Plant diets of *Gryllus bimaculatus* and *Locusta migratoria* revealed by trnL metabarcoding

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Research Article

Keywords: trnL metabarcoding, Edible insects, Locusta migratoria, Gryllus bimaculatus, Diet assessment

Posted Date: May 22nd, 2026

DOI: https://doi.org/10.21203/rs.3.rs-9767050/v1

Additional Declarations: The authors declare no competing interests.

Version of Record: A version of this preprint was published at Journal of Insects as Food and Feed on June 17th, 2026. See the published version at https://doi.org/10.1163/23524588-bja10428.

Abstract

Edible orthopterans represent promising sustainable protein sources, yet their natural plant diets remain poorly characterized, limiting optimized feed development in farms. Here we applied chloroplast *trn*L metabarcoding to gut contents of *Gryllus bimaculatus* and *Locusta migratoria* sampled in Iberian field sites to provide a descriptive account of the plants ingested by two economically relevant species. Results revealed that *L. migratoria*'s diet is dominated by Poaceae grasses and Asteraceae forbs, notably *Dactylis, Cynodon, Sorghum, Bromus* and *Tragopogon dubius*. In contrast, *G. bimaculatus* displayed a richer plant diversity, dominated by Asteraceae and Poaceae with contributions from Malvaceae and Amaranthaceae; including *Oxytropis campestris, Cynodon dactylon* and *Dactylis glomerata*. These findings document the plant fraction of their diets (as *trn*L targets chloroplast DNA), complementing previous omnivory reports, and identify candidate taxa for palatability testing and feed formulation. Additionally, detection of potentially toxic plants (e.g. *Oxytropis, Mercurialis*) suggests the need for safety evaluations. Overall, this study provides actionable insights into orthopteran foraging ecology to inform sustainable insect farming practices.

INTRODUCTION

The pursuit of sustainable protein alternatives has positioned edible insects as a promising solution to address environmental concerns associated with conventional livestock production. These arthropods demonstrate superior resource conversion efficiency while generating substantially reduced greenhouse gas emissions compared to traditional animal farming (van Huis, 2022). Within the diverse realm of entomophagy, members of the Orthoptera order represent a particularly noteworthy category, encompassing roughly 340 documented species suitable for human consumption (Jongema, 2017; Magara et al., 2021).

Despite this taxonomic diversity, commercial insect farming operations have concentrated their efforts on a limited selection of orthopteran species. The industry predominantly cultivates *Acheta domesticus, Gryllodes sigillatus, Gryllus bimaculatus, G. assimilis*, and *Locusta migratoria* for large-scale production (van Huis, 2020). These particular species have established themselves as dietary staples around the world and demonstrate varying regulatory status within European markets. While *L. migratoria* and *A. domesticus* have already received approval as a novel food in Europe, the other species are currently undergoing the authorization process (Halloran, 2017; 2018; Magara et al., 2021). Market analyses indicate substantial growth potential for this sector, with economic projections estimating the edible Orthoptera market value will reach USD 3.5 billion within the next decade (Meticulous Research, 2022).

However, commercial orthopteran production faces significant economic challenges, with feed procurement constituting the largest operational cost. The industry's reliance on conventional poultry feeds imposes substantial financial burdens on producers (Hanboonsong & Durst, 2020; Harsányi et al., 2020). These feeds directly influence critical production parameters, such as development time, final biomass, and the nutritional profile of the harvested insects (Hackewitz, 2020; Oloo et al., 2019).

Consequently, developing cost-effective feeding strategies is essential to improve the economic viability of orthopteran farming.

Numerous research efforts have focused on developing artificial diets incorporating various local plant-based materials to reduce reliance on costly poultry feed (Anagonou et al., 2025; Magara et al., 2025; Orinda et al., 2025; Runyambo et al., 2023; Sorjonen et al., 2019; Miech et al., 2016). However, these formulations often lack a thorough understanding of the insect's natural feeding behaviors, which may their effectiveness. This knowledge gap is due to the limited research on the feeding ecology of orthopteran species (Fontanetti and Zefa, 2000). Gaining detailed insight into their natural dietary diversity could improve animal welfare (Chatpongcharoen et al., 2021) and enhance intake and productivity (Hesselmann et al., 2025).

Current knowledge indicates that crickets are non-specialized omnivores, consuming plant material, fungi, and occasionally scavenging on dead insects or engaging cannibalism. Similarly, *L. migratoria* primarily exhibits graminivorous feeding pattern, with grasses as it main food source in natural habitats (van Peer et al., 2021). However, these dietary characterizations are based on visual observations (Collavo et al., 2005; Gangwere, 1961; Gutiérrez et al., 2020; Hanboonsong et al., 2013) rather than new generation analytical approaches.

DNA metabarcoding analysis of fecal material or digestive tract contents represents a transformative methodology that enables higher taxonomic resolution and improved accuracy in dietary studies compared to traditional observational methods (Taberlet et al., 2018; Ando et al., 2013; Galimberti et al., 2016; Dunn et al., 2018). This molecular approach has successfully reconstructed natural feeding preferences across diverse animal taxa, including various insect species, providing unprecedented insights into ecological dietary relationships (Kartzinel et al., 2025; Stenhouse et al., 2023; McClenaghan et al., 2019; Thomsen et al., 2019). To address this critical knowledge gap and provide evidence-based foundations for improved feeding strategies in commercial orthopteran production, we employed DNA metabarcoding techniques to comprehensively characterize the natural diets of two economically important edible orthopteran species: *G. bimaculatus* and *L. migratoria*. This investigation aims to elucidate the taxonomic diversity and compositional patterns of their natural food sources, information that could be relevant for the development of more effective feeding protocols for industrial insect farming operations.

