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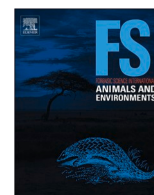

Assessing the impact of habitat and captivity status on volatilome profiles of the illegally traded shingleback, *Tiliqua rugosa*

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ABSTRACT

Volatilomics is the study of the total biogenic volatile organic compounds (BVOCs) produced by an organism. This field has been used to assess organism and ecosystem health, as well as determine BVOC biomarkers for forensic purposes, including the detection of human remains, ignitable liquid residues and illicit drugs. For volatilomics to be applied in wildlife-victim casework (*e.g.* the illegal wildlife trade) a large reference database must be collected across each targeted species range. Adequate sample sizes must be collected from different habitats spanning across the species range of the targeted species to evaluate volatilome variability associated with different environmental and dietary characteristics. This will allow for assessment of chemical diversity and the determination of BVOC biomarkers that are relevant to wildlife forensic cases (*e.g.* detection, species identification, geographic origin assessment). This study collected the first live animal volatilome database, using the highly trafficked and widely distributed Australian shingleback lizard (*Tiliqua rugosa*). Optimised thermal desorption and analysis methods were used to examine 127 wild shingleback volatilome samples collected from sites across New South Wales, South Australia and Western Australia and 28 volatilome samples from captive shinglebacks. The results demonstrated that volatilome profiles and chemical diversity differed across each sampling region, potentially related to habitat and diet changes. At least 7 volatilome samples were required to capture chemical diversity in a sampling region. Forty-four tentatively identified BVOCs were shared across all sampling regions and captive animals which may aid in detection purposes. Bioregion-specific BVOCs were also identified, which will also aid in geographic assignment of confiscated individuals. This work demonstrates the importance of sample sizes in capturing chemical diversity within Bioregions prior to downstream volatilome analysis for the establishment of wildlife forensic databases and biomarker selection.

1. Introduction

Biogenic volatile organic compounds (BVOCs) are low molecular weight, carbon-based compounds that are produced by living [1] and deceased [2] organisms. The study of the collective BVOCs produced by an organism or group of organisms is known as volatilomics [3]. BVOCs can originate from endogenous processes, including biological processes related to metabolism or genetic processes [4,5], through active chemical communication [6,7] and through specialised organs [8]. Additionally, BVOCs found within volatilomes can also be produced by exogenous sources to the organism, such as gut or skin microbiomes [3], secretions [9], diet [10,11], interactions with stimuli [12] or environmental pollutants [13]. Regardless of source, unconjugated BVOCs disperse ambiently into surrounding environments [14] and can be assessed and targeted for a variety of interests, including the evolution of chemical signalling, disease diagnosis or for detection purposes [15-18].

Volatilomes have been evaluated in forensic contexts to aid in the detection of illicit drugs, ignitable liquid residues (in relation to arson), human remains and environmental crimes [19-22]. The chemical constituents of volatilomes are identified using sophisticated analytical instruments, including gas chromatography coupled with mass spectrometry (GC-MS), or two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GC×GC-ToFMS) [23]. After target BVOCs are identified, less specific methods, such as specialised detection dogs [24] and electronic noses [25,26] are used for detection in the field. The BVOCs that have been primarily attributed to specific volatilomes (*i.e.* biomarkers) have been used as evidence in criminal proceedings [27] and to increase detection efficacy rates in

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cadaver detection dogs [28]. This precedent supports the use of BVOC biomarker detection as an avenue for exposing other illicit activities, including the illegal wildlife trade (IWT).

The illegal wildlife trade encompasses crimes relating to the unlawful harvesting, poaching, killing and trafficking wild flora and fauna from their respective native habitats. It is sustained through demand for numerous wild animal and plant species, with perceived (but not always scientifically supported) medical benefits, enhanced social status through luxury foods or ornaments and as exotic pets [29,30]. In order to prosecute crimes related to the IWT, criminal acts must first be detected and then relevant evidence (*e.g.* species identification, geographic origin, pedigree [31]) must be collected and forensically analysed. The burden of proof required to prosecute suspected perpetrators continues to be a significant hurdle for law enforcement agencies due to the diverse array of species with differing levels of protections as well as the available validated analytical methods for evidentiary purposes [32].

Volatilome assessments could be a relevant and effective tool in the detection of illegally trafficked wildlife in transit (*e.g.* post, air and shipping routes) through the detection of BVOCs that are specific to wildlife targets. Additionally, total volatilome assessments may be informative for the determination of geographic origin(s) of targeted wildlife post confiscation. It has been established that volatilomes are influenced by dietary, genetic and environmental factors, hence making them suitable targets for investigations of geographic origin, [33-35]. In order to determine whether volatilome assessment can be used to detect targeted wildlife species, or to identify the geographic origin of the species of interest, the chemical differences in volatilomes across the geographic distribution of the targeted species must be adequately assessed. Similar to other "-omic" studies, adequate sample sizes are required to capture the diversity and variability of compounds found within a population before other statistical judgements are made [36, 37]. Large enough sample sizes must be collected from each known habitat of the targeted species to evaluate the potential volatilome variability associated with the unique environmental characteristics from where the animals are sourced. Instances of intra-species volatilome investigations have been limited, especially in species whose distributions extend across large geographic ranges that include a range of different habitat types. Thus, guidance with regards to sufficient volatilome sample sizes for live animals have not been defined.

The highly trafficked shingleback lizard (*Tiliqua rugosa*) serves as an appropriate candidate for assessment of volatilomes across a species range. The shingleback is a mid-sized, blue-tongued squamate lizard that is endemic to Australia and is one of the most illegally trafficked live animals from this country [38]. The shingleback distribution extends over one million square kilometres [39,40] ranging from the east coast Australia states (Queensland, New South Wales, and Victoria) through South Australia, to the barrier islands off the coast of Western Australia. They are habitat generalists and can be found inhabiting diverse environments, including deserts, saltbush, grasslands, mallee, shrublands, coastal dunes and woodlands [41,42]. Despite their wide distributions, shinglebacks have limited, but stable, home ranges which are driven by the availabilities of both food and water [43]. Shinglebacks are omnivores who primarily eat vegetation but have been reported to opportunistically scavenge carrion and invertebrates [43-45]. As with other opportunistic scavenging lizards [46] shinglebacks have varied diets which are expected to change seasonally [43]. As volatilomes are impacted by both metabolic status and changes in diet, access to different food items is suspected to influence BVOC profiles. In addition to wild poached individuals, captive bred, or housed shinglebacks are also found to be illegally trafficked [38]. To date, no study has determined the volatilome profile differences between captive and wild members of the same species, warranting further investigation.

It was the aim of this study to use optimised reptile volatilome collection [47] and comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry analysis methods to assess volatilome diversity across the shingleback's species range. This work investigated the required sample sizes to capture volatilome chemical diversity and how volatilome profiles differed across different habitats. This work set the foundation for future shingleback volatilome statistical analysis and the determination of shingleback BVOC biomarkers that can be used for detection purposes.

2. Materials and methods

2.1. Sampling areas

Volatilome samples were collected from across the shingleback species range, including New South Wales (NSW), South Australia and Western Australia (Fig. 1) as well as from captive animals located at Featherdale Wildlife Park (FWP; Sydney, Australia). Samples were collected in the Australian spring (September – November), summer (December – February) and autumn (March – May) when shinglebacks are most active. Bioregions from each sampling location were determined using the SEED Central Resource for Sharing and Enabling Environmental Data in NSW [48]. When sampling occurred on Bioregion borders, the Bioregion was selected by visual assessment of both vegetation and soil composition from photos captured during each sample collection point. All GPS data points from sampling locations were plotted using ArcGIS online 2.21 (ESRI; Redlands, California).

2.2. Shingleback sampling

Wild shinglebacks were searched for visually on roadside verges from a car, or on foot in suitable habitat. All individuals (wild and captive) were caught by hand while wearing powder free latex gloves (Premier examination gloves, USA) and placed in new 300 x 450 mm draw string calico bags (Prospectors Supplies, Sydney, Australia). A blood sample was then taken from the ventral tail vein of each captured shingleback after the respective area was sanitized with an alcohol spray (Isocol, Australia) and dried using generic cotton swabs for future DNA analysis. Shinglebacks were then placed in the sampling container for volatilome sample collection.

2.3. Volatilome collection

Volatilomes were collected through optimised methods as described in [47] using a purpose-built aluminium container (60 cm(length) × 50 cm(width) × 50 cm(height)) aluminium sampling container with a polycarbonate viewing window and a side vent (Fig. S1). Prior to the capture of each individual shingleback, the sampling container was sterilised with acetone and dried to mitigate persistent VOCs and a sample blank of the container was taken to determine VOCs present in the environment that could later be removed. Individual shinglebacks were acclimated for 20 min. Following, a pre-conditioned dual sorbent tube (Tenax® and Carbograph 5DT Markes International Ltd, UK; parts number C2-AAXX-5149) was then connected to a sampling port located at the top of the box following acclimation. Two 15-minute sampling replicates were taken in tandem at a flow rate of 100 ml/min using an ACTI-VOC pump (Markes International Ltd., UK). Volatilomes were only collected if the internal sampling container temperature could be maintained below 30°C for the safety of the animal. If escape behaviour, such as persistent gaping or climbing on the viewing window was observed, sampling was abandoned, and the animal was released.

