A Review of Chemically Based Communication in Miridae, with a Focus on Two Sympatric Species of *Eccritotarsus*[1]

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Abstract This report is a review of pertinent scientific literature on the chemical communication of the Miridae (Order Hemiptera; Suborder Heteroptera), with a focus on the sympatric species *Eccritotarsus catarinensis* Carvhalho and *Eccritotarsus eichhorniae* Henry. Both species have been deployed as biological control agents of water hyacinth, *Pontederia* (*Eichhornia*) *crassipes* (Martius) Solms-Laubach, in over 30 sites in South Africa. Our aim in this review was to gather and assimilate information on the chemical communication and chemical ecology of mirids, with an ultimate goal of enhancing the effectiveness of biological control agents of water hyacinth, particularly *E. catarinensis* and *E. eichhorniae*. Many hemipterans have highly developed metathoracic scent glands (MTGs), Brindley's glands, and secretory setae that store and emit chemicals that may act as allomones, kairomones, or pheromones. The preponderance of the available literature dealt with sex pheromones. Hexyl butyrate, (E)-4-oxo-2-hexenal, and (E)-2-hexenyl butyrate are reported to be sex pheromones in many mirids: for example, *Adelphocoris fasciaticollis* Reuter*, Apolygus spinolae* Meyer-Dür*, Liocoris tripustulatus* F.*, Lygocoris pabulinus* L., several *Lygus* spp. (*Lygus hesperus* Knight, *Lygus lineolaris* Beauvois, *Lygus elisus* van Duzee, *Lygus pratensis* L., and *Lygus rugulipennis* Poppius), and *Stenotus rubrovittatus* Matsumura. Hexyl butyrate is the most prevalent sex pheromone in Miridae, while (E)-4-oxo-2-hexenal is the most prevalent sex pheromone in Heteroptera. Both pheromones co-occur in some species and exhibit many functionalities. The constancy of sex pheromones linked to behavioral patterns in Miridae suggest a punctuated equilibrium pattern of evolution (dating back to the Triassic), similar to morphological characters of many insects.

Key Words pheromones, Hemiptera, Heteroptera, Miridae, chemical communication

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Chemical communication in insects and other invertebrates is a field of study that is likely too large to comprehensively review (Ali and Morgan 1990). There are large numbers of semiochemicals that serve as intraspecific and interspecific chemical signals when emitted by an individual, which affect the behavior of other conspecific

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and heterospecific individuals. These chemicals are of different compositions, have specific and general functions, and vary in structural complexity.

Among all taxonomic orders of insects, the Lepidoptera and social insects in Hymenoptera have the greatest amount of published information on their semiochemicals, and their use in Integrated Pest Management (IPM) programs. Knowledge of chemical communication in mirids, heteropterans, and hemipterans is often restricted to those chemical compounds stored, produced, and released by the metathoracic glands (MTGs) that occur on most species. Our interest in conducting this review of pertinent literature on chemical communication focusing on mirids arose from our efforts to successfully deploy and utilize the sympatric species *Eccritotarsus catarinensis* Carvhalho and *Eccritotarsus eichhorniae* Henry as biological control agents of water hyacinth, *Pontederia (Eichhornia) crassipes* (Martius) Solms-Laubach. To date, the mirids have been established in over 30 sites in South Africa to control water hyacinth. Our ultimate goal in this review was to enhance all aspects of the classical biological control of this noxious aquatic invasive weed using these two mirids. The review is organized into sections addressing (a) chemicals relative to taxonomic divisions, (b) types of semiochemicals, (c) functional groups of chemicals, (d) the MTG function, (e) antennae function, and (f) chemicals of *Eccritotarsus*.

Semiochemicals Relative to Taxonomic Divisions

Most animal lineages use semiochemicals in various behavioral contexts such as finding mates, food resources, habitats, and egg-laying sites. Communication is an intricate phenomenon. The innate ability of animals to detect chemicals must match the growth and development of their anatomical, biochemical, physiological, and molecular processes.

Pheromones are intraspecific chemicals used by invertebrates and vertebrates. They include aggregation, alarm, trail, epideictic, invitation, dispersal, territorial, surface, and sex pheromones. They are prominently used in long-range intrasexual communication, while substrate-borne vibrational signals are prominently used in short-range intersexual communication. They have a long evolutionary history, playing an integral part in insect communication and mate recognition (Teal and Tumlinson 1992). Essentially, all pheromones induce stereotypical behaviors. They are chemicals emitted to stimulate behavioral and physiological responses (Teal and Tumlinson 1992). Releaser pheromones induce behavioral responses, whereas primer pheromones induce physiological responses (Teal and Tumlinson 1992). Chemically, they are grouped into lipids, proteins, and peptides. Protein and peptide pheromones are used by both aquatic and terrestrial invertebrates and vertebrates. Despite their relatively large size, protein and peptide pheromones are highly soluble, but nonvolatile, chemicals.