METHODOLOGY

Insects

Wild adult specimens were collected from four distinct Spanish agroecosystems (Table 1), with sampling yielding 3–4 individuals per location. Upon capture, insects were individually housed in sterile plastic containers and immediately transferred to a portable cooling system equipped with ice packs for transport to laboratory facilities. Specimens were subsequently preserved at -20°C pending molecular

and dietary analyses. Species identification was confirmed through molecular barcoding procedures involving the removal of a single hind limb from each specimen. Genomic DNA extraction was performed utilizing the DNeasy Blood and Tissue Kit (Qiagen Sciences) according to standard manufacturer specifications. Amplification of the mitochondrial cytochrome oxidase I (COI) region was achieved using primer pairs LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3'), generating amplicons of approximately 708 base pairs. Sequencing was conducted via Sanger methodology, with resulting sequences subjected to comparative analysis against the NCBI GenBank database to verify taxonomic identity of *G. bimaculatus* and *L. migratoria* specimens.

Table 1

Species	Geographic Location	Coordinates
L. migratoria	Sant Guim de Freixenet, Lerida, Spain	41°38'04.5"N 1°26'11.0"E
	Palomares del Rio, Sevilla, Spain	37°18'37.4"N 6°02'30.8"W
	Torce, Asturias, Spain	43°32'01.1"N 6°46'26.1"W
G. bimaculatus	Salamanca, Castilla y León, Spain	40°29'24.3"N 5°40'40.6"W
	Rota, Andalucia, Spain	36°38'01.8"N 6°23'22.9"W
	Castelldefels, Cataluña, Spain	41°17'00.9"N 1°57'58.0"E

DNA extraction and amplicon sequencing.

For dietary DNA analysis, insect specimens underwent surface sterilization through a sequential decontamination protocol involving immersion in sterile distilled water (15 seconds), followed by 70% ethanol treatment (15 seconds), and concluding with a final sterile water rinse. Complete digestive systems were aseptically dissected from each individual and transferred to 2 ml of 0.9% saline solution. Gut contents were subsequently released through homogenization using sterile plastic micro-pestles, creating a uniform tissue slurry.

Individual gut analyses were conducted using 1 ml aliquots of the homogenized material for plant DNA extraction, following the Power Soil Pro kit protocols (Qiagen Sciences). Amplification targeted the P6 loop region of the chloroplast *trnL* intron, a highly variable and taxonomically informative sequence, using the universal primer sets from Taberlet et al. (2007): Forward (g) 5'-GGGCAATCCTGAGCCAA-3' and Reverse (h) 5'-CCATTGAGTCTCTGCACCTATC-3'. To facilitate sample multiplexing during high-throughput sequencing, unique 8-base pair molecular tags, differing by a minimum of 4 nucleotides, were incorporated at the 5' terminus of each primer (Taberlet et al., 2012) (Appendix Table S1)

PCR amplifications were performed in duplicate under the following thermal cycling conditions: initial denaturation at 95°C for 10 minutes, followed by 40 cycles of denaturation (95°C, 30 seconds), annealing (55°C, 30 seconds), and extension (72°C, 30 seconds), with a final extension step at 72°C for 5 minutes.

The resulting 18 amplicon samples, each bearing distinct forward and reverse primer tags, were combined into a single pooled library. Purified PCR products were mixed at equimolar concentrations and submitted to Novogen (UK) for library preparation and paired-end sequencing on an Illumina PE250 platform.

Bioinformatic analysis

Quality assessment of raw reads was performed with FastQC (v0.12.1) and summarized using MultiQC (v1.21). It showed that most reads contained Illumina adaptor sequences, tags, primers and other 5' and 3' errors given that the sequencing length was three times as the expected amplicon length (c. 50 bp), but the following pipeline helped recover the majority of reads. Adapter and primer sequences were removed using cutadapt (v4.4) in paired-end mode and allowing up to one mismatch (-e 1). Paired-end reads were then merged with leeHom (v1.3.1) in automatic adapter detection mode (--auto) as this produced a more satisfactory pairing of reads than vsearch for our dataset, according to quality assessment of merged reads. This produced one output of merged reads per sample which were processed in parallel as follows. Merged reads were dereplicated with OBItools2 (obiuniq), and low-abundance sequences (count < 2) were removed (obigrep). Remaining sequences were sample-tagged (obiannotate), denoised with obiclean (parameters: -r 0.05, -H), non-informative OBITools tags were stripped, and sequences were sorted by abundance (obisort). To work with one fasta file for all samples before taxonomic assignment, all per-sample datasets were concatenated into a single dataset, dereplicated by sample (obiuniq -m sample), and sorted by count. A final sequence table was generated with obitab for downstream analysis in R.

The resulting dataset was subjected to BLASTn (v2.16.0) against a curated *trn*L reference database (accessible from Zenodo). The BLAST was performed with blastn algorithm (-task blastn) keeping a maximum of 50 aligned sequence targets (-max_target_seqs 50) and most importantly we deactivated the default masking of low-complexity regions (-dust no) since the *trn*L UAA region is rich in these features (homopolymers, tandem repeats) and can be used by MEGAN to better trace common ancestry of sequences. The BLAST output was imported into MEGAN (v6.25.10) for taxonomic assignment using the weighted LCA algorithm with the following parameters: min score = 75, max expected = 0.01, min percent identity = 87, top percent = 10, min support = 1, min read length = 10, percent to cover = 80. Both BLAST and MEGAN parameters were empirically selected based on the analysis of the BLAST output using a custom R script (v4.2.3) (see Appendix Figure S1 and Table S2).

Because no extraction/PCR blanks were available, we applied three conservative cleaning steps before ecological analyses. First, ASVs assigned to plant families kown to appear as environmental or reagent background in *trn*L metabarcoding runs were removed globally. Second, to limit cross-sample "tag-jump" and very low-frequency artefacts, we imposed a per-ASV per-sample minimum abundance of 0.1% of that ASV's total run count (i.e. reads in a sample < 0.001 × total reads of that ASV across the run were set to zero). Third, after collapsing ASVs to the target taxonomic rank (order, family, genus, species), we expressed abundances as relative read abundance (RRA, %) per sample and zeroed taxa with RRA <

0.1%, which we defined empirically (Appendix, Figure S2). This produced a conservative matrix that was used for the descriptive diet plots. Ambiguous assignments (e.g., sequences reaching family but not genus/species) were reported at the highest reliable rank. A detailed list of the removed taxa at each of the cleaning steps is provided in Appendix Table S3. All the bioinformatic scripts, the reference database, and the R scripts for the final curation and visualizations are available in Zenodo. The figures were produced in R (v.4.2.3) and curated using Inkscape (v1.0).