2.4. Volatilome analysis

2.4.1. Sample desorption

Samples were analysed using optimised reptile volatilome analysis methods as described in [47]. Prior to analysis, 0.2 µl solution of 10 ppm d_5-chlorobenzene (CAS number 3114–55–4; Merck, AUS) was injected into each sorbent tube to serve as an internal standard to assess

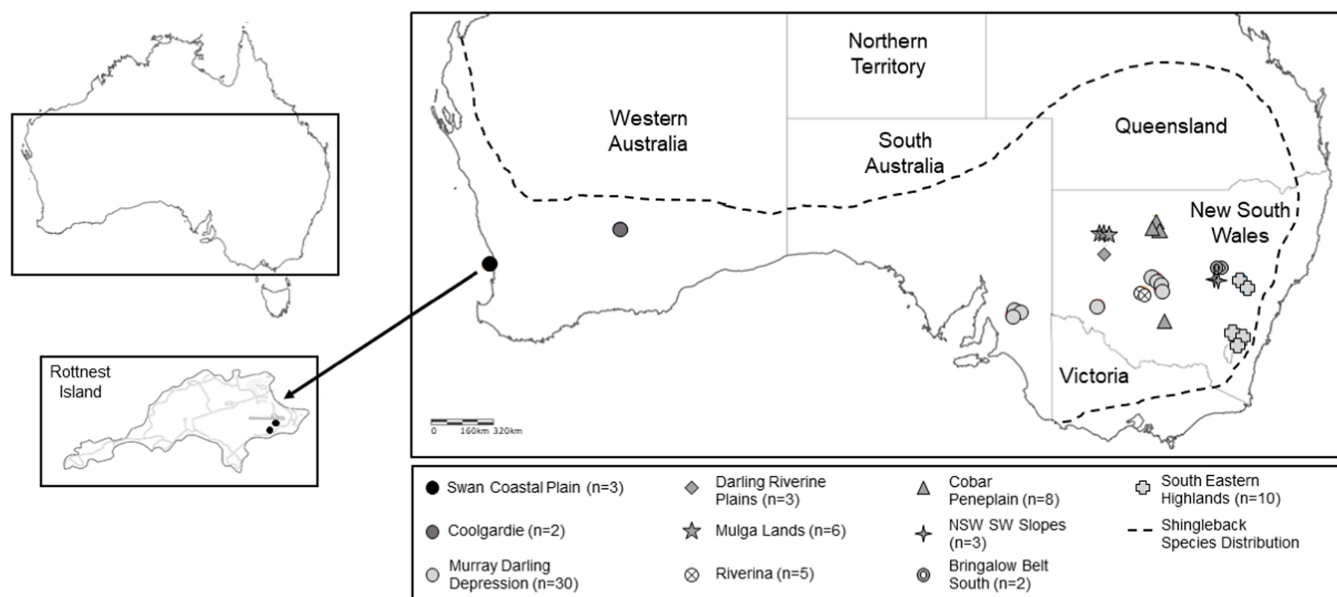

Fig. 1. GPS locations of volatilome samples collected from individual wild shinglebacks across mainland Australia. Each colour represents the Bioregion from where that sample was sourced, and n represents the number of individuals sampled from each Bioregion. Paired animals shared the same GPS location and were represented by one circle.

instrument reproducibility and to serve as a reference for compound normalisation [49]. Samples were desorbed using Markes Unity 2 Thermal Desorber and Series 2 ULTRA multi-tube autosampler (Markes International Ltd, UK) with a Markes General Purpose Carbon C4/5-C30/32 cold trap (parts number U-T11GPC-2S). The samples were desorbed using the following parameters: the pre-desorption pre-purge time was set to 2 min, the flow path temperature was 150̊C, the tube desorption time was 3 min with a flow rate of 20 ml/min, the desorption temperature was 300̊C, the maximum trap temperature was 300̊C, the minimum trap temp was −15 ̊C, the trap hold was 4 min and the trap flow was 25 ml/min.

2.4.2. GC×GC-ToFMS analysis

Comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (Pegasus 4D GC×GC-ToFMS (LECO Australia Pty Ltd, Australia)) was used to analyse all samples. Samples were analysed using both optimised column and separation methods described in [47]. A 30 m Rxi-624 Sil MS (Restek, Catalog number 13868) primary column and a 2 m Stabilwax secondary column (Restek, Catalog number 10623) were used for analysis. The carrier gas was high purity helium at a flow rate of 1 ml/ min for the entire run. The initial oven temperature was set to 35̊C and held for 2 min. The temperature was then ramped at 7̊C/ min until the temperature reached 80̊C. Following, the temperature was ramped at 10̊C/ min until a maximum temperature of 240̊C was reached with a 5 min hold. The secondary oven was offset 10̊C relative to the GC oven and the modulator was offset 30̊C to the secondary oven temperature. There was a 5 s modulation period with a 1 s hot pulse time and a cooling time of 1.5 s between stages. The transfer line to the ion source was held at 250̊C and the ion source was held at 200̊C with an electron ionisation energy at 70 eV. The acquisition rate was 100 spectra/ s with a target mass range of 29–550 amu. The detector voltage was offset 200 V to the optimised detector voltage. Alkane standards and purified isomers (*e.g.* p-xylene, o-xylene, m-xylene) were run periodically to assess separation and detection quality.

2.5. Data processing

ChromaTOF (version 4.51.6.0; LECO) was used to analyse and align the resulting chromatograms. A signal-to-noise ratio of 150 with a 0.8 baseline offset was used to align samples. A 30 s first dimension peak width and a 0.15 s second dimension peak width were required for peak identification and sub-peaks required a minimum S/N of 6 for identification. Compound identification was completed using a minimum similarity match of 75 % in The National Institute of Standards and Technology (NIST) Mass Spectral Library [32,47]. Compounds with a below 80 % similarity matches (but above 75 %) were manually compared to histograms where compounds had a high match similarity to tentatively identify, or reject, compound assignment. The ChromaTOF Statistical Compare function was used to align peaks across samples from the same region using a 60 % match threshold. The data was then exported to Microsoft Excel (2010), where it was manually filtered to remove contaminants (*i.e.* column bleeds, internal standard, alcohol spray and solvents). Container blanks were removed from each shingleback sample using a peak area threshold value of 50 % [47]. Unidentified analytes were included in downstream analysis.

2.6. Chemical diversity assessment

Chemical richness and diversity were determined through methods defined in Baeckens et al., [50]. Chemical richness was defined as the average number, with standard deviation, of compounds found within a geographic locale. Chemical diversity was calculated using chemical classes as described Baeckens et al. [50] and using the Shannon-Weiner Diversity Index [51]. This calculates the diversity of compounds within a chemical class for each Bioregion. A species accumulation curve was adapted to "chemical abundance curves" where incidences of new compounds were recorded per Bioregion. For this analysis, each shingleback volatilome replicate was treated as an independent sample. This analysis was completed to determine the benefit of additional sampling efforts for each Bioregion. Volatilome profiles were graphed as the average summed peak area from each represented chemical class for visualization purposes. Additionally, compounds that were shared across all Bioregions, or that were found to be unique to a certain Bioregion, were also assessed.

2.7. Impacts of bioregion and captivity

Unique compounds specific to Bioregions or captivity were identified and their potential origin was determined through a literature search. Compounds that were shared across all Bioregions were also defined and the strength of their odours [52] were determined to assess for their potential as shingleback BVOC biomarkers for detection.

3. Results and discussion

3.1. Sampling

A total of 72 wild shinglebacks were sampled from 15 locations across 10 Bioregions for a total of 127 volatilome sample replicates (Fig. 1). Sample sizes from each Bioregion ranged from 4 to 54 sample replicates. Twelve captive shinglebacks were sampled from the FWP collection for a total of 28 sample replicates.

3.2. Compound classification

1407 unique compounds were tentatively identified across all samples (n = 155). Of those, 153 compounds were removed from analysis either due to them being known contaminants or due to them being tentatively identified as a hormone. This work focused on determining biomarkers that could be produced and released metabolically by the animals, and did not target compounds that are generated as a response to a stimulus. As hormones are signalling molecules related to development or behaviour, it is unlikely that they will be produced in stressful trafficking scenarios. Additionally, because the production of hormones is highly variable, they are not likely to be relevant for biomarker detection nor aid in determining the geographic origin of trafficked shinglebacks. For these reasons, potential hormones were not included in volatilome analysis for either potential BVOC biomarkers for detection purposes or for the assessment of geographic origin.

