied *Drosera*. A possible explanation for this may be the relatively
1021 high abundance of Cicadellidae in tropical habitats (Nielson & Knight, 2000) compared to
1022 subtropical or temperate habitats where above-mentioned previous *Drosera* prey spectra studies
1023 were conducted.

1024

Of these five most commonly detected orders, Lepidoptera (butterflies and moths) generally comprised the largest prey items in terms of body size or wingspan, respectively. This prey order was exceptionally common in *D. finlaysoniana* (present in 100% of samples and also visually conspicuous in the *in-situ* photographs). Since this *Drosera* species had by far the largest trapping leaves measured among the three species studied (average leaf length of 10.4 cm; Appendix S7) it is possible that this represents an example of large prey items being more easily captured by species with larger trapping leaves (via "differential escape"; Gibson, 1991). Alternatively, Fleischmann (2016) suggested that captured Lepidoptera themselves could attract further individuals of the same species by pheromone release, potentially explaining the very high numbers of this insect order observed in many *D. finlaysoniana* (curiously, this phenomenon may even be apparent in herbarium specimens of this species at the Western Australian Herbarium (PERTH), several of which are covered with a large number of butterflies and moths; T. Krueger, pers. obs.).

3.4.3. Differences among observed prey spectra

Comparison of prey spectra between the three studied *Drosera* species revealed significant differences at arthropod family-level but not at the higher level of arthropod orders, indicating that at a coarse taxonomic resolution the same five arthropod orders (Diptera, Hemiptera, Hymenoptera, Lepidoptera and Thysanoptera) generally comprise most of the prey in *D.* sect. *Arachnopus*, regardless of given *Drosera* species or habitat. However, as strong differences were discovered in the ANOSIM comparison at family-level, it can be concluded that differences might likely increase with finer taxonomic resolution of prey taxa (a conclusion also reached by the CP prey spectra meta-analysis from Ellison & Gotelli, 2009). While these differences may be partially attributed to different morphological traits of the three species (*D. finlaysoniana* and half of the studied *D. margaritacea* individuals produced scented traps potentially olfactorily attracting prey (Fleischmann, 2016; Krueger et al., 2020; Chapter 2),

1051 while *D. hartmeyerorum* is odourless but possesses potentially visually attractive, eglandular
1052 appendages on its leaves (Hartmeyer & Hartmeyer, 2006). The very high ANOSIM R-values
1053 returned indicate that the most likely explanation is very different available prey spectra at the
1054 three study sites. Indeed, significant differences among different study sites, even within the
1055 same species, were previously reported for *Drosera rotundifolia* by Lekesyte et al. (2018) and
1056 for four species from *D.* sect. *Arachnopus* by Krueger et al. (2020). As shown by Krueger et al.
1057 (2020) and Chapter 2, the effect of scent on prey spectra appears to be restricted to one or
1058 perhaps a few prey groups and is thus unlikely to account for very high ANOSIM R-values. In
1059 addition, SIMPER analysis showed so many differences at prey family-level in the pairwise
1060 species comparisons that even Hemiptera–Aleyrodidae (which was present in 90% of *D.*
1061 *margaritacea* samples compared to 0% of *D. finlaysoniana* and 10% of *D. hartmeyerorum*
1062 samples; Table 5) did not contribute more than 5% to total prey spectra dissimilarity (Table 6).
1063
1064 Analyses indicate there is likely very little specialisation in prey capture by the three studied
1065 *Drosera* species. Rather, it can be hypothesised that the three studied *Drosera* species were
1066 exposed to very different available prey spectra, as they were growing at three separate study
1067 sites featuring different habitat types and climate regimes (Figure 4). For example, the relatively
1068 high detection rate of Lepidoptera in the samples of *D. finlaysoniana* and *D. hartmeyerorum*
1069 compared to *D. margaritacea* may be explained by the lake margin habitats of the former two
1070 species, while the latter species was found in a completely dry drainage channel lacking any
1071 nearby waterbodies (Figure 4). Lepidoptera are likely to occur in much higher concentrations
1072 near water sources, especially during the dry season (May to November) when the surrounding
1073 areas are lacking other water sources (G. Bourke in Fleischmann, 2016).
1074