RESULTS

Analysis of chloroplast trnL sequencing data

The *trn*L UAA (g–h) barcode region was successfully amplified from eDNA extracted from nine wild *G. bimaculatus* and nine *L. migratoria* gut samples. Following initial quality control procedures, including read trimming, filtering, and paired-end merging, a total of 4,384,124 reads were recovered across all 18 digestive tract samples, yielding an average sequencing depth of 225,426 reads per sample (range 42,469–572,744). When reads were summed by host, *G. bimaculatus* contributed 2,076,542 reads (median 209,812; range 42,469–455,402) and *L. migratoria* contributed 2,307,582 reads (median 241,041; range 114,651–572,744).

Dietary analysis

L. migratoria

In wild *L. migratoria* (9 individuals) we detected up to 12 plant families, again mostly Magnoliopsida (~ 100%). By order (RRA), the diet was clearly grass-biased, with Poales at 56.6% and Asterales at 32.9%; Caryophyllales (2.9%), Fabales (2.8%) and Malvales (2.1%) contributed smaller fractions (Supplementary Figure S3A).At the family level (RRA) the pattern was very similar: Poaceae 56.6%, Asteraceae 32.9%, Amaranthaceae 2.8%, Fabaceae 2.8% and Malvaceae 2.1% (Fig. 1A). At the genus level (RRA), *Tragopogon* (35.6%), *Dactylis* (23.6%), *Cynodon* (16.0%), *Sorghum* (12.5%), *Bromus* (3.5%), *Amaranthus* (3.0%) and *Oxytropis* (2.8%) were the main components (Fig. 1B). At the species level (RRA) the signal was again more concentrated, with *Tragopogon dubius* (45.3%), *Dactylis glomerata* (30.1%), *Cynodon dactylon* (20.4%) and *Oxytropis campestris* (3.5%) as the dominant species (Fig. 1C). The differences with respect to the genus-level plots arise because not all reads assigned to a given genus could be resolved with the same confidence to species, or they fell below the species-level reporting threshold.

G. bimaculatus

Across the 9 wild *G. bimaculatus* individuals, we recovered up to 20 plant families and a comparable number of genera, belonging mainly to Magnoliopsida (100%). At the order level (RRA), the diet was dominated by Asterales (34.8%), Poales (26.1%), Malvales (13.4%), Caryophyllales (13.3%) and Fabales (8.6%) (Supplementary Figure S2B). At the family level (RRA), the leading groups were Asteraceae

(34.8%), Poaceae (26.1%), Malvaceae (13.4%), Amaranthaceae (12.7%) and Fabaceae (8.6%) (Fig. 2A). At the genus level (RRA), the most represented genera were *Tragopogon* (43.5%), *Amaranthus* (15.9%), *Oxytropis* (10.7%), *Bromus* (8.6%), *Cynodon* (7.4%) and *Dactylis* (6.8%) (Fig. 2B). At the species level, only those reads that could be confidently assigned to species were kept, so the list is shorter and more concentrated; under this criterion, *Tragopogon dubius* (62.2%), *Oxytropis campestris* (15.4%), *Cynodon dactylon* (10.6%) and *Dactylis glomerata* (9.8%) comprised most of the species-level signal (Fig. 2C). The apparent difference between genus- and species-level plots (e.g. *Amaranthus* present at genus but not in the species panel) reflects the fact that the short *trn*L marker cannot resolve *Amaranthus* taxa at the species level or fell below the species-level reporting threshold.

DISCUSSION

This study provides a description of the plant taxa foraged by two relevant edible orthopterans, *G. bimaculatus* and *L. migratoria*, using trnL-based DNA metabarcoding of gut contents. Taking advantage of the short *trn*L marker, ideal for degraded eDNA as found in gut contents, and its satisfactory taxonomic resolution (Espinosa Prieto et al., 2024), we were able to document the breadth and structure of their plant intake at order, family, genus and, when possible, species level, overcoming some of the limitations of purely observational or morphology-based diet studies. Understanding which plants are actually consumed by these two species can inform the selection of plant ingredients and sensory cues for artificial diets in insect farming systems.

To date, most knowledge of the natural diet of *L. migratoria* comes from field observations or crop-damage reports and is therefore biased toward conspicuous, cultivated grasses. These sources consistently state that *L. migratoria* feeds primarily on Poaceae such as wheat, barley, oat, rye, maize, rice, *Panicum*, and sorghum (Belhadj et al., 2024; Djamel et al., 2023) and may occasionally exploit legumes and horticultural crops (alfalfa, clover, peas/legumes, soybean, cucurbits), likely because these plants are abundant and monitored. Our *trn*L metabarcoding data broadly corroborate this graminoid core: Poaceae was the dominant family, with *Dactylis, Cynodon, Sorghum*, and *Bromus* among the most represented genera. Crucially, the data also show that the field diet is not restricted to cereal-like grasses. We detected a substantial contribution of Asteraceae (≈ 33% RRA), a group seldom mentioned in agronomic checklists (Lecoq 2023) because many of its members are non-crop forbs; we also recovered Amaranthaceae, Fabaceae (including *Oxytropis*), and Malvaceae at low but recurrent relative abundances. Within Poaceae, *D. glomerata* occurred alongside the expected pasture grasses, and within Asteraceae, *T. dubius* was clearly represented, even though this species is rarely reported as a food plant for *L. migratoria*, likely because it is not routinely surveyed. Taken together, these results expand the list of plant resources actually exploited in situ, adding ruderal and wild dicotyledonous taxa typically missed by visual methods, and support the view that this orthopteran uses a broader, more heterogeneous vegetation matrix than suggested by crop-focused records. Beyond corroborating the graminoid core reported in host-plant lists and datasheets, our *trn*L results extend those compilations by documenting recurrent use of Asteraceae like *T. dubius* with additional low but consistent signals from Amaranthaceae, Fabaceae (*Oxytropis*), and Malvaceae.