3.3. Chemical richness and chemical diversity

The Riverina Bioregion (Fig. 1) exhibited the highest chemical richness (Fig. 2), while the lowest chemical richness was observed in the South Eastern Highlands, Bringalow Belt South and NSW SW Slopes Bioregions. The disparity in chemical richness may be partially explained by the environmental conditions present while sampling. Numerous samples in the NSW SW Slopes and in the South Eastern Highlands Bioregions were taken during the coldest temperatures experienced during field collection (average sampling temperatures 19°C and 23.3°C respectively), with overnight temperatures averaging 3.5°C and 6.3°C, respectively (Table S1). These shinglebacks likely had reduced metabolic states, as they are likely in torpor under 17°C [53] and will have reduced glucose metabolism [54]. Furthermore, as BVOC dispersal is facilitated by increased temperature, the lower temperatures could indicate a slower dispersal of BVOCs within the container. Some of the warmest average sampling temperatures were recorded in the Coolgardie (27.3°C), Mulga Lands (30.7°C), Riverina (26.5°C) and the Murray-Darling Depression (27.2°C) Bioregions (Table S1). These regions exhibited two of the highest overnight minimum temperatures (Riverina: 21.8°C, Coolgardie: 18.1°C) and two mid-range overnight minimum temperatures (Mulga Lands: 11.8°C, Murray Darling Depression 11°C) (Table S1). The high average sampling temperatures coincided with some of the highest averages of chemical richness, which may be in part due to increased BVOC volatility. In physiological studies, it was determined that shinglebacks record the most water loss, and are thus most metabolically active, in the first two hours of exposure periods with temperatures ranging from 30 to 37°C [55] and are most active between 24 and 34°C [53]. As these animals were sampled at temperatures within this range, it is likely that they were more metabolically active, which contributed to the disparity in chemical richness. A comparison of chemical richness in Bioregions and captivity between areas where average sampling temperatures were above 25°C and below 25°C were compared, confirming that environmental temperature did significantly impact the average number of BVOC samples recovered (Fig. S2). BVOC sampling of wild animals will always be subject to changing environmental conditions that will impact certain data analyses, especially for ectothermic taxa. However, trafficking scenarios will also present the same challenge of unstable thermal conditions. Although changes in environmental temperature may impact chemical richness and diversity assessments, analysing shinglebacks in different metabolic states is relevant in identifying compounds that will be present in a diverse range of trafficking scenarios. As one of the aims of this study was to identify compounds that are shared across all Bioregions, sampling shinglebacks in different metabolic states also allows for the identification of compounds shared across a majority of metabolic states. The captive shinglebacks had the largest standard deviation in chemical richness, despite their controlled diets. This variability may be attributed to the varied life histories of the sampled animals (*i.e.* captive bred *versus* wild caught) or transition into captivity as some had been in captivity for shorter periods of time.

A similar, but less apparent, pattern was observed in chemical diversity (Table 1). Chemical diversity is a measure of compound diversity that is found within each chemical class within each Bioregion. This analysis demonstrated that although the number of compounds differ per region, the number of compounds detected within each chemical class do not vary significantly. The only notable exceptions to this

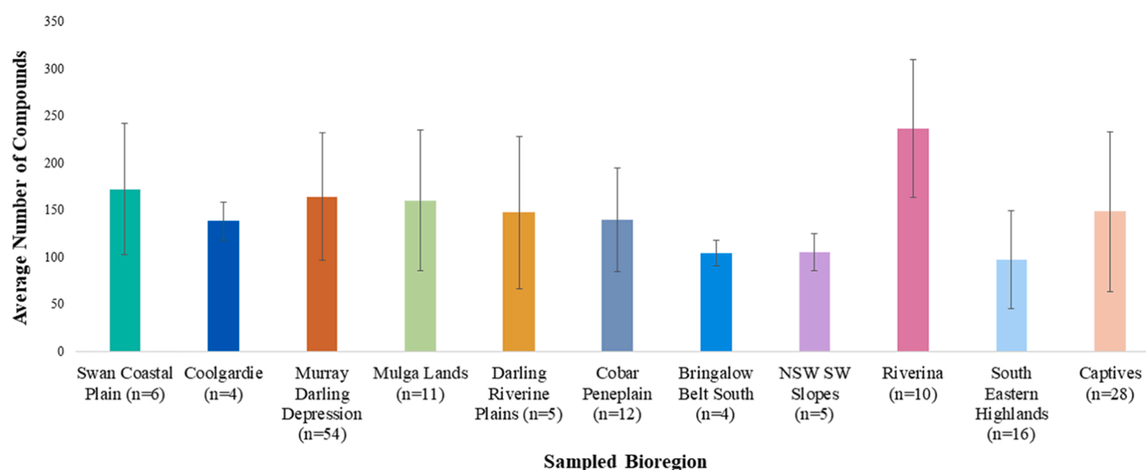

Fig. 2. Average Chemical Richness with standard deviations retrieved per Bioregion. Sample replicates are denoted beneath each Bioregion.

Table 1
Shannon-Weiner Diversity Index per Bioregion and Captivity.

Shannon-Weiner Diversity Index	
South Eastern Highlands	2.24
Murray Darling Depression	2.22
Bringalow Belt South	2.16
Cobar Peneplain	2.25
Coolgardie	2.24
Mulga Lands	2.25
Swan Coastal Plain	2.31
Darling Riverine Plains	2.32
Riverina	2.35
NSW SW Slopes	2.21
Captives	2.16

observation occurred in the Bringalow Belt South Bioregion and captive animals as compared to the Riverina Bioregion. The consistency in chemical diversity may indicate that shinglebacks share similar biogenic pathways [56] and that the diversity of compounds within each chemical class may instead be impacted by access to varieties or diversities of food sources.

The differences in chemical richness may not be directly associated with sample size. Chemical abundance curves, which measure chemical diversity through the accumulation of newly observed compounds between samples, demonstrate that chemical diversity begins to plateau from biotypes which had greater than seven replicates (Fig. 3). Chemical diversity appears to have been captured from five of the 10 bioregions sampled: the Murray Darling Depression, Mulga Lands, Cobar Peneplain, Riverina and South Eastern Highlands Bioregions. This may indicate that biotypes have innate differences in chemical diversities due to environmental, dietary and physiological factors which respond to differing climates. Sample sizes should be increased from other Bioregions to ensure that the sampling asymptote is reached. As this was a foundational and exploratory study, the data from each Bioregion was analysed in the same manner, even if chemical abundance curves did not plateau. Samples taken from captive animals were not included due to the diverse life histories of the animals found within this group.

The visualisations of average summed peak area per compound class demonstrated that the Murray Darling Depression, Cobar Peneplain and Riverina Bioregions were dominated (42–52 %) by N-containing compounds (Fig. 4). The other two regions whose volatilome profile was dominated by one chemical class were the NSW SW Slopes (Unidentified - 62 %) and the Mulga lands (ketones – 47 %) Bioregions (Fig. 4). The NSW SW Slopes Bioregion exhibited the lowest summed peak areas per compound that could have contributed to the limited compound identification by the mass spectrometer. This could be attributed to the air temperature and reduced metabolic status and BVOC diffusion associated with the lower temperatures observed when sampling in this region (Table S1). It is not immediately clear the origin of the ketone compounds found in shingleback volatilome profiles from the Mulga Lands. However, it is likely that dietary preferences for these shinglebacks differ due to availability of differing vegetation types in this area (Table S2). The captive profiles, when presented collectively, shared the most similarities to the Bringalow Belt South and Darling Riverine Plain Bioregions (Fig. 4). The most distinct differences between these three sample groups were the lower abundance of ester, acid, and alcohol compounds found in captive profiles. Interestingly, the genetic lineages (data not shown) of the captive animals supported the premise that a majority of the captive shinglebacks did originate from the North Eastern (n = 6) lineages [57], which can be distributed across the Bringalow Belt South and Darling Riverine Plains Bioregions. The captive animal group also had the presence of a manganese-based compound that was not found in either Bioregion, which may be attributed to the controlled diet of captive animals (Supplemental data). The profile of one confiscated captive individual was analysed to determine whether the volatilome profile of this animal more resembled a captive profile or the profile from the Bioregion where it was confiscated from (Fig. S3). This animal was selected as its precise Bioregion of origin was known due to the animal's distinctive morphological features (*i.e.* orange coloration; Coolgardie). This shinglebacks' profile was found to more closely resemble the averaged captive profile as compared to the averaged Bioregion profile from which it originated (Fig. S3). At the time of analysis, this individual had been in captivity for over a year, indicating that a change in diet and habitat influenced the total volatilome profiles. However, larger sample sizes and finer scale investigations of individuals that transition from a known wild Bioregion into captivity are required before definitive conclusions are drawn.