1075 **3.4.4. Differences in total prey capture**

1076 In addition to the compositional prey analysis by DNA-metabarcoding, the *in-situ* macro
1077 photography method facilitated accurate estimation of prey quantity per sample. In contrast to
1078 Krueger et al. (2020), who generally found more prey items on larger trapping leaves in *D.* sect.
1079 *Arachnopus* species (even when values were compared as per cm of trapping leaf length), the
1080 species with the largest leaves studied here (*D. finlaysoniana*) captured significantly less prey
1081 items than the smaller-leaved species *D. margaritacea* and *D. hartmeyerorum* (Figure 7).
1082 However, while Krueger et al. (2020) was able to compare sympatric species (thus minimising
1083 any potential effects of the habitat or region on prey spectra), the three species in this study
1084 were studied at three different, geographically distant sites. While it is possible that overall prey
1085 abundance in the habitat was much lower at the *D. finlaysoniana* study site (Site 1), it can be
1086 hypothesised that the low total prey capture observed in this species may be due to the very
1087 large and extremely dense population resulting in strong intraspecific competition for prey (see
1088 Figure 4 and section 3.2.1.). This effect of population structure on prey capture has also been
1089 observed by Tagawa & Watanabe (2021) who found a significant negative correlation between
1090 total prey capture and population density in *D. serpens* Planch. (reported as *D. makinoi* Masam.;
1091 T. Krueger & A. Fleischmann, pers. obs.).
1092

1093 ## 3.4.5. Prey spectra comparison of scented and unscented *D. margaritacea*

1094 This study could not confirm the hypothesis of significant differences in the prey spectra
1095 between sympatric scented and unscented individuals of *D. margaritacea*. This may be a
1096 consequence of the small sample size of n = 5 for each "scent morph" as the sampled population
1097 of this species consisted of only ca. 100 individuals (i.e. 10% were sampled). While scented
1098 individuals captured a higher average number of prey items per cm of leaf length than unscented
1099 ones (Figure 7), this difference was revealed as statistically non-significant. The prey orders
1100 Hymenoptera and Lepidoptera were previously observed to be more commonly captured by
1101 scented species of *D.* sect. *Arachnopus* compared to unscented ones (Fleischmann, 2016;

Krueger et al., 2020). However, of these groups only Lepidoptera were detected in more samples of scented *D. margaritacea* compared to unscented samples (4:1 ratio; Appendix S6).

An alternative hypothesis of why no significant differences were detected among scented and unscented individuals in the population could be that trap scent primarily functions as a long-distance attraction strategy in *D. margaritacea* (similar to long-distance attraction of pollinators in flowers; Wilmer 2011) and therefore also benefits sympatric non-scented plants (like in scentless flowers, or deceit flowers; e.g., Wilmer 2011).

4. Conclusions

This study identified prey spectra of five species of carnivorous sundews in *D.* sect. *Arachnopus* in their natural habitats in Western Australia, discovering differential prey selectivity and possible scent-based prey attraction among two morphologically similar species (*D. cucullata* and *D. fragrans*; Chapter 2). Furthermore, a novel *in-situ* photography-controlled DNA-metabarcoding approach for accurate prey spectra analyses in CPs was successfully tested (Chapter 3).