Of the three, lipid pheromones are most prominent in insects. They have varied solubilities and vapor pressures, which are suitable in varying ecological conditions (Teal and Tumlinson 1992). Lipid pheromones include aggregation, alarm, aromatic, trail, and volatile chemicals (Teal and Tumlinson 1992). The complexity of chemical blends is strongly correlated with an evolutionary pecking order (Teal and Tumlinson 1992). Highly evolved taxa use multicomponent blends synthesized by numerous biochemical pathways (e.g., Hymenoptera), while less evolved taxa use single-component blends synthesized by a single biochemical pathway (e.g., Lepidoptera) (Zhang and Aldrich 2004).

Hemiptera. This insect order includes true bugs. Its fossil record dates back to the Carboniferous (Moscovian) (Nel et al. 2013). It comprises well over 80,000 species. Most hemipterans extract plant fluids from their hosts using their piercing–sucking mouthparts. Some exhibit hematophagy while others are predatory, feeding on small invertebrates and other insects (Grimaldi and Engel 2005). Like many other groups of insects, hemipterans occupy diverse habitats. They are mostly terrestrial, while others are found in, or on the surface of, freshwater. They are hemimetabolous (i.e., undergoing incomplete metamorphosis with three developmental stages of egg, nymph, and adult), while some are capable of parthenogenesis, which enables them to reproduce extremely rapidly in a very short period of time, especially under favorable environmental conditions (Grimaldi and Engel 2005).

Many species of Hemiptera have highly developed MTGs, Brindley's glands, and secretory setae that produce, store, and emit chemicals (Zhang et al. 2014). In the MTG, chemicals are produced, stored, and emitted from the pleura of the anteroventral cavity, which is located between the middle and posterior coxal pits (Heshula., unpubl. data.). Other parts of thorax that are not part of the MTG have also been reported to emit chemicals (Millar et al. 1997). So far, no abdominal region of hemipterans has been reported to emit chemicals.

Heteroptera. The Heteroptera first appeared in the Triassic period (Nel et al. 2013). The production and release of defensive scents from glands is common in most of its members. The oily secretions produced by the glands include aliphatic aldehydes, alkanes, esters, ketoaldehydes, and several other biosynthetically related chemical compounds (Knight et al. 1984). Nearly all heteropterans emit pheromones for various physiological, behavioral, and ecological reasons (Aldrich 1988). Esters are considered to be important in the biosynthesis of aldehydes and ketoaldehydes which, in turn, serve as primary irritants (Knight et al. 1984). Released from the MTG, esters are usually present in small amounts which vary in quantity (Knight et al. 1984). Furthermore, heteropterans usually produce alcohol and acid as byproducts of the esters, as well as aldehydes that correspond with alcohol components (Knight et al. 1984).

Miridae. Well over 11,000 species are described within 1,400 genera of mirids (Zhang and Aldrich 2004), making it the largest heteropteran family. It is known to be represented by insects that suck juices from the meristematic plant tissues. In past years, it was widely reported and accepted that only females emitted short- and long-range chemicals, while males responded accordingly (Aldrich 1988). However, some studies also have shown that males of some species attract females (e.g., Moraes et al. 2005). Furthermore, it is not unusual for females and males to have identical chemical compositions, within and between species (and with similar or identical functionalities), although the evolutionary reason for that is still a subject of some debate, particularly in some species (Zhang et al. 2015).

Although interchangeable, it has been reported that chemicals that females use as sex pheromones are instead used by their conspecific males as allomones and defense pheromones, or by heterospecific females and males as kairomones (Millar et al. 1997). Thus, pheromones used by females of a particular species in one way are often used by conspecific males and heterospecific females and males in another way. When chemical compounds have been reported in both sexes, there has been a failure of either sex to attract its conspecific (Aldrich 1988). This finding has been proposed to have massive implications for mate recognition.