3.4. Unique geographic compounds

Compounds that were found to be unique to each specific Bioregion were further assessed (Table S3). No tentatively identified compounds were unique to the Darling Riverine Plains or to the Murray Darling Depression Bioregions. This may be due to the fact that these regions are adjacent to one another, and the selected sampling areas were on the border of these two regions [48]. Five tentatively identified compounds were unique to Bringalow Belt South Bioregion, which were primarily related to vegetation [58-61]. Thirty-five tentatively identified compounds were unique to the Cobar Peneplain Bioregion. These

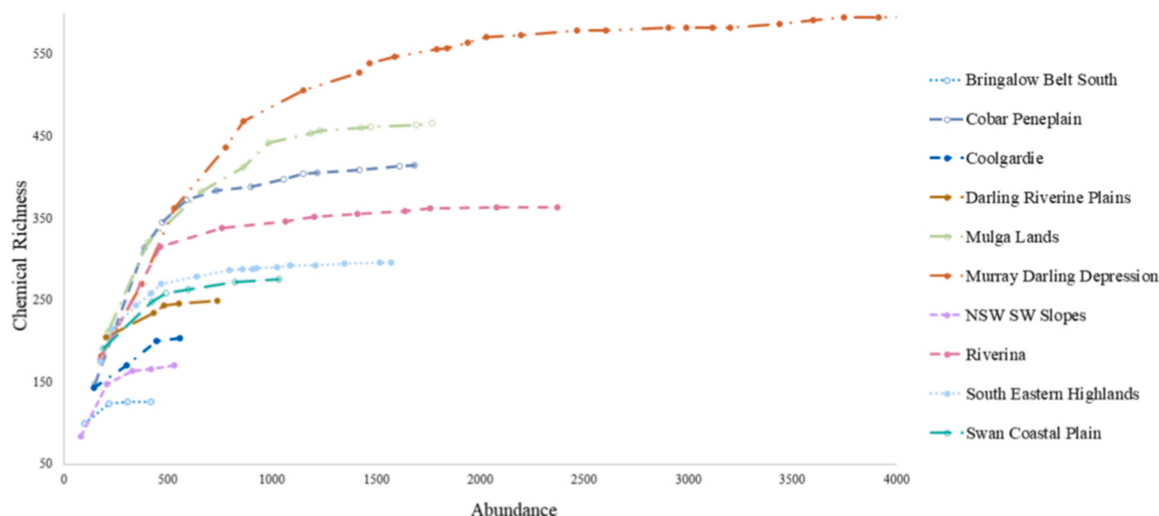

Fig. 3. Chemical abundance curve from each sampling location. Abundances were scaled to 4000 on the x-axis due to sample size disparity.

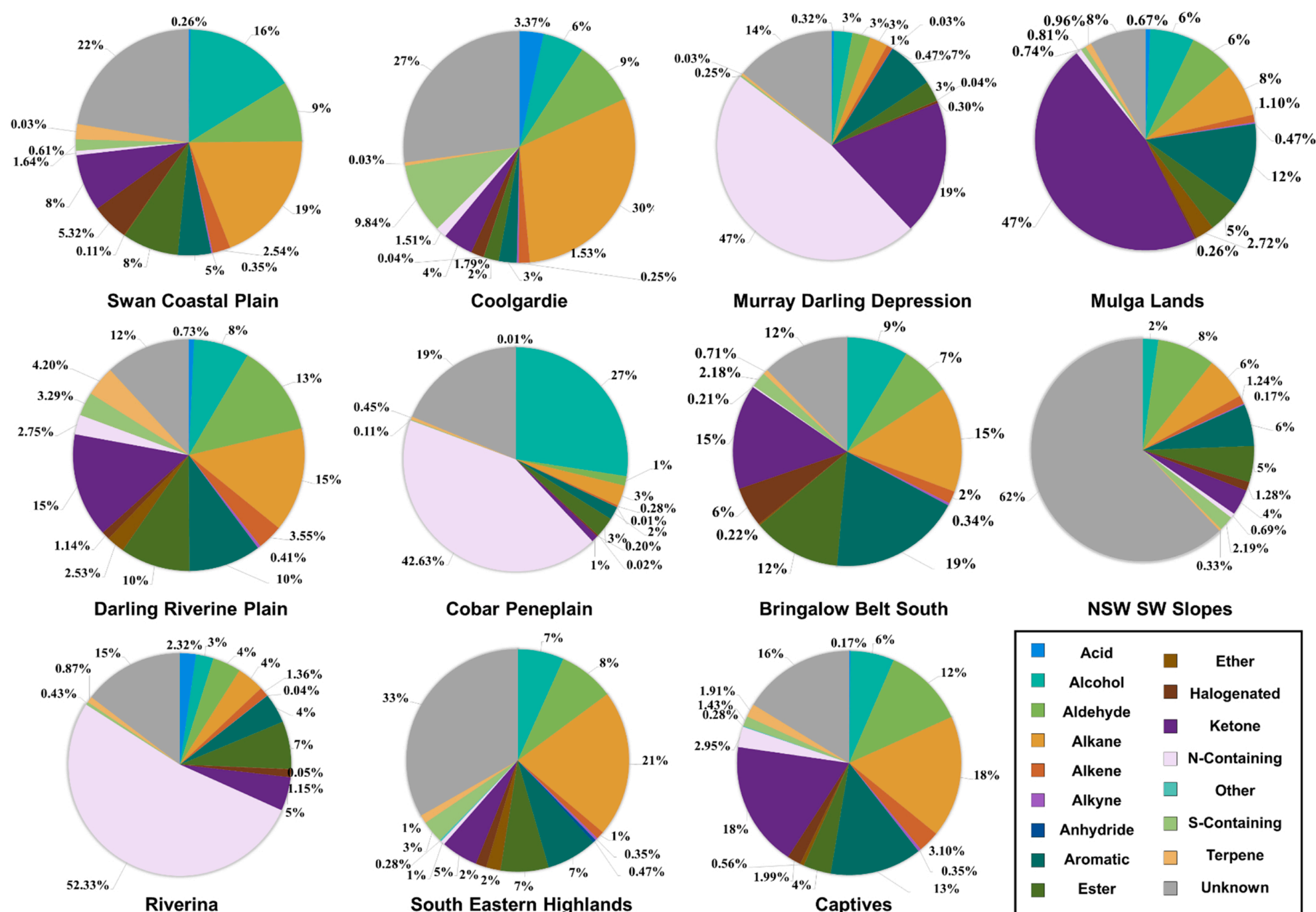

Fig. 4. Volatilome profiles of sampled shingleback areas represented by average summed areas per compound class.

compounds included VOCs related to vegetation, fruits, fungi, larvae and meats [62-67] with the most significant classes represented being esters (n = 8); and N-containing compounds (n = 7) which suggest a more variable diet in this region. There were ten tentatively identified compounds unique to the Coolgardie Bioregion. The most represented unique compounds were alcohols (n = 3) and alkanes (n = 3), which included compounds that have been detected both from meat, plant oils and seeds [68-72]. The Mulga lands Bioregion had two tentatively identified unique alcohols which have been previously attributed to fruits [73,74]. The NSW SW Slopes Bioregion had three tentatively identified unique compounds potentially related to volatiles released by fruit and fungi [75-77]. The Riverina Bioregion had 22 tentatively identified unique compounds, most of which were acids (n = 5) that have been attributed to fruit [78-80] and N-containing compounds (n = 4) from vegetation and unknown origin [81,82]. Although the Riverina and Cobar Peneplain all had high proportions of N-containing compounds in their volatilome profiles (Fig. 4), the individual compound constituents differed (Table S3). This may indicate dietary differences, as two N-containing compounds appeared to have originated from meat in the Cobar Peneplain Bioregion. The remaining unique N-containing compounds appear to have originated from differing flowering plants. The South Eastern Highlands Bioregion had 19 unique tentatively identified compounds that potentially originated from insects, cyanobacteria and raw meats [70,83-85], with the predominate chemical class of the unique compounds being alkanes (n = 6). The Swan Coastal Plain Bioregion had 32 tentatively identified compounds, ten of which were esters related to tropical fruits such as bananas, mangos, plums and starfruit [86-90]. that differentiated it from other regions. These shinglebacks were sampled on the popular tourist destination, Rottnest Island, and were found in close proximity to the main tourist infrastructure on the island. This may have provided access to a range of different fruits *via* intentional feeding by tourists or disposal of waste. Finally, captive animals had the highest number of tentatively identified compounds (n = 39) that were unique to captivity. A majority of these compounds were unique ketones (n = 8) associated with plant-based dietary compounds, including banana flowers [91-93], that distinguished captive animals from their wild counterparts. This finding may suggest that changes in the captive diet, including access to chicory, kale, endive, carrots, sweet potatoes, zucchini, apples, pears, grapes and strawberries (FWP, personal comm) distinguishes them from their wild counterparts.

The investigation into potential BVOC origins was high-level and preliminary with the main purpose being to determine whether these compounds were naturally occurring or synthetic. The origins of these compounds could potentially be misconstrued, as VOC profiling for most Australian flora and fauna has not been conducted, which limits the interpretation of potential dietary origination. Further shingleback diet analysis is required to determine the potential origin dietary origins of these BVOCs. The compounds identified from potential dietary sources should be interpreted as naturally occurring compounds that could have also been generated from native plants and fruits.