For *G. bimaculatus*, this is, to our knowledge, the first description of the plant component of the gut content based on DNA metabarcoding. Most of the available information on “field crickets” is generic and refers to crickets as opportunistic or omnivorous orthopterans that feed on weed seeds, seedlings, decaying plant material and, when available, dead or living arthropods, but it does not resolve the botanical composition of the diet of *G. bimaculatus* specifically (El-Damanhouri, 2011; Ogita et al., 2021). Our data refine that view by showing which vascular plant lineages are actually ingested in the field.

The *trn*L signal of *G. bimaculatus* was more even and taxonomically richer than that of *L. migratoria*. We detected two plant classes, 16 orders and 22 families in only nine individuals, with four families (Asteraceae, Poaceae, Malvaceae and Amaranthaceae) accounting for most of the relative read abundance. This pattern is consistent with a ground-dwelling, generalist forager that exploits a mosaic of ruderal, pasture and synanthropic plants, rather than a strict graminivore. Asteraceae was the most abundant family (≈ 35% RRA), with *Tragopogon* as the leading genus, followed by Poaceae (≈ 26%), and then Malvaceae (≈ 13%) and Amaranthaceae (≈ 13%). The presence of Fabaceae (≈ 9%) further indicates access to herbaceous legumes.

At genus and species level, the diet became more structured around a small set of taxa. In *G. bimaculatus*, genera were led by *Tragopogon, Amaranthus, Oxytropis, Bromus, Cynodon* and *Dactylis*, while species-level assignments concentrated the signal in *T. dubius*, *O. campestris*, *C. dactylon* and *D. glomerata*. All these genera and species are common in disturbed Mediterranean plant assemblages and are phenologically available in open habitats (Castroviejo 1986–2012), so the composition retrieved by metabarcoding is ecologically plausible for Iberian field sites, especially given that our sampling localities were near highly disturbed, human-impacted areas.

The fact that *Tragopogon* is strongly represented in *G. bimaculatus* and also present in *L. migratoria* suggests that this forb is both readily available and broadly palatable across orthopteran guilds in the study area. Likewise, the recurrent detection of pasture grasses such as *Dactylis* and *Cynodon* in both datasets is consistent with foraging within the low herbaceous layer. However, *G. bimaculatus* retains non-poaceous families at comparatively high proportions, whereas *L. migratoria* expresses a more pronounced graminoid bias patterns coherent with their ecological strategies (generalist/omnivorous collector vs. grass-oriented feeder).

When plant taxa were grouped by functional type, a clear contrast emerged between the two orthopterans. In *G. bimaculatus*, the *trn*L signal was dominated by non-legume herbaceous dicotyledons (≈ 61%), with grasses contributing a smaller but substantive share (≈ 26%) and legumes accounting for a minor fraction (≈ 9%); woody plants were negligible. In *L. migratoria*, by contrast, grasses formed the core of the diet (≈ 57%), while non-legume herbaceous dicots contributed ≈ 38% and legumes ≈ 3%, again with a negligible woody component. Functionally, this indicates that *G. bimaculatus* maintains a broader dicot herb layer within its foraging profile, whereas *L. migratoria* concentrates more strongly on graminoids.

It is important to note that our metabarcoding marker targets chloroplast DNA; thus, the results document the plant portion of the diet and do not contradict previous reports of animal matter or

detritus consumption in field crickets. Rather, they complement them by showing that, when plant DNA is quantified, the species is not restricted to one or two crop plants but routinely ingests several herbaceous families. This has two practical consequences. First, it shows that *G. bimaculatus* ingests taxonomically diverse plant material, suggesting that formulated feeds could include ingredients from Asteraceae, Poaceae or Malvaceae. Second, the recurrence of certain taxa across individuals (Asteraceae and Poaceae in particular) suggests that these plants are commonly consumed by this species and could be considered when designing captive diets.

As concrete opportunities, *T. dubius* is a widely distributed ruderal weed whose leaves and stems are used for food and traditional remedies (Ahmad et al., 2024). Although it is not a conventional crop, its availability, reported edibility, and strong signal in our data make it a sensible candidate for feed trials with *G. bimaculatus*. Likewise, *C. dactylon* and *D. glomerata* are cosmopolitan pasture grasses noted for heat tolerance, modest water requirements, and established use in animal feeding systems (Gaier et al., 2024; Garelt et al., 2023; Severoglu and Gullap, 2023); both are agronomically manageable and thus practical entries for formulation screens with orthopterans.

By contrast, two taxa detected in our material, *O. campestris* and *M. annua* warrant caution. Several *Oxytropis* ("locoweed") species contain swainsonine and are toxic to grazing vertebrates; *M. annua* is a ruderal Euphorbiaceae linked to livestock poisoning and allergenic pollen in humans (Mahillon et al., 2024; Guan et al., 2021). Their occurrence here does not imply suitability as ingredients, but it does raise testable hypotheses: i) whether *G. bimaculatus* can detoxify or tolerate alkaloid-bearing forbs without adverse effects, and ii) whether any plant toxins or metabolites persist in the insect and could carry over to edible products.

An additional implication of our results is that having a resolved list of plant taxa actually ingested by *G. bimaculatus* and *L. migratoria* gives a realistic starting set of sensory targets (odours, surface chemicals, and textures) that these insects are capable of detecting and accepting. Crickets, including *G. bimaculatus*, have well-developed olfactory and contact-chemosensory systems on the palps and mouthparts, and they can rapidly learn to associate an odour with a reward and reverse that association, showing fast acquisition and long retention of olfactory memories (Matsumoto and Mizunami 2000). This means that at least in crickets, plant-derived volatiles or surface cues can be used as conditioning signals, so plant species that we detected in the gut represent cues that the insects have already accepted in nature. *L. migratoria* show a comparable but more "mechanical" testing sequence: they palpate, bite, and then either continue feeding or reject the plant, and this rejection stage can shift with experience, which shows that contact chemoreceptors guide acceptance of particular plant surfaces (Blaney et al., 1985). In *L. migratoria* there is also antennal machinery tuned to host-plant volatiles antenna-specific P450s that modulate perception of wheat/gramineous volatiles, confirming that host recognition is, at least in part, odour-driven (Wu et al., 2022)

From animal nutrition is known that "palatability enhancers" are routinely used to increase intake by modifying smell and taste, often with plant extracts or natural flavouring components, and this approach

is especially useful when intake is depressed or when novel ingredients are introduced (Perera and Ravindran, 2025). Translating that logic to edible-insect farming, our diet list tells us which botanical lineages the two orthopterans already recognize and consume in the field, so electrophysiological or behavioural assays can now focus on volatiles and surface extracts from exactly those taxa, rather than screening arbitrary plants. In practice, this could help formulators consider plant ingredients from taxa already consumed by these orthopterans, or test volatiles and surface extracts from these plants for their effects on diet acceptance.