In Miridae, chemical compounds include esters, monoterpenes, sesquiterpenoids, and acetogenins, while defensive chemical compounds are alcohols, aldehydes, alkanes, alkenals, monoterpenes, or esters (Millar 2005). Environmental parameters such as temperature, humidity, light, and photoperiod modulate sex pheromone responses. Despite this, traps baited with pheromones continue to be a reliable and effective tool for monitoring and detecting insect pest populations, and these are increasingly employed in various IPM programs (Zhang et al. 2014). Pheromone-baited trapping and monitoring of agricultural pest population fluctuations are useful in the implementation of IPM strategies. Furthermore, by inundating the environment with sex pheromone levels exceeding the natural levels of the target species, normal chemical signaling and reproduction of the pest may be disrupted, reduced, and impaired (Dicken et al. 1991). In Miridae, the assessment, identification and application of pheromones in IPM programs is still developing.

Chemical Composition and Function

Sex pheromones. To date, a four-pheromone blend has been detected in the MTG of the following hemipterans: *Adelphocoris fasciaticollis* Reuter (Yang et al. 2014, Zhang et al. 2015), *Apolygus spinolae* Meyer-Dür (Yang et al. 2014), *Lygus lineolaris* Beauvois (Aldrich 1988), *Lygus hesperus* Knight, *Lygus lineolaris* and *Lygocoris pabulinus* L. (Byers et al. 2013 Ho and Millar 2002), *Lygus rugulipennis* Poppius (Innocenzi et al. 2004, 2005), *Lygus pratensis* (L.), *Lygus rugulipennis* and *Liocoris tripustulatus* F. (Fountain et al. 2014), *Lygos pabulinus* L. (Drijfhout and Groot 2001 Drijfhout et al. 2002), *Phytocoris difficilis* Fallén (Zhang and Aldrich 2004), and *Stenotus rubrovittatus* Matsumura (Oku and Yasuda 2010, Yasuda et al. 2008).

One of the most notable chemicals produced by heteropterans is (E)-4-oxo-2-hexenal. This chemical compound is ubiquitous in this group. It is also a constant in extracts of several mirids (Zhang et al. 2015). It has been reported to function as a defensive chemical (Zhang and Aldrich 2004), kairomone (Innocenzi et al. 2005), and sex pheromone (Yasuda et al. 2008). It is yet to be reported to function as synomone, allomone, aggregation, trail, alarm, or dispersal pheromone. It is already disregarded as an apneumone, because it is relatively unstable, decomposes rapidly, and has a weak response on mass spectrometry and flame ionization detectors (Innocenzi et al. 2005). In many cases, it co-occurs with hexyl butyrate and (E)-2-hexenyl butyrate.

Hexyl butyrate is the only chemical compound recorded in high quantities in both females and males of all the mirids that have been assessed thus far (Zhang and Aldrich 2003). It also serves as an antisex pheromone that inhibits an attraction of sympatric species, or prevents conspecific females from producing chemical compounds (Millar et al. 1997). Its functions have been reported in *Ph. difficilis*, *Lygo. pabulinus*, and *Lygu. lineolaris*. It has been postulated that the four-pheromone mixture combining hexyl butyrate, (E)-2-hexenyl butyrate, (E)-4-oxo-2-

hexenal, and (E)-2-octenyl butyrate will also be major constituents of chemical blends that are yet to be discovered in various species of Miridae (Millar et al. 1997).

According to Yasuda et al. (2008), there is no significant difference in captures of traps baited with (E)-4-oxo-2-hexenal and hexyl butyrate in male *S. rubrovittatus*. This pattern also has been observed in many other mirids listed above. The basis for this is yet to be fully understood, although several speculations are being offered by several researchers.

NonSex Pheromones. Aggregation pheromones are often combined with sex pheromones to produce a semiochemical attractant. This occurs in *Anthonomus rubi* Herbst (Coleoptera: Curculionidae), *Lygu. rugulipennis* (Hemiptera: Miridae), and *Byturus tomentosus* de Geer (Coleoptera: Byturidae). Combining semiochemicals is used as an approach of monitoring and trapping insect pests. In *Ph. difficilis*, hexyl butyrate and (E)-2-hexenyl butyrate reportedly function as antisex pheromones. Traps baited with the aggregation pheromone (methyl [2E,4E,6Z]-decatrienoate) of *Plautia stali* Scott (Hemiptera: Pentatomidae) are also mentioned by Aldrich et al. (2012).