For the first time, prey selectivity among morphologically similar *Drosera* species was demonstrated (Chapter 2). Winged Hymenoptera, the most common prey group in *D. fragrans*, was shown to contribute most to prey spectra dissimilarities with the sympatric, phylogenetically related (both belong to the same affinity, *Drosera* sect. *Arachnopus*) and morphologically congruent *D. cucullata* at multiple study sites. This apparent prevalence of *D. fragrans* in capturing winged Hymenoptera was most likely caused by the honey-like trap scent

1126 present in this species, thus supporting the existence of a specialised scent-based prey attraction
1127 strategy in some members of *D.* sect. *Arachnopus*.
1128

1129 Chapter 2 used an *in-situ* macro photography-based approach (similar to Krueger et al., 2020)
1130 which can be preferable to DNA-metabarcoding at extremely remote study sites that are only
1131 accessible by air travel (where transporting flammable liquids, such as ethanol – necessary for
1132 conservation of arthropod DNA – would violate safety protocols). The photography-based
1133 method is also comparatively less time-intensive, allows for non-invasive, non-destructive
1134 sampling of studied plants, therefore does not require collection licences for biological
1135 specimens, and provides sufficient data to assess certain hypotheses such as the question of
1136 scent-based prey attraction in *D.* sect. *Arachnopus*. However, as Chapter 3 demonstrates, DNA-
1137 metabarcoding, especially when combined with controls such as *in-situ* macro photography, is
1138 clearly superior in terms of taxonomic resolution and completeness for analysis of
1139 environmental bulk samples (containing different organisms in highly variable states of
1140 preservation), as used here for the reconstruction of prey spectra of CPs. The capability of this
1141 method increases with new reference barcodes being regularly added to DNA barcode libraries
1142 (such as BOLD) and it thus has the potential to become the standard methodology for future
1143 CP prey spectra research.
1144
1145

1146 # 5. Future directions for research

1147 Further experimental studies are needed to examine whether *D. fragrans* gains any advantages
1148 by attracting more winged hymenopteran prey via scent emission (as reported in Chapter 2),
1149 and whether it gains nutritive or biological advantage over non-scented congeners by this prey
1150 attraction strategy. Although it seems possible that *D. fragrans* captures an overall greater

1151 number of total prey items (total prey capture was significantly predicted by the variable
1152 '*species*'; Table 3), this difference was not consistent among all sites (Appendix S1) and it thus
1153 remains unclear if this species increases the amount of captured biomass by scent attraction.
1154 Alternatively, olfactory prey attraction in *D. fragrans* may reduce interspecific competition
1155 with sympatric non-scented *Drosera* by resource partitioning, if prey is a limited resource in
1156 the habitat (as it is the case for food resources in co-occurring closely related animals; e.g., the
1157 well-studied Darwin finches; Grant & Grant, 2006).
1158
1159 Additional studies are also needed to investigate whether there is a seasonal bias for certain
1160 prey groups, if winged Hymenoptera are the only insect group being attracted as prey by the
1161 specific scent of *D. fragrans*, or if other insect groups such as Lepidoptera show a similar
1162 behaviour, given high seasonal abundance in the habitat. Crucially, the precise role of scent
1163 attraction in *D.* sect. *Arachnopus* should also be studied by identifying the chemical volatiles
1164 responsible for the species' honey-like trap scent and by analysing the prey spectra of other
1165 scented species from the same affinity.
1166
1167 Continuative research, ideally involving extensive sampling of sites with sympatric occurrences
1168 of scented and unscented species, or scented and odorless individuals of a given species, is
1169 clearly needed to better understand the ecological role of trap scent in *D.* sect. *Arachnopus*.
1170 Similarly, potential functions of the unique eglandular appendages ("emergences") found in *D.*
1171 sect. *Arachnopus* should be investigated further. For example, manipulation experiments
1172 involving the removal of all yellow blackberry-shaped appendages of *D. hartmeyerorum*
1173 (which have been hypothesised to function as visual prey attractants; Hartmeyer & Hartmeyer,
1174 2006) and subsequent prey spectra comparisons of mutilated plants lacking emergences with
1175 control plants are proposed.
1176

Potential effects of population density on prey spectra (as hypothesised in Chapter 3) could be studied by comparing prey spectra of individual plants from within mass populations (such as the *D. finlaysoniana* population at Site 1; Chapter 3) with more exposed-growing individuals of the same population.