3.5. Tentative biomarker selection

No compound was shared across every individual shingleback sampled. However, 44 tentatively identified compounds were shared across all Bioregions and captive shinglebacks (Table 2, Table S4; Supplemental data). Of these, 16 are considered to have high odour strength

Table 2
Shingleback BVOCs that were found across all Bioregions and Captive shinglebacks ordered by odour strength.

Odour Strength [52]	Compound Name	Class	Potential Origin	Citation
High	Benzaldehyde	Aldehyde	Fruit	[78]
High	Benzeneacetaldehyde	Aldehyde	Meat	[94]
High	Butanal	Aldehyde	Vegetation	[95]
High	Decanal	Aldehyde	Fruit	[78]
High	Heptanal	Aldehyde	Meat	[70]
High	Hexanal	Aldehyde	Fruit	[78]
High	Octanal	Aldehyde	Fruit	[78]
High	Pentanal	Aldehyde	Meat	[70]
High	Methyl benzoate	Ester	Flowers	[96]
High	2-Butanone	Ketone	Meat	[94]
High	2-Heptanone	Ketone	Meat	[70]
High	2-Hexanone	Ketone	Meat, vegetation, fruit	[94,97]
High	2-Pentanone	Ketone	Meat and Flowers	[70,98]
High	Acetophenone	Ketone	Meat	[70]
High	Benzothiazole	S-Containing	Flowers	[98]
High	Carbon disulfide	S-Containing	Meat	[99]
Medium	1-Butanol	Alcohol	Fruit	[80]
Medium	1-Propanol	Alcohol	Meat	[94]
Medium	2-Ethylhexanol	Alcohol	Fruit	[75]
Medium	Furfural	Aldehyde	Fruit	[75]
Medium	2-Methylfuran	Aromatic	Fruit	[87]
Medium	2-Pentylfuran	Aromatic	Fruit	[78]
Medium	6,10-Dimethyl-5,9-undecadien-2-one	Ketone	Meat	[70]
Medium	6-Methyl-5-hepten-2-one	Ketone	Fruit	[78]
Medium	Benzophenone	Ketone	Fruit	[100]
Medium	Cyclohexanone	Ketone	Fruit	[101]
Low	Tetradecane	Alkane	Fruit	[80]
Undefined	2-Ethyl hexanal	Aldehyde	Meat	[102]
Undefined	n-Hexane	Alkane	Fruit	[75]
Undefined	Decane	Alkane	Fruit	[80]
Undefined	Hexadecane	Alkane	Fruit	[80]
Undefined	Tridecane	Alkane	Meat	[70]
Undefined	Phenylethyne	Alkyne	Meat	[103]
Undefined	à-Methylstyrene	Aromatic	Meat	[104]
Undefined	(1-Butylhexyl)benzene	Aromatic	Seed	[105]
Undefined	(1-Butyloctyl)benzene	Aromatic	Meat	[106]
Undefined	(1-Propylnonyl)benzene	Aromatic	Seed	[105]
Undefined	Ethylbenzene	Aromatic	Fruit	[65]
Undefined	Hemimellitene	Aromatic	Flower	[97]
Undefined	Propylbenzene	Aromatic	Animal product	[107]
Undefined	3-Methylheptyl acetate	Ester	Vegetation	[95]
Undefined	Trichloromonofluoromethane	Halogenated	Fruit	[87]
Undefined	2,4-Dimethyl-3-pentanone	Ketone	Fruit	[108]
Undefined	Benzonitrile	N-Containing	Meat	[94]

(recommended smelling in solution of less than 1 %; [52]), 10 have medium odour strength, one has low odour strength and 17 are undefined (Table 2). The 16 high odour strength compounds are favourable odour targets from a biomarker detection perspective. The presence of these high odourous compounds are commonly found in natural environments. The abundance of these compounds was higher in shingleback volatilome samples as compared to environmental blanks. Thus, targeting these compounds, especially in varying concentrations, with a wildlife detection dog or electronic nose could be beneficial when trying to detect illegally trafficked shinglebacks as they were found across all habitats, captivity conditions and metabolic statuses Alternatively, as odour strength in this work is defined as it relates to humans as opposed to dogs, it may be relevant to analyse all 44 compounds for detection dog purposes. This may allow for the most robust detection of illegally trafficked shinglebacks with various life histories or geographic origins.

4. Conclusion

This study was able to assess shingleback volatilomes across their species range and was the largest live animal volatilome study to date. No single compound was shared across all sampled shinglebacks. This may be due to the increased sample size of animals analysed for this study, or the changes in metabolic states from every Bioregion. The complexities of volatilome profiling of living biological specimens should be thoroughly addressed when developing chemical detection methods to ensure adequate biomarkers that represent the majority of individuals within a species are targeted. For example, this work determined that increased sample sizes are required to capture the chemical volatilome diversity within a Bioregion, prior to answering questions regarding species identity or geographic origin assessment. This discovery is important as volatilome profiling of living animals is expanding [109-111]. This work highlights that shingleback volatilome profiles naturally differ among Bioregions, potentially due to dietary and habitat influences. Despite these changes, shinglebacks had similar chemical diversities within each chemical class for each Bioregion.

Captivity appeared to influence total shingleback volatilome profiles, as they exhibited the lowest chemical diversity Other variables that are not present in wild animals, including the presence different life stages (*e.g.* how long the animal has been in captivity, whether the animal was born captive or wild) or the fact that they were sourced from different Bioregions require further evaluation of captive profiles. This could aid in determining which Bioregions are more targeted in the illegal pet trade and build hot spot intelligence and facilitate anti-poaching efforts. The volatilome profile of the only captive shingleback with known origin more closely aligned with the captive profiles than the Bioregion from where it was sourced. Identifying captive *versus* wild sourced animals will also help determine whether wild shinglebacks are being illegally sourced for breeding programs, which has been

gaining more focus in wildlife forensic science [112]. Further work analysing the transition from the wild to captivity is warranted to determine how captivity influences volatilome changes and at what rate. This type of work is also warranted to determine how quickly a confiscated shingleback should be analysed prior to assessing the Bioregion origin.

Shingleback volatilomes appear to be influenced by changes in metabolic status as seen in other taxa [110]. However, despite changes in metabolomic status, potential BVOC biomarkers can still be defined for detection targets. This work highlighted 16 BVOCs that should be further investigated for the detection of illegally trafficked shinglebacks. These highly odourous compounds were found across animals from all Bioregions and captivity status. For this reason, these are better suited for the broad detection of illegally trafficked shinglebacks as opposed to post-confiscation Bioregion assessment. This study determined that most Bioregions had unique BVOCs, with the exception of when sampling sites bordered Bioregions. This caveat is shared with other wildlife forensic techniques (*e.g.* genetics) and should be coupled with other forensic methods (*e.g.* morphology) to enhance Bioregion determination [113].

This study has set the foundation for further statistical and investigative work for live animal volatilome analysis. It is recommended that future work assess the influences of other biological factors (*e.g.* sex, age, disease status) prior to selecting BVOC detection biomarkers or to using volatilome analysis for geographic origin purposes. This is particularly important when sample sizes of animals of different classes (*e.g.* males with parasites *versus* males without parasites) differ. Similar to human volatilome research, this investigation supports that large, cross-validated and peer-reviewed volatilome databases must be generated prior to using animal volatilomes for both detection and evidentiary contexts [27].

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.fsiae.2023.100071.

References

[1] A. Guenther, P. Zimmerman, M. Wildermuth, Natural volatile organic compound emission rate estimates for US woodland landscapes, Atmos. Environ. 28 (6) (1994) 1197–1210.

[2] M.E. Cablk, E.E. Szelagowski, J.C. Sagebiel, Characterization of the volatile organic compounds present in the headspace of decomposing animal remains, and compared with human remains, Forensic Sci. Int. 220 (1–3) (2012) 118–125.

[3] R. Cumeras, Volatilome metabolomics and databases, recent advances and needs, Curr. Metab. 5 (2017) 9–89.

[5] S.B. Watson, P. Monis, P. Baker, S. Giglio, Biochemistry and genetics of taste-and odor-producing cyanobacteria, Harmful Algae 54 (2016) 112–127.

[4] F. Buljubasic, G. Buchbauer, The scent of human diseases: a review on specific volatile organic compounds as diagnostic biomarkers, Flavour Fragr. J. 30 (2015) 5–25.

[6] E. Pichersky, Biosynthesis of plant volatiles: nature's diversity and ingenuity, Science 311 (2006) 808–811.

[7] R. Schmidt, V. Cordovez, W. de Boer, J. Raaijmakers, P. Garbeva, Volatile affairs in microbial interactions, ISME J. 9 (2015) 2329–2335.