The chemicals reported in this review are not only sex pheromones, although most are presented as such. The same chemicals serve as aggregation, alarm, trail, epideictic, invitation, dispersal, territorial, and surface pheromones. Moreover, the same chemicals can serve as allelomones: that is, allomones, kairomones, synomone, and apneumone. For example, chemical profiles released by disturbed and undisturbed females and males of *Lygu. lineolaris* had in common six chemical compounds: (E)-2-hexenal, 1-hexanol, (E)-2-hexenol, hexyl butyrate, (E)-2-hexenyl butyrate, and (E)-2,4-oxohexenal. Some are mentioned in other sections of this review as sex pheromones, but also serve as alarm or epideictic and defensive allomones. However, in Miridae, apart from aggregation and sex pheromones, very little research has been reported on the role of other types of chemical compounds.

Role of Functional Groups in Chemicals

Functional groups are extremely important for accurate detection in various ecosystems. Some insects only respond when a particular functional group is present and detected. Other insects have been reported to be hindered by identical pheromones that have dissimilar functional groups. Once there is an addition or substitution of atoms or functional groups to a chain of a pheromonal blend, the chemical nature of that particular pheromone inevitably changes drastically, and almost instantly (Millar et al. 1997). Therefore, it has become evident that the presence and preciseness of components of all pheromone blends play a key role in insect communication. They dictate the presence or absence of an outcome, in a form of a behavioral or physiological response. The presence and/or absence of any response relies on whether or not threshold measures or levels have been reached.

The nomenclature of chemical compounds depends on a carbon chain and its length, as well as on the name and the position of the functional group (Millar et al. 1997). The interconversion of functional groups showcases differentiation in the pheromone blends, including those used by closely related species (Millar et al.

1997). It is well demonstrated that chemical cues such as the number, position, and geometry of double bonds affect the pheromone efficacy (Dickens et al. 1991). In addition, the substitution of fluorine, halogen, proton, or a methyl group also affects the pheromone efficacy (Dickens et al. 1991). Lastly, the shortening of an alkyl chain and length of the carbon skeleton equally affects the pheromone efficacy (Dickens et al. 1991). An example of such findings was reported by Dickens et al. (1991), who assessed the neurons in both sexes of the boll weevil, *Anthonomus grandis* Boheman.

Furthermore, deleting isoprene units containing a benzyl or hydrocarbon group distorts the pheromone efficacy in various insect groups (Xue et al. 1991). Currently, much remains unknown about pheromones, requiring further investigation. However, to date, it is clear from currently published studies that, in general, pheromone receptors of various insect groups (especially mirids) are highly specific, and play an integral part in mate recognition, attraction, and defense.

Metathoracic Scent Glands

In Miridae, adults have well-developed MTGs that play important roles in both intraspecific and interspecific communication, by producing, storing, and emitting semiochemicals, which can be distinguished as pheromones (shared among members of the same species) and allelochemicals (shared among members of different species) (Zhang et al. 2014). These pheromones are grouped into primer and releaser, and include defense, aggregation, alarm, trail, dispersal, and sex pheromones. Allelochemicals include allomones, kairomones, synomones, and apneumones. There are two kinds of MTGs, namely, the diastomian and the omphalian (Zhang et al. 2014). These are distinguished based on the number of openings on them. There tends to be diverse structures in the opening and evaporation area of the MTG in the different families of Hemiptera, including the Miridae.

MTG anatomy. Located in the pleura of the anteroventral cavity, between the middle and posterior coxal pit (Heshula., unpubl. data), the MTG is reported to be composed of a well-developed reservoir and paired lateral glands. These are considered to be important diagnostic features in Miridae (Zhang et al. 2014). The reservoir is reportedly long and bag-shaped (Zhang et al. 2014). Its walls have sac-like secretory cells and collecting canals (Zhang et al. 2014). Chemical secretions move from the sac-like secretory cells into the collecting duct before being transferred to the reservoir (Zhang et al. 2014). The collecting duct connects the reservoir to multitubular lateral glands, and the chemical bouquet of a MTG is thought to be emitted through paired openings, spreading over the evaporative areas that surround both openings (Zhang et al. 2014).

The paired lateral glands have openings that are located on the metathoracic basisternum, particularly on the metepisternum of the metapleuron, opening between the mesocoxae and metacoxae (Zhang et al. 2014). There is also a lengthy depressed channel that extends downward through the middle of the metathorax (Zhang et al. 2014). Because families of the Hemiptera have diverse structures in the opening and evaporation areas, diagnostic features in Miridae exist due to suprageneric subtle differences recorded in the position of the peritreme

(Zhang et al. 2014). However, there is usually no sexual dimorphism, although this often depends on the nutritional content of the insect.