The novel *in-situ* photography-controlled DNA-metabarcoding approach presented in this study (Chapter 3) should also be tested for other CP genera, especially those possessing different trap types. Within Western Australia, three additional trap types occur: snap traps (*Aldrovanda*), suction traps (*Utricularia*) and pitfall traps (*Cephalotus*). In particular, it might be expected that *in-situ* photography will not work as well for the extremely small, typically submerged traps of *Aldrovanda* and *Utricularia* (which also completely enclose their captured, microscopic prey items), potentially necessitating usage of alternative control methods for DNA-metabarcoding data.

Finally, DNA-metabarcoding may be used to investigate (klepto-)parasitic or mutualistic relationships with CPs such as *Setocoris* (Hemiptera–Miridae) inhabiting *Drosera* traps (Lowrie, 2014) or larvae of *Badisis* (Diptera–Micropezidae) living in the digestive fluid of *Cephalotus* pitchers (Yeates, 1992).

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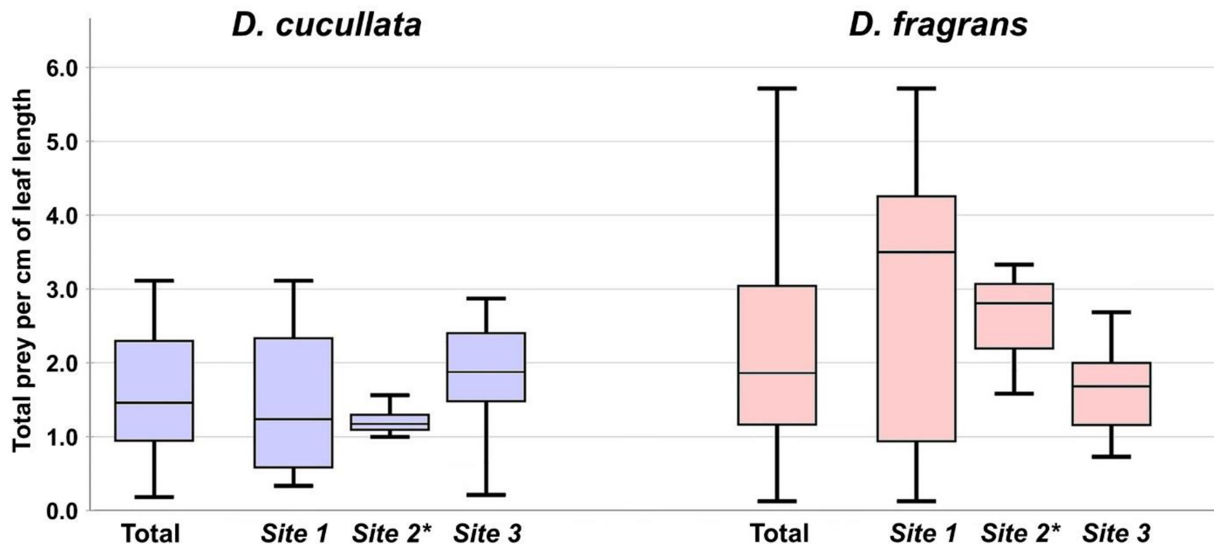
1398 would be pleased to hear from any copyright owner who has been omitted or incorrectly
1399 acknowledged.