[8] A. Leguet, M. Gibernau, L. Shintu, S. Caldarelli, S. Moja, S. Baudino, J.C. Caissard, Evidence for early intracellular accumulation of volatile compounds during spadix development in *Arum italicum L.* and preliminary data on some tropical Aroids, Naturwissenschaften 101 (8) (2014) 623–635.

[9] E.F. Kean, C.T. Müller, E.A. Chadwick, Otter scent signals age, sex, and reproductive status, Chem. Senses 36 (6) (2011) 555–564.

[11] S. Fischer, A. Bergmann, M. Steffens, P. Trefz, M. Ziller, W. Miekisch, J. S. Schubert, H. Köhler, P. Reinhold, Impact of food intake on in vivo VOC concentrations in exhaled breath assessed in a caprine animal model, J. Breath. Res. 9 (4) (2015), 047113.

[10] O.A. Ajibola, D. Smith, P. Španěl, G.A. Ferns, Effects of dietary nutrients on volatile breath metabolites, J. Nutr. Sci. 2 (2013).

[12] S.J. Himanen, J.D. Blande, T. Klemola, J. Pulkkinen, J. Heijari, J.K. Holopainen, Birch (Betula sp.) leaves adsorb and re-release volatiles specific to neighbouring plants—a mechanism for associational herbivore resistance? N. Phytol. 186 (2010) 722–732.

[13] J. Labows, G. Preti, E. Hoelzle, J. Leyden, A. Kligman, Analysis of human axillary volatiles: compounds of exogenous origin, J. Chromatogr. B: Biomed. Sci. Appl. 163 (3) (1979) 294–299.

[14] R.A. Raguso, Start making scents: the challenge of integrating chemistry into pollination ecology, Èntomol. Exp. Appllicata 128 (1) (2008) 196–207.

[15] Braun, B. (2013). Wildlife detector dogs—a guideline on the training of dogs to detect wildlife in trade. WWF Germany, Berlin, 1–16.

[18] T. Zarra, M.G. Galang, F. Ballesteros Jr, V. Belgiorno, V. Naddeo, Environmental odour management by artificial neural network–a review, Environ. Int. 133 (2019), 105189.

[16] K.G. Furton, N.I. Caraballo, M.M. Cerreta, H.K. Holness, Advances in the use of odour as forensic evidence through optimizing and standardizing instruments and canines, Philos. Trans. R. Soc. B: Biol. Sci. 370 (1674) (2015) 20140262.

[17] F. Lough, J.D. Perry, S.P. Stanforth, J.R. Dean, Detection of exogenous VOCs as a novel in vitro diagnostic technique for the detection of pathogenic bacteria, TrAC Trends Anal. Chem. 87 (2017) 71–81.

[19] A. Deo, S.L. Forbes, B.H. Stuart, M. Ueland, Profiling the seasonal variability of decomposition odour from human remains in a temperate Australian environment, Aust. J. Forensic Sci. 52 (6) (2020) 654–664.

[20] S.L. Forbes, A.N. Troobnikoff, M. Ueland, K.D. Nizio, K.A. Perrault, Profiling the decomposition odour at the grave surface before and after probing, Forensic Sci. Int. 259 (2016) 193–199.

[21] B. Gruber, B.A. Weggler, R. Jaramillo, K.A. Murrell, P.K. Piotrowski, F.L. Dorman, Comprehensive two-dimensional gas chromatography in forensic science: a critical review of recent trends, TrAC Trends Anal. Chem. 105 (2018) 92–301.

[22] A. Sampat, M. Lopatka, M. Sjerps, G. Vivo-Truyols, P. Schoenmakers, A. van Asten, Forensic potential of comprehensive two-dimensional gas chromatography, TrAC Trends Anal. Chem. 80 (2016) 345–363.

[23] M.A. Iqbal, K.D. Nizio, M. Ueland, S.L. Forbes, Forensic decomposition odour profiling: a review of experimental designs and analytical techniques, TrAC Trends Anal. Chem. 91 (2017) 112–124.

[24] J. Ensminger, Police and military dogs: Criminal detection, forensic evidence, and judicial admissibility, CRC Press, 2011.

[26] A.D. Wilson, Electronic-nose applications in forensic science and for analysis of volatile biomarkers in the human breath, J. Forensic Sci. Criminol. 1 (1) (2014) 1–21.

[25] M. Ferreiro-González, G.F. Barbero, M. Palma, J. Ayuso, J.A. Álvarez, C. G. Barroso, Determination of ignitable liquids in fire debris: direct analysis by electronic nose, Sensors 16 (5) (2016) 695.

[27] J. Ensminger, M.A. Ferguson, L.E. Papet, Was there a body in the trunk: volatile organic compounds in the trial of casey anthony and the evolving search for a chemical profile for human decomposition, SMU Sci. Technol. Law Rev. 19 (2016) 275.

[28] R. Dargan, S.L. Forbes, Cadaver-detection dogs: a review of their capabilities and the volatile organic compound profile of their associated training aids, Wiley Interdiscip. Rev.: Forensic Sci. (2020), e1409.

[29] S. Broad, T. Mulliken, D. Roe, The nature and extent of legal and illegal trade in wildlife. The trade in wildlife, Routledge, 2014, pp. 25–44.

[30] S. Busilacchi, J.R. Butler, I. van Putten, M. Cosijn, J. Posu, R. Fitriana, A. Slamet, Why does illegal wildlife trade persist in spite of legal alternatives in transboundary regions? Hum. Dimens. Wildl. (2021) 1–18.

[31] J.E. Huffman, J.R. Wallace, Wildlife forensics: methods and applications, Vol. 6, John Wiley & Sons, 2012.

[32] A.O. Brown, G.J. Frankham, L. Bond, B.H. Stuart, R.N. Johnson, M. Ueland, An overview of risk investment in the transnational illegal wildlife trade from stakeholder perspectives, Wiley Interdiscip. Rev.: Forensic Sci. 3 (2) (2021), e1397.

[33] G.L. Feudo, B. Macchione, A. Naccarato, G. Sindona, A. Tagarelli, The volatile fraction profiling of fresh tomatoes and triple concentrate tomato pastes as parameter for the determination of geographical origin, Food Res. Int. 44 (3) (2011) 781–788.

[34] A.M. Gioacchini, M. Menotta, M. Guescini, R. Saltarelli, P. Ceccaroli, A. Amicucci, E. Barbieri, G. Giomaro, V. Stocchi, Geographical traceability of Italian white truffle (*Tuber magnatum* Pico) by the analysis of volatile organic compounds, Rapid Commun. Mass Spectrom.: Int. J. Devoted Rapid Dissem. -to--Minute Res. Mass Spectrom. 22 (20) (2008) 3147–3153.

[35] S. Medina, R. Perestrelo, R. Santos, R. Pereira, J.S. Camara, Differential volatile organic compounds signatures of apple juices from Madeira Island according to variety and geographical origin, Microchem. J. 150 (2019), 104094.

[36] D. Nedelkov, Population proteomics: addressing protein diversity in humans, Expert Rev. Proteom. 2 (2005) 315–324.

[37] D. Nedelkov, U.A. Kiernan, E.E. Niederkofler, K.A. Tubbs, R.W. Nelson, Population proteomics: The concept, attributes, and potential for cancer biomarker research, Mol. Cell. Proteomics 5 (2006) 1811–1818.

[38] S. Heinrich, A. Toomes, C.R. Shepherd, O.C. Stringham, M. Swan, P. Cassey, Strengthening protection of endemic wildlife threatened by the international pet trade: the case of the Australian shingleback lizard, Anim. Conserv. (2021).

[39] Ehmann, H., 1992. Encyclopedia of Australian animals. Reptiles. Angus & Robertson, Australia.

[40] K. Griffiths. Frogs and reptiles of the Sydney region, second ed.,, Reed New Holland, Australia, 2006.

[42] S. Wilson, G. Swan. A complete guide to reptiles of Australia, Reed New Holland, Australia, 2003.

[41] H.G. Cogger, Reptiles and Amphibians of Australia, CSIRO Publishing, Collingwood, 2014.

[43] G. Norval, M.G. Gardner, The natural history of the sleepy lizard, *Tiliqua rugosa* (Gray, 1825)–Insight from chance observations and long-term research on a common Australian skink species, Austral Ecol. 45 (4) (2020) 410–417.

[44] G. Norval, J. Clayton, R.D. Sharrad, M.G. Gardner, Notes on the stomach contents of a juvenile Sleepy Lizard, *Tiliqua rugosa* (Gray 1825), killed by an Eastern Brown Snake, *Pseudonaja textilis* (Duméril, Bibron, and Duméril 1854) in South Australia, Reptiles Amphib. 25 (3) (2018) 200–203.

[45] R.C. O'Brien, S.L. Forbes, J. Meyer, I.R. Dadour, A preliminary investigation into the scavenging activity on pig carcasses in Western Australia, Forensic Sci. Med. Pathol. 3 (3) (2007) 194–199.