In conclusion, *trn*L metabarcoding of gut contents revealed that wild *G. bimaculatus* and *L. migratoria* routinely ingest a limited set of herbaceous plant lineages common to disturbed Mediterranean mosaics, with *G. bimaculatus* exhibiting a broader dicot component and L. migratoria a stronger grass bias. These descriptive profiles identify concrete genera and species that can guide palatability assays and the assembly of plant-based, nutritionally equivalent feed ingredients, while flagging taxa that merit targeted safety evaluation. Because our sample size per species was moderate and lacked broad spatial and seasonal replication, the inferences are necessarily descriptive and specific to the Iberian context sampled. Future work should expand sampling across habitats and seasons to capture geographic and phenological variation in diet, thereby completing a fuller picture of foraging breadth and refining candidate ingredients for formulated diets in edible-insect production.

Declarations

Data Accessibility and Benefit-Sharing

*trn*L metabarcoding sequences and bioinformatic scripts, the reference database, and the R scripts for the final curation and visualizations are available in Zenodo with the DOIs: 10.5281/zenodo.18355000 and 10.5281/zenodo.18354624 respectively.

Author contributions: CRediT

Diego Cruz: Writing-original draft, Formal analysis, Data curation, Funding acquisition. **Armando Espinosa:** Writing-original draft, Formal analysis, Data curation. **Paula Garcia-Fraile:** Supervision, Writing-review *and* editing, Funding acquisition.

Conflict of interest

No potential conflict of interest was reported by the authors.

Funding

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 101034371, and funds received by "Escalera de Excelencia" CLU-2025-2-04 co-funded by Consejería de Educación de Castilla y León and FEDER Funds 2021–2027.

References

1. Ahmad, S.S., Garg, C., Kour, R., Bhat, A.H., Raja, V., Gandhi, S.G., Ataya, F.S., Fouad, D., Radhakrishnan, A. and Kaur, S., 2024. Metabolomic insights and bioactive efficacies of *Tragopogon dubius* root fractions: Antioxidant and antiproliferative assessments. Heliyon 10: e34746. https://doi.org/10.1016/j.heliyon.2024.e34746
2. Anagonou, C.M., Loko, Y.L.E., Dassou, A.G., Azalhouet, R., Tokannou, R., Amadji, G.L., Segnon, A.C. and Tamò, M., 2025. Plant material and by-product diets for the mass-rearing of house crickets (*Acheta domesticus* L. (Orthoptera: Gryllidae)) in the Republic of Benin. International Journal of Tropical Insect Science 45: 669-680. https://doi.org/10.1007/s42690-025-01459-x
3. Ando, H., Setsuko, S., Horikoshi, K., Suzuki, H., Umehara, S., Inoue-Murayama, M. and Isagi, Y., 2013. Diet analysis by next-generation sequencing indicates the frequent consumption of introduced plants by the critically endangered Red-headed Wood Pigeon (*Columba janthina nitens*) in oceanic island habitats. Ecology and Evolution 3: 4057-4069. https://doi.org/10.1002/ece3.773
4. Blaney, W.M., Winstanley, C. and Simmonds, M.S.J., 1985. Food selection by locusts: An analysis of rejection behaviour. Entomologia Experimentalis et Applicata 38: 35-40.
5. Belhadj, H., Belkouche, S. and Achouche, A., 2024. Host plants of three species of locusts from Algeria. Indian Journal of Entomology Online e24959. https://doi.org/10.55446/IJE.2024.1959
6. Castroviejo, S. (coord. gen.). 1986-2012. *Flora iberica* 1-8, 10-15, 17-18, 21. Real Jardín Botánico, CSIC, Madrid.
7. Chatpongcharoen, P., Campera, M., Laithong, P., Gibson, N.L. and Nekaris, K.A.I., 2021. Naturalising diet to reduce stereotypic behaviours in slow lorises rescued from wildlife trade. Applied Animal Behaviour Science 242: 105413. https://doi.org/10.1016/j.applanim.2021.105413
8. Collavo, A., Glew, R., Huang, Y., Chuang, L., Bosse, R. and Paoletti, M., 2005. House cricket small-scale farming. In: Paoletti, M.G. (ed.) Ecological implications of minilivestock. CRC Press, Boca Raton, FL, USA, pp. 533-558.
9. Djamel Eddine, L., Abdelmadjid, B., Imen, L., Badis, A. and Akkal, S., 2023. Review on *Locusta migratoria cinerascens* (Fabricius, 1781). Journal of Bioresource Management 10(4).
10. El-Damanhouri, H.I.H., 2011. Studies on the influence of different diets and rearing conditions on the development and growth of the two-spotted cricket *Gryllus bimaculatus* de Geer. Doctoral dissertation, Universität Bayreuth, Germany. Available at: https://epub.uni-bayreuth.de/id/eprint/310/1/Diss.pdf
11. Espinosa Prieto, A., Hardion, L., Debortoli, N., Beisel, J.-N., 2024. Finding the perfect pairs: A matchmaking of plant markers and primers for multi-marker eDNA metabarcoding. Molecular Ecology Resources e13937. https://doi.org/10.1111/1755-0998.13937
12. Fontanetti, C.S. and Zefa, E., 2000. Morphological characterization of the proventriculus of *Gryllus assimilis* Fabricius (Orthoptera, Gryllidae). Revista Brasileira de Zoologia 17: 193-198.