1400 # Appendix

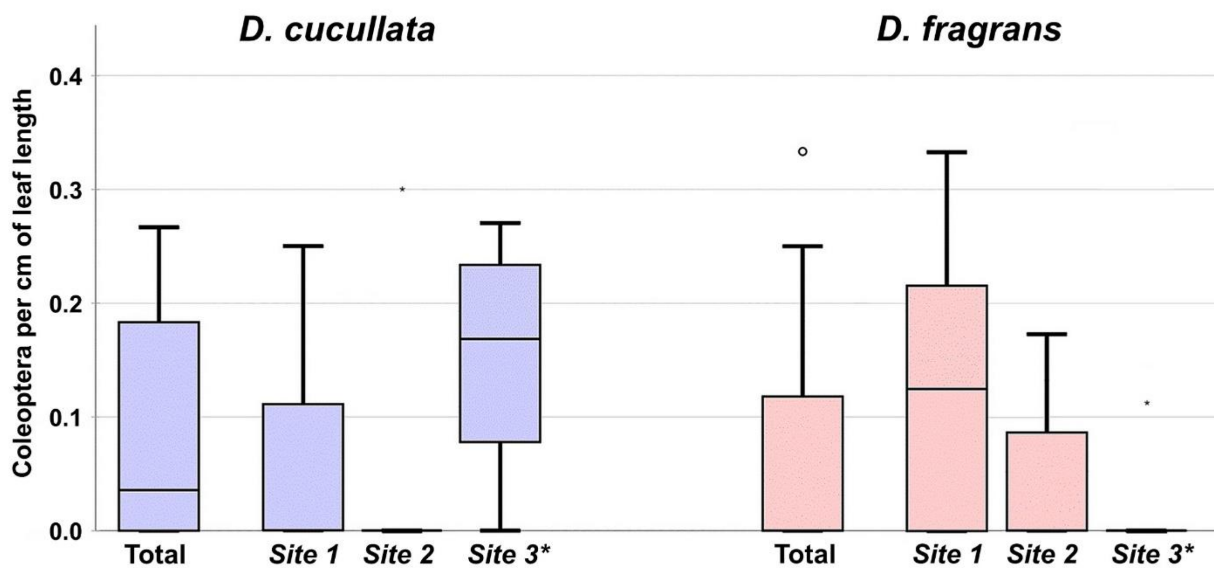
1401



1402

1403 **Appendix S1. Comparison of total prey capture per cm of leaf length between sympatric**
1404 ***Drosera cucullata* and *D. fragrans*.** Data is presented for each study site in the northern
1405 Kimberley region and for the combined data from all three sites. Statistical significance ($P <$
1406 0.05) is determined by Mann-Whitney U tests and indicated in the graphic by asterisks.

1407

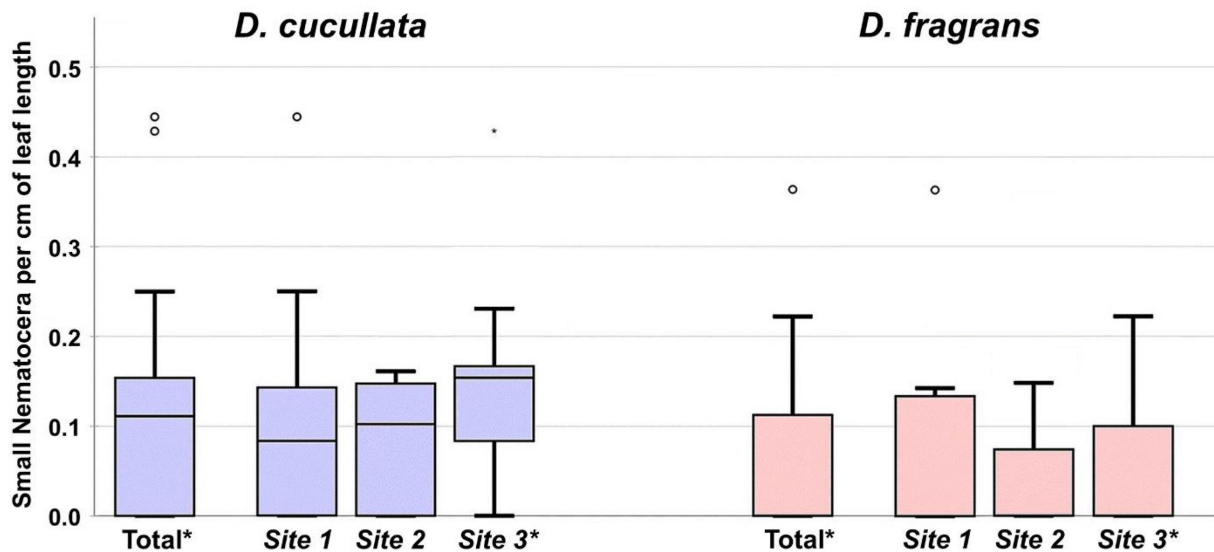


1408

1409 **Appendix S2. Comparison of captured Coleoptera per cm of leaf length between**
1410 **sympatric *Drosera cucullata* and *D. fragrans*.** Data is presented for each study site in the