[46] Z.S. Atkins, N. Clemann, M. Schroder, D.G. Chapple, N.E. Davis, W.A. Robinson, J. Wainer, K.A. Robert, Consistent temporal variation in the diet of an endangered alpine lizard across two south-eastern Australian sky-islands, Austral Ecol. 43 (3) (2018) 339–351.

[47] A.O. Brown, G.J. Frankham, B.H. Stuart, M. Ueland, Reptile volatilome profiling optimisation: a pathway towards forensic applications, Forensic Sci. Int.: Anim. Environ. 1 (2021), 100024.

[48] SEED The Central Resource for Sharing and Enabling Environmental Data in NSW. (2021). Retrieved from: 〈https://geo.seed.nsw.gov.au/Public_Viewer/index.html?viewer=Public_Viewer&locale=en-AU&runWorkflow=AppendLayerCatalog&CatalogLayer=SEED_Catalog.140.IBRA7%20regions〉.

[49] A.C. Schrimpe-Rutledge, S.G. Codreanu, S.D. Sherrod, J.A. McLean, Untargeted metabolomics strategies-challenges and emerging directions, J. Am. Soc. Mass Spectrom. 27 (12) (2016) 1897–1905.

[50] S. Baeckens, J. Martín, R. García-Roa, R. Van Damme, Sexual selection and the chemical signal design of lacertid lizards, Zool. J. Linn. Soc. 183 (2) (2018) 445–457.

[51] C.E. Shannon, A mathematical theory of communication, Bell Syst. Tech. J. 27 (1948) 379–423.

[52] Good Scents Company. (2021). Providing information for the flavor, fragrance, food and cosmetic industries. Retrieved from: 〈http://www.thegoodscentscompany.com/〉.

[53] A. Titmuss, Husbandry manual for the shingleback lizard *Tiliqua rugosa* GRAY, 1825 Reptilia: Scincidae, University of Western Sydney, Hawkesbury, 2007. Retrieved from, https://aszk.org.au/wp-content/uploads/2020/04/Reptiles.-Shingleback-Lizard-2007AT.pdf.

[54] M. Guppy, S.D. Bradshaw, B. Fergusson, I.A. Hansen, C. Atwood, Metabolism in lizards: low lactate turnover and advantages of heterothermy, Am. J. Physiol. -Regul., Integr. Comp. Physiol. 253 (1) (1987) R77–R82.

[55] M.R. Warburg, The influence of ambient temperature and humidity on the body temperature and water loss from two Australian lizards, *Tiliqua rugosa* (Gray) (Scincidae) and *Amphibolurus barbatus* cuvier (Agamidae), Aust. J. Zool. 13 (2) (1965) 331–350.

[56] J. Laothawornkitkul, J.E. Taylor, N.D. Paul, C.N. Hewitt, Biogenic volatile organic compounds in the Earth system, N. Phytol. 183 (1) (2009) 27–51.

[57] M.H. Ansari, S.J. Cooper, M.P. Schwarz, M. Ebrahimi, G. Dolman, L. Reinberger, K.M. Saint, S.C. Donnellan, C.M. Bull, M.G. Gardner, Plio-Pleistocene diversification and biogeographic barriers in southern Australia reflected in the phylogeography of a widespread and common lizard species, Mol. Phylogenetics Evol. 133 (2019) 107–119.

[61] J. Zhao, Z.H. Hu, P.S. Leng, H.X. Zhang, F.Y. Cheng, Fragrance composition in six tree peony cultivars, Hortic. Sci. Technol. 30 (6) (2012) 617–625.

[58] S.S. El-hawary, M.E. El-Tantawy, M.A. Rabeh, W.K. Badr, Chemical composition and antimicrobial activity of volatile constituents of cladodes, fruits peel and fruits pulp from *Opuntia ficus indica* (L.) Mill.(Prickly Pear) growing in Egypt, Egypt. J. Chem. 64 (1) (2021) 437–444.

[59] H. Hafsah, I. Iriawati, T.S. Syamsudin, Dataset of volatile compounds from flowers and secondary metabolites from the skin pulp, green beans, and peaberry green beans of robusta coffee, Data Brief. 29 (2020), 105219.

[60] K.A. Shaheed, M.A. Alsirraj, S.A. Allaith, N.A. Noori, M.H. Obaid, Z.M. Mouhsan, The biological activities of seeds extracts for fenugreek and black cumin and its inhibitory influences towards some pathogens, Iraq Med. J. 2 (2) (2018).

[66] Y. Wu, J. Zhou, T. Ming, S. Tang, H. Bu, Y. Chen, J. Jiang, W. Tian, X. Su, Analysis of volatile components of potato from different habitats by electronic nose and GC-MS, Food Sci. 24 (2016).

[67] N.H. Zainol Hilmi, A.S. Idris, M.N. Mohd Azmil, Headspace solid-phase microextraction gas chromatography–mass spectrometry for the detection of volatile organic compounds released from *Ganoderma boninense* and oil palm wood, For. Pathol. 49 (4) (2019), e12531.

[63] Y.M. García, J. Rufini, M.P. Campos, M.N. Guedes, R. Augusti, J.O. Melo, SPME fiber evaluation for volatile organic compounds extraction from acerola, J. Braz. Chem. Soc. 30 (2019) 247–255.

[64] H. Pan, Y. Lu, C. Xiu, H. Geng, X. Cai, X. Sun, Y. Zhang, L. Williams III, K. A. Wyckhuys, K. Wu, Volatile fragrances associated with flowers mediate host plant alternation of a polyphagous mirid bug, Sci. Rep. 5 (1) (2015) 1–11.

[62] X.H. Bai, L.H. Teng, D.Q. Lü, H.Y. Qi, Co-treatment of EFF and 1-MCP for enhancing the shelf-life and aroma volatile compounds of oriental sweet melons (*Cucumis melo var. makuwa* Makino), J. Integr. Agric. 13 (1) (2014) 217–227.

[65] M. Suarez, C. Duque, Volatile constituents of lulo (*Solanum vestissimum* D.) fruit, J. Agric. Food Chem. 39 (8) (1991) 1498–1500.

[72] A. Tateo, A. Maggiolino, R. Domínguez, J.M. Lorenzo, F.R. Dinardo, E. Ceci, R. Marino, A.D. Malva, A. Bragaglio, P.D. Palo, Volatile organic compounds, oxidative and sensory patterns of vacuum aged foal meat, Animals 10 (9) (2020) 1495.

[68] H.S. Choi, Chemical composition of the essential oils from *Solidago virga-aurea var. asiatica* Nakai with different harvesting area, Korean J. Food Nutr. 33 (3) (2020) 257–265.

[69] Y. Duan, F. Zheng, H. Chen, M. Huang, J. Xie, F. Chen, B. Sun, Analysis of volatiles in Dezhou Braised Chicken by comprehensive two-dimensional gas chromatography/high resolution-time of flight mass spectrometry, LWT-Food Sci. Technol. 60 (2) (2015) 1235–1242.

[70] S. Petričević, N.M. Radovčić, K. Lukić, E. Listeš, H. Medić, Differentiation of dry-cured hams from different processing methods by means of volatile compounds, physico-chemical and sensory analysis, Meat Sci. 137 (2018) 217–227.

[71] H. Seifi, S. Masoum, S. Seifi, E.H. Ebrahimabadi, Chemometric resolution approaches in characterisation of volatile constituents in *Plantago ovata* seeds using gas chromatography–mass spectrometry: methodology and performance assessment, Phytochem. Anal. 25 (3) (2014) 273–281.

[73] L. Kamaliroosta, M. Zolfaghari, S. Shafiee, K. Larijani, M. Zojaji, Chemical identifications of citrus peels essential oils, J. Food Biosci. Technol. 6 (2) (2016) 69–76.

[74] S.D. Kim, S. Odagiri, T. Ito, Separation and identification of volatile components of apple fruits after harvest, Appl. Biol. Chem. 32 (2) (1989) 143–147.

[75] M. de Sousa Galvão, N. Narain, M.D.S.P. dos Santos, M.L. Nunes, Volatile compounds and descriptive odor attributes in umbu (*Spondias tuberosa*) fruits during maturation, Food Res. Int. 44 (7) (2011) 1919–1926.

[76] R. Sasikumar, D. Das, C. Saravanan, S.C. Deka, GC-HRMS screening of bioactive compounds responsible for antimicrobial and antioxidant activities of blood fruit (*Haematocarpus validus* Bakh. F. Ex Forman) of North-East India, Arch. Microbiol. 202 (10) (2020) 2643–2654.

[77] M. Sinha, A. Sørensen, A. Ahamed, B.K. Ahring, Production of hydrocarbons by *Aspergillus carbonarius* ITEM 5010, Fungal Biol. 119 (4) (2015) 274–282.

[78] W. Aboshora, S.E. Idriss, K.A. Omar, H.A. Hassanin, J. Yu, M. Abdalla, H. Raza, L. Zhang, Volatile flavor compounds of peel and pulp from Doum (*Hyphaene thebaica* L.) fruit, Am. J. Food Sci. Nutr. Res. 4 (5) (2017) 165–169.