13. Gaier, L., Poetsch, E.M., Graiss, W., Klingler, A., Herndl, M. and Krautzer, B., 2024. The effect of drought on agronomic and plant physiological characteristics of cocksfoot (*Dactylis glomerata* L.) cultivars. Agriculture 14(7): 1116. https://doi.org/10.3390/agriculture14071116
14. Galbraith, J.A., Beggs, J.R., Jones, D.N. and Stanley, M.C., 2015. Supplementary feeding restructures urban bird communities. Proceedings of the National Academy of Sciences 112: 2648-2657. https://doi.org/10.1073/pnas.1501489112
15. Gangwere, S.K., 1961. A monograph on food selection in Orthoptera. Transactions of the American Entomological Society 87: 67-230.
16. Garelt, S., Mendoza, A., Bello, N.M., Lattanzi, F.A., Fariña, S.R. and Savian, J.V., 2023. Effects of grazing management and concentrate supplementation on intake and milk production of dairy cows grazing orchardgrass. Animal Feed Science and Technology 301: 115668. https://doi.org/10.1016/j.anifeedsci.2023.115668
17. Guan, H., Liu, X., Mur, L.A.J., Fu, Y., Wei, Y., Wang, J. and He, W., 2021. Rethinking of the roles of endophyte symbiosis and mycotoxin in *Oxytropis* plants. Journal of Fungi 7(5): 400. https://doi.org/10.3390/jof7050400
18. Gutiérrez, Y., Fresch, M., Ott, D., Brockmeyer, J. and Scherber, C., 2020. Diet composition and social environment determine food consumption, phenotype and fecundity in an omnivorous insect. Royal Society Open Science 7: 200100. https://doi.org/10.1098/rsos.200100
19. Hackewitz, L., 2020. Sustainable and cost-efficient feed ingredients for optimum breeding of house crickets (*Acheta domesticus*) for human consumption in Thailand. Master's thesis, Swedish University of Agricultural Sciences, Uppsala, Sweden.
20. Hanboonsong, Y., Jamjanya, T. and Durst, P.B., 2013. Six-legged livestock: edible insect farming, collection and marketing in Thailand. FAO, Regional Office for Asia and the Pacific, Bangkok, Thailand.
21. Hesselmann, M., Thorne, S., Vitra, A., Steiner, A.K., Leiber, F. and Dittmann, M.T., 2025. Foraging preferences of dairy cows grazing on contrasted multispecies swards. Veterinary and Animal Science 28: 100439. https://doi.org/10.1016/j.vas.2025.100439
22. Jongema, Y., 2017. List of edible insects of the world (1 April 2017). Wageningen UR, Wageningen, The Netherlands. Available at: https://www.wur.nl/en/Research-Results/Chair-groups/Plant-Sciences/Laboratory-of-Entomology/Edible-insects/Worldwide-species-list.htm
23. Kartzinel, T.R., Chen, P.A., Coverdale, T.C., Erickson, D.L., Kress, W.J., Kuzmina, M.L., Rubenstein, D.I., Wang, W. and Pringle, R.M., 2015. DNA metabarcoding illuminates dietary niche partitioning by African large herbivores. Proceedings of the National Academy of Sciences 112(26): 8019-8024. https://doi.org/10.1073/pnas.1503283112
24. Magara, H.J., Niassy, S., Ayieko, M.A., Mukundamago, M., Egonyu, J.P., Tanga, C.M., Kimathi, E.K., Ongere, J.O., Fiaboe, K.K., Hugel, S., Orinda, M.A., Roos, N. and Ekesi, S., 2021. Edible crickets (Orthoptera) around the world: distribution, nutritional value, and other benefits - a review. Frontiers in Nutrition 7: 537915. https://doi.org/10.3389/fnut.2020.537915

25. Magara, H.J.O., Hugel, S. and Fisher, B.L., 2025. Growth performance and nutritional content of tropical house cricket (*Gryllodes sigillatus* (Walker, 1969)) reared on diets formulated from weeds and agro by-products. Insects 16(6): 600. https://doi.org/10.3390/insects16060600
26. Mahillon, M., Brodard, J., Schoen, R., Botermans, M., Dubuis, N., Groux, R., Pannell, J.R., Blouin, A.G. and Schumpp, O., 2024. Revisiting a pollen-transmitted ilarvirus previously associated with angular mosaic of grapevine. Virus Research 344: 199362. https://doi.org/10.1016/j.virusres.2024.199362
27. Matsumoto, Y. and Mizunami, M., 2000. Olfactory learning in the cricket *Gryllus bimaculatus*. The Journal of Experimental Biology 203: 2581-2588.
28. McClenaghan, B., Gibson, J.F., Shokralla, S. and Hajibabaei, M., 2019. Discrimination of grasshopper (Orthoptera: Acrididae) diet and niche overlap using next-generation sequencing of gut contents. Ecology and Evolution 9(5): 2651-2662. https://doi.org/10.1002/ece3.4927
29. Miech, P., Berggren, Å., Lindberg, J., Chhay, T., Khieu, B. and Jansson, A., 2016. Growth and survival of reared Cambodian field crickets (*Teleogryllus testaceus*) fed weeds, agricultural and food industry by-products. Journal of Insects as Food and Feed 2: 285-292. https://doi.org/10.3920/JIFF2016.0028
30. Ogita, S., Tanaka, Y. and Kuriwada, T., 2021. Effect of diet on body size and survival of omnivorous crickets. Entomological Science 24(4): 347-353. https://doi.org/10.1111/ens.12487
31. Okweche, S.I., Eyo, K.I. and Effa, E.B., 2022. Food preference and nutrient composition of African cricket *Brachytrupes membranaceus* L. (Dury) in Cross River State Nigeria. Journal of Insects as Food and Feed 8(12): 1501-1510. https://doi.org/10.3920/JIFF2021.0198
32. Oloo, J.A., Ayieko, M.A. and Nyongesah, J.M., 2019. *Acheta domesticus* (cricket) feed resources among smallholder farmers in Lake Victoria region of Kenya. Food Science & Nutrition 8: 69-78. https://doi.org/10.1002/fsn3.1276
33. Perera, W.N.U. and Ravindran, V., 2025. Role of feed additives in poultry nutrition: Historical, current and future perspectives. Animal Feed Science and Technology 326: 116371. https://doi.org/10.1016/j.anifeedsci.2025.116371
34. Orinda, M.A. and Aboge, D.O., 2025. The influence of plant-based diets on growth, development, and nutritional quality in *Acheta domesticus* and *Gryllus bimaculatus* crickets. International Journal of Tropical Insect Science 45: 1225-1235. https://doi.org/10.1007/s42690-025-01509-4
35. Runyambo, I., Andika, D., Watako, A., Mwonga, S. and Mweresa, C., 2023. Feeding preferences of the field cricket *Scapsipedus icipe* (Orthoptera: Gryllidae) for different species of *Commelina*. European Journal of Entomology 120: 115-127. https://doi.org/10.14411/eje.2023.013
36. Severoglu, S. and Gullap, M.K., 2023. Determination of feed yield and quality parameters of bermudagrass (*Cynodon dactylon* L. (Pers.)) populations collected from natural flora. Agronomy 13(6): 1471. https://doi.org/10.3390/agronomy13061471
37. Sorjonen, J.M., Valtonen, A., Hirvisalo, E., Karhapää, M., Lehtovaara, V.J., Lindgren, J. and Roininen, H., 2019. The plant-based by-product diets for the mass-rearing of *Acheta domesticus* and *Gryllus bimaculatus*. PLoS ONE 14(6): e0218830. https://doi.org/10.1371/journal.pone.0218830