1411 northern Kimberley region and for the combined data from all three sites. Statistical
1412 significance ($P < 0.05$) is determined by Mann-Whitney U tests and indicated in the graphic by
1413 asterisks.

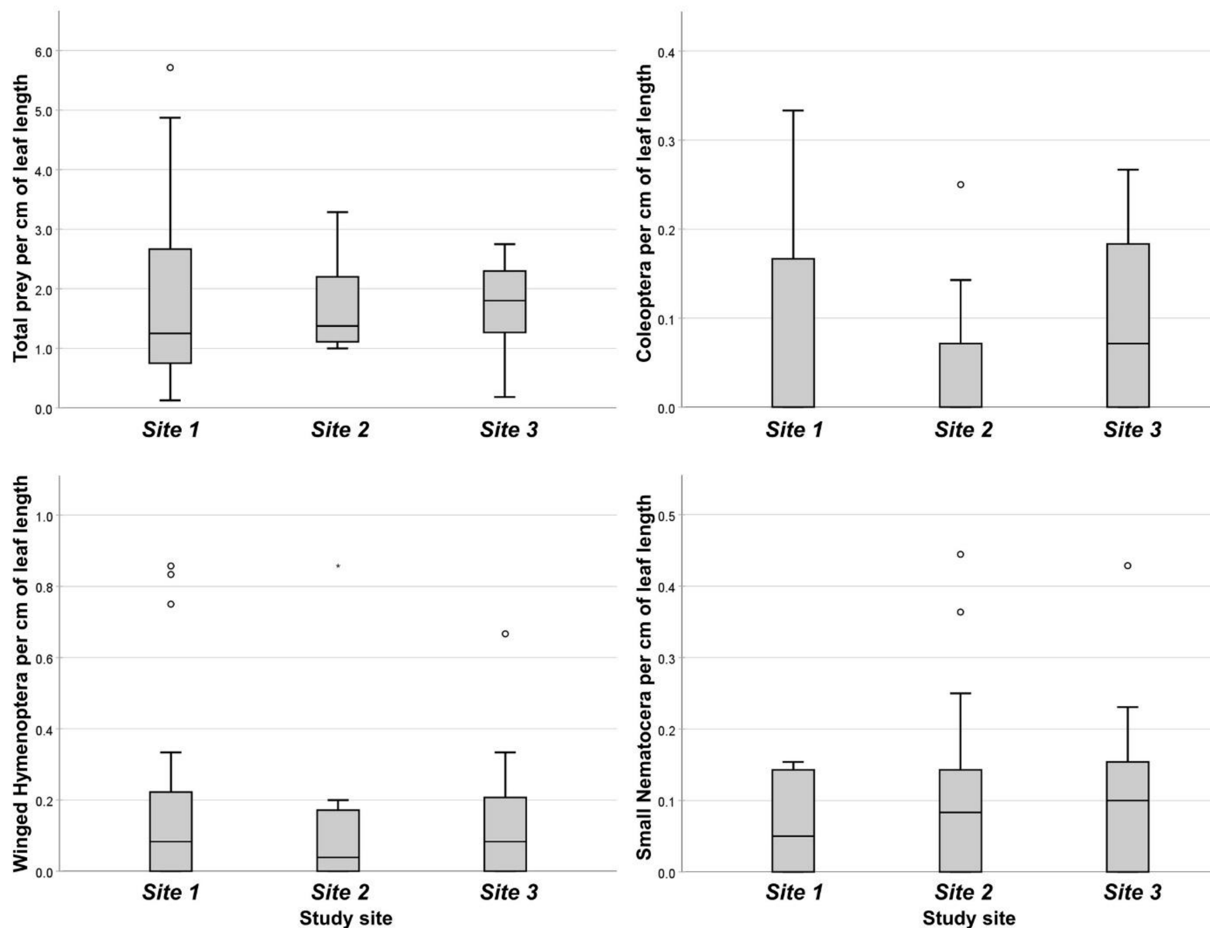
1414



1415

1416 **Appendix S3. Comparison of captured Small Nematocera per cm of leaf length between**
1417 **sympatric *Drosera cucullata* and *D. fragrans*.** Data is presented for each study site in the
1418 northern Kimberley region and for the combined data from all three sites. Statistical
1419 significance ($P < 0.05$) is determined by Mann-Whitney U tests and indicated in the graphic by
1420 asterisks.

1421



1422

1423 **Appendix S4. Among-site comparisons of total prey, Coleoptera, Winged Hymenoptera**
1424 **and Small Nematocera per cm of leaf length captured by *Drosera cucullata* and *D.***
1425 ***fragrans*.** Pooled data from both studied *Drosera* species in the northern Kimberley region is
1426 presented. Statistical significance ($P < 0.05$) is determined by Mann-Whitney U tests and
1427 indicated in the graphic by asterisks.

1428

1429 **Appendix S5. OTU table displaying DNA-metabarcoding raw read count and**
1430 **identification data for prey samples of three species from *D.* sect. *Arachnopus*.**

1431

1432 **Appendix S6. Processed OTU table displaying DNA-metabarcoding read count and**
1433 **identification data (pooled to arthropod family and subjected to pictorial quality control)**