[79] P.J. Gilliver, H.E. Nursten, The source of the acyl moiety in the biosynthesis of volatile banana esters, J. Sci. Food Agric. 27 (2) (1976) 152–158.

[80] A.L. Morales, C. Duque, Free and glycosidically bound volatiles in the mammee apple (*Mammea americana*) fruit, Eur. Food Res. Technol. 215 (3) (2002) 221–226.

[81] Å.E. Dahl, A.B. Wassgren, G. Bergström, Floral scents in Hypecoum sect. Hypecoum (Papaveraceae): chemical composition and relevance to taxonomy and mating system, Biochem. Syst. Ecol. 18 (2–3) (1990) 157–168.

[82] M.S. Karawya, S.A. Wahab, M.M. El-Olemy, N.M. Farrag, Diphenylamine, an antihyperglycemic agent from onion and tea, J. Nat. Prod. 47 (5) (1984) 775–780.

[83] V. Gkarane, M. Ciulu, B.A. Altmann, A.O. Schmitt, D. Mörlein, The effect of algae or insect supplementation as alternative protein sources on the volatile profile of chicken meat, Foods 9 (9) (2020) 1235.

[84] R. Makarow, S. Schäfer, S. Albrecht, S. Robert, S. Schütz, P. Kaul, Investigation of volatile organic compounds emitted by *Anoplophora glabripennis* (Moschulsky) using thermal desorption and gas chromatography-mass spectrometry, Microchem. J. 146 (2019) 142–148.

[85] J. Román-Kustas, J.B. Hoffman, D. Alonso, J.H. Reed, A.E. Gonsalves, J. Oh, S. Hong, K.D. Jo, C.E. Dana, M. Alleyne, N. Miljkovic, Analysis of cicada wing surface constituents by comprehensive multidimensional gas chromatography for species differentiation, Microchem. J. 158 (2020), 105089.
[86] G. MacLeod, J.M. Ames, Volatile components of starfruit, Phytochemistry 29 (1) (1990) 165–172.
[87] M. Moalemiyan, A. Vikram, A.C. Kushalappa, Detection and discrimination of two fungal diseases of mango (cv. Keitt) fruits based on volatile metabolite profiles using GC/MS, Postharvest Biol. Technol. 45 (1) (2007) 117–125.
[88] J.A. Pino, C.E. Quijano, Study of the volatile compounds from plum (*Prunus domestica* L. cv. Horvin) and estimation of their contribution to the fruit aroma, Food Sci. Technol. 32 (1) (2012) 76–83.
[89] R.B. Watharkar, Y. Pu, B.B. Ismail, B. Srivastava, P.P. Srivastav, D. Liu, Change in physicochemical characteristics and volatile compounds during different stage of banana (*Musa nana* Lour vs. Dwarf Cavendish) ripening, J. Food Meas. Charact. 14 (4) (2020) 2040–2050.
[90] H.V. de Vasconcelos Facundo, D. dos Santos Garruti, C.T. dos Santos Dias, B. R. Cordenunsi, F.M. Lajolo, Influence of different banana cultivars on volatile compounds during ripening in cold storage, Food research international 49 (2) (2012) 626–633.
[93] Y. Wang, X. Wang, F. Xu, H. Wu, Determination of the Volatile Constituents in Banana Flowers and Bracts by Headspace Solid-Phase Microextraction and Gas Chromatography-Mass Spectrometry. 2016 International Conference on Engineering and Technology Innovations, Atlantis Press, 2016.
[91] R.J. Bryant, A.M. McClung, Volatile profiles of aromatic and non-aromatic rice cultivars using SPME/GC–MS, Food Chem. 124 (2) (2011) 501–513.
[92] R.G. Buttery, W.J. Orts, G.R. Takeoka, Y. Nam, Volatile flavor components of rice cakes, J. Agric. Food Chem. 47 (10) (1999) 4353–4356.
[94] L.O. Sunesen, V. Dorigoni, E. Zanardi, L. Stahnke, Volatile compounds released during ripening in Italian dried sausage, Meat Sci. 58 (1) (2001) 93–97.
[95] X. Liu, S. Wang, S. Tamogami, J. Chen, H. Zhang, Volatile profile and flavor characteristics of ten edible oils, Anal. Lett. (2020) 1–16.
[96] K.E. Rusanov, N.M. Kovacheva, I.I. Atanassov, Comparative GC/MS analysis of rose flower and distilled oil volatiles of the oil bearing rose Rosa damascena, Biotechnol. Biotechnol. Equip. 25 (1) (2011) 2210–2216.
[97] C.B. Adeosun, O.L. Bamigbade, A. Osho, O. Atolani, Volatile composition of the leaf, flower and fruit of Cordia sebestena (L.), J. Essent. Oil Bear. Plants 18 (4) (2015) 976–981.
[98] J. Ren, L. Yang, Y. Wang, H. Yao, Chemical profile of floral scent at different flower developmental stages of rose de rescht (*Rosa damascena* Mill.) cultivated in Beijing, J. Essent. Oil Bear. Plants 19 (2) (2016) 433–443.
[99] D.U. Ahn, C. Jo, D.G. Olson, Analysis of volatile components and sensory characteristics of irradiated raw pork, Iowa State Univ. Anim. Ind. Rep. 1 (1) (2000).
[100] J.A. Pino, J. Mesa, Y. Muñoz, M.P. Martí, R. Marbot, Volatile components from mango (Mangifera indica L.) cultivars: Journal of Agricultural and Food Chemistry 53 (6) (2005) 2213–2223.
[101] E. Alissandrakis, D. Daferera, P.A. Tarantilis, M. Polissiou, P.C. Harizanis, Ultrasound-assisted extraction of volatile compounds from citrus flowers and citrus honey, Food Chem. 82 (4) (2003) 575–582.
[102] G. Ripoll, M.D.G. Córdoba, M.J. Alcalde, A. Martín, A. Argüello, R. Casquete, B. Panea, Volatile organic compounds and consumer preference for meat from suckling goat kids raised with natural or replacers milk, Ital. J. Anim. Sci. 18 (1) (2019) 1259–1270.
[103] K. Sha, Z. Ze-Jun, S. Bao-Zhong, L. Hai-Peng, S. Huan-Lu, L. Yu-Miao, L. Yuan-Hua, L. Hong-D, Z. Yang, Investigation of physicochemical and textural characteristics and volatile compounds of Kazakh dry-cured beef, Food Sci. Technol. Res. 23 (2017) 375–383.
[104] R. Domínguez, M. Gómez, S. Fonseca, J.M. Lorenzo, Influence of thermal treatment on formation of volatile compounds, cooking loss and lipid oxidation in foal meat, LWT-Food Sci. Technol. 58 (2) (2014) 439–445.
[105] J.F. Mwatseteza, N. Torto, Profiling volatile compounds from Mucuna beans by solid phase microextraction and gas chromatography-high resolution time of flight mass spectrometry, Food Chem. 119 (1) (2010) 386–390.
[106] C. Li, H. Lichao, J. Guofeng, M. Sumin, W. Wenmin, G. Lan, Effect of different irradiation dose treatment on the lipid oxidation, instrumental color and volatiles of fresh pork and their changes during storage, Meat Sci. 128 (2017) 68–76.
[107] A.J. Macleod, J.S. Cave, Volatile flavour components of eggs, J. Sci. Food Agric. 26 (3) (1975) 351–360.
[108] S.D. Johanningsmeier, R.F. McFeeters, Detection of volatile spoilage metabolites in fermented cucumbers using nontargeted, comprehensive 2–dimensional gas chromatography-time-of-flight mass spectrometry (GC× GC-TOFMS), J. Food Sci. 76 (1) (2011) C168–C177.
[109] K. Deshpande, K.G. Furton, K. De Etta, The equine volatilome: volatile organic compounds as discriminatory markers, J. Equine Vet. Sci. 62 (2018) 47–53.
[110] C.A. Lawson, M. Possell, J.R. Seymour, J.B. Raina, D.J. Suggett, Coral endosymbionts (*Symbiodiniaceae*) emit species-specific volatilomes that shift when exposed to thermal stress, Sci. Rep. 9 (1) (2019) 1–11.
[111] A. Olander, C.A. Lawson, M. Possell, J.B. Raina, M. Ueland, D.J. Suggett, Comparative volatilomics of coral endosymbionts from one-and comprehensive two-dimensional gas chromatography approaches, Mar. Biol. 168 (5) (2021) 1–13.
[112] F. Jiguet, K.J. Kardynal, K.A. Hobson, Stable isotopes reveal captive vs wild origin of illegally captured songbirds in France, Forensic Sci. Int. 302 (2019), 109884.
[113] R. Ogden, A. Linacre, Wildlife forensic science: a review of genetic geographic origin assignment, Forensic Sci. Int.: Genet. 18 (2015) 152–159.