38. Stenhouse, E.H., Bellamy, P., Vaughan, I., Kirby, W.B., Symondson, W.O.C. and Orozco-terWengel, P., 2023. Using DNA metabarcoding to explore spatial variation in diet across European Hawfinch populations. Journal of Field Ornithology 94(1): 1-12. https://doi.org/10.5751/JFO-00254-940112
39. Taberlet, P., Coissac, E., Pompanon, F., Gielly, L., Miquel, C., Valentini, A., Vermat, T., Corthier, G., Brochmann, C., Willerslev, E., 2007. Power and limitations of the chloroplast trnL (UAA) intron for plant DNA barcoding. Nucleic Acids Research 35(3), e14. https://doi.org/10.1093/nar/gkl938
40. Taberlet, P., Coissac, E., Hajibabaei, M. and Rieseberg, L.H., 2012. Environmental DNA. Molecular Ecology 21(8): 1789-1793. https://doi.org/10.1111/j.1365-294X.2012.05542.x
41. Taberlet, P., Bonin, A., Zinger, L. and Coissac, E., 2018. Environmental DNA: for biodiversity research and monitoring. Oxford University Press, Oxford, UK. https://doi.org/10.1093/oso/9780198767220.001.0001
42. Thomsen, P.F. and Sigsgaard, E.E., 2019. Environmental DNA metabarcoding of wild flowers reveals diverse communities of terrestrial arthropods. Ecology and Evolution 9(4): 1665-1679. https://doi.org/10.1002/ece3.4809
43. Van Huis, A., 2020. Edible crickets, but which species? Journal of Insects as Food and Feed 6(2): 91-94. https://doi.org/10.3920/JIFF2019.0048
44. Van Peer, M., Frooninckx, L., Coudron, C., Berrens, S., Álvarez, C., Deruytter, D., Verheyen, G. and Van Miert, S., 2021. Valorisation potential of using organic side streams as feed for *Tenebrio molitor*, *Acheta domesticus* and *Locusta migratoria*. Insects 12(9): 796. https://doi.org/10.3390/insects12090796
45. Wu, H., Liu, J., Liu, Y., Abbas, M., Zhang, Y., Kong, W., Zhao, F., Zhang, X. and Zhang, J., 2022. CYP6FD5, an antenna-specific P450 gene, is potentially involved in the host plant recognition in *Locusta migratoria*. Pesticide Biochemistry and Physiology 188: 105255. https://doi.org/10.1016/j.pestbp.2022.105255