1434 **for prey samples of three species from *D.* sect. *Arachnopus*.** Microorganisms, marine taxa,
1435 fungi and other obvious contaminants (as well as the ubiquitous phytophagous mealybugs and

1436 mites of Pseudococcidae, Trombidiformes and Mesostigmata) are not shown. Prey taxa
1437 confirmed by pictorial quality control are highlighted in green, and those excluded by this
1438 method are highlighted in red.

1439

1440 **Appendix S7. Table of measured average leaf lengths and observed total prey per cm of**
1441 **leaf length in three sampled species of *D*. sect. *Arachnopus*.**

Sample ID/species	Average leaf size (cm)	Number of captured prey per cm of leaf length
margaritacea 1 Scented	9	1.96
margaritacea 2 Scented	7.5	2.51
margaritacea 3 Scented	6.1	2.00
margaritacea 4 Scented	6.3	2.48
margaritacea 5 Scented	8.7	3.63
margaritacea 6 Unscented	7.7	1.58
margaritacea 7 Unscented	5.5	2.95
margaritacea 8 Unscented	8.1	1.88
margaritacea 9 Unscented	5.9	1.69
margaritacea 10 Unscented	6.2	1.81
finlaysoniana 1	11	0.53
finlaysoniana 2	10.2	0.63
finlaysoniana 3	10.7	0.82
finlaysoniana 4	10.8	0.81
finlaysoniana 5	9.6	1.17
finlaysoniana 6	9.3	1.33
finlaysoniana 7	11.1	0.65
finlaysoniana 8	10.1	0.69
finlaysoniana 9	10.7	1.07
finlaysoniana 10	10.9	0.44
hartmeyerorum 1	4.9	2.20
hartmeyerorum 2	7	1.26
hartmeyerorum 3	6.8	1.50
hartmeyerorum 4	5.4	2.52
hartmeyerorum 5	3.4	2.41
hartmeyerorum 6	5.4	1.93
hartmeyerorum 7	5.2	1.81
hartmeyerorum 8	5.6	1.18
hartmeyerorum 9	4.3	1.21
hartmeyerorum 10	5.4	1.96

1442