Figures

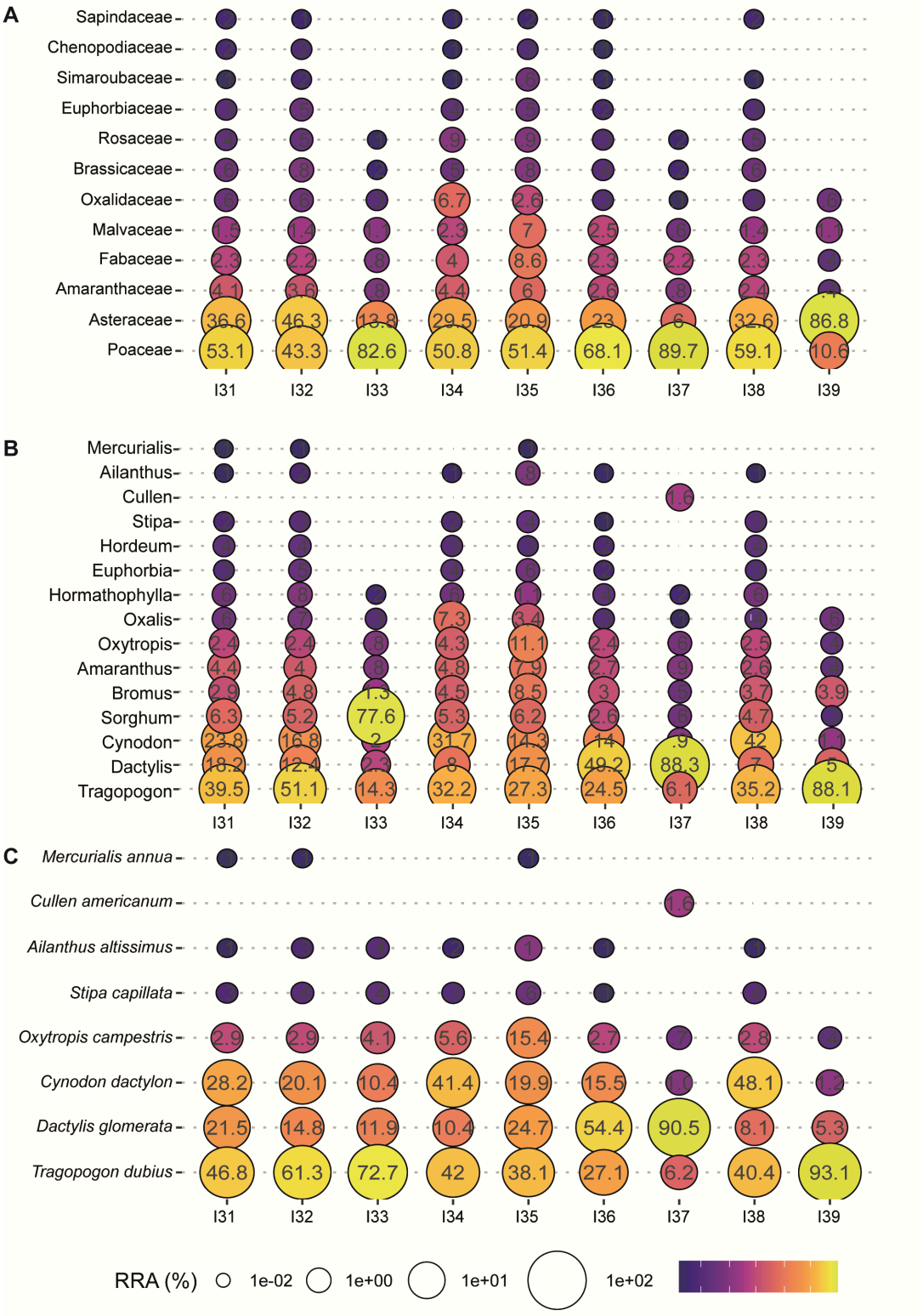

Figure 1

Plant taxa detected in *L. migratoria* gut contents at family (A), genus (B), and species (C) levels. Values represent relative read abundance (RRA, %). Bubble size is square-root scaled; color is log10-scaled. Sample IDs correspond to individual specimens listed in Table S1.

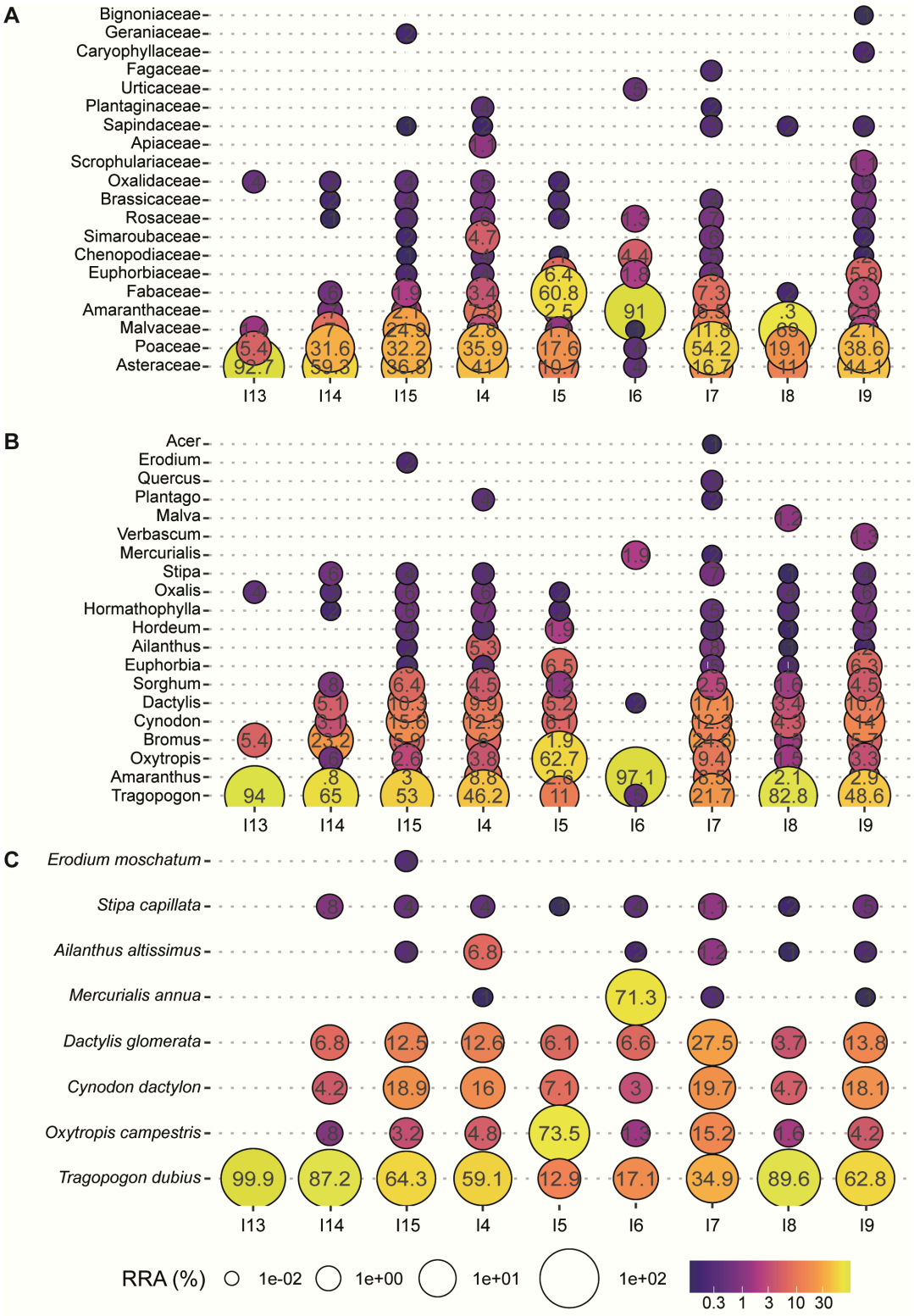

Figure 2

Plant taxa detected in *G. bimaculatus* gut contents at family (A), genus (B), and species (C) levels. Values represent relative read abundance (RRA, %). Bubble size is square-root scaled; color is log10-scaled.