Produced, stored, and released MTG pheromones. Chemical compounds produced, stored, and released from the MTG influence insect behavior, depending on the quantitative and qualitative measures of the profiles. In many mirids that have been assessed thus far, hexyl butyrate has been recorded as a major pheromone in MTG secretions, reportedly with various functions (Zhang and Aldrich 2003). It has been reported to be a major pheromone in the MTGs of *Adelphocoris suturalis* Jakovlev and *Pilophorus perplexus* Douglas & Scott, while in *Harpocera thoracica* Fallén, the major pheromones were terpenoids (Aldrich 1988). Butyl butanoate and hexyl butanoate were major pheromones in *Pi. perplexus*, while major pheromones in *Blepharidopterus angulatus* Fallén were hexyl hexanoate and (E)-2-hexenyl hexanoate. Major pheromones in *Ph. difficilis*, such as hexyl butyrate and (E)-2-hexenyl butyrate, are only produced by males (Zhang and Aldrich 2003).

The coexistence of certain pheromones stored and emitted from the MTG has been reported by several researchers (e.g., Byers et al. 2013; Drijfhout and Groot 2001; Drijfhout et al. 2002; Fountain et al. 2014; Groot et al. 1999, 2001; Ho and Millar 2002; Innocenzi et al. 2001, 2004, 2005; MacLeod et al. 1975; Millar et al. 1997; Oku and Yasuda 2010; Yang et al. 2014, 2015; Yasuda et al. 2008; Zhang and Aldrich 2004, 2008; T. Zhang et al. 2015; Z. Zhang et al. 2014). For example, the MTG secretion in several hemipterans includes acetic acid, hexanal, hexanol, and hexyl acetate combined. According to Knight et al. (1984), these are hydrolyzed from esters and match free alcohols and acids as byproducts. This then results in a subsequent oxidation of the free alcohols into aldehydes. Research on the regulation of pheromone production in the MTG of *E. catarinensis* and *E. eichhorniae* is in a later section.

Function of Antennae

Antennal grooming. In mirids, chemical compounds are detected by olfactory receptor neurons (ORN), which are confined within the cuticular sensilla of antennae, or in mouthparts (Anton et al. 2007). The axons of the ORNs transmit signals along an antennal nerve to innervate and stimulate an antennal lobe (AL) of the brain that is considered to be the main olfactory center (Anton et al. 2007). The axons make synaptic contact with both intrinsic and extrinsic AL neurons. The intrinsic AL neurons are termed local interneurons, while the extrinsic AL neurons are termed projection neurons. The combination of these neurons transfers electrical impulses and chemical signals to the brain, in the lateral protocerebrum and mushroom bodies (Anton et al. 2007). The information is then transmitted from the brain to the remainder of the central nervous system through the nerve cord. Modulatory neurons originating from various sections of the central nervous system extend their axons into the intrinsic and extrinsic ALs (Anton et al. 2007). Both the intrinsic and extrinsic ALs have an abundance of a species-specific globular neuropil, which is also known as the glomeruli (Anton et al. 2007).

Oftentimes, substances may accumulate on ungroomed antennae in the course of routine insect activity, covering the sensillar pores. Cuticular hydrocarbons (CHC) and waxes are found on the epicuticular surface of insects. In such insects, they become species-, sex-, and nestmate-recognition signaling cues (Böröczky et al. 2013). They tend to be deposited on antennal surfaces (Böröczky et al. 2013). To ensure an optimal function in the chemo-detection described above, insects habitually groom their antennae. Grooming is, therefore, a physical removal of CHCs that helps to maintain a fresh layer on the epicuticular surface. This act leads to a fresh and more reliable chemical signal that is free from extrinsic contaminants, thus, subsequently improving the efficacy of the chemical communication.

Grooming allows insects to remove foreign materials from their body surfaces. According to Böröczky et al. (2013), an ungroomed antenna accumulates more CHCs than a groomed antenna. An ungroomed antenna reportedly blocks and affects the accurate detection of chemicals, thereby affecting mate recognition (Böröczky et al. 2013). In addition, an ungroomed antenna reportedly accumulates significantly more pollutants from surfaces and the atmosphere than a groomed antenna (Böröczky et al. 2013), with the implication that ungroomed antennae tend to be less effective in responding to chemicals, particularly sex pheromones, than groomed antennae (Böröczky et al. 2013).

The advantage of antennal grooming includes removing excess cuticular lipids and foreign material. These physically and chemically interfere with olfaction (Böröczky et al. 2013). Coincidentally, grooming maintains the olfactory acuity of an antenna. For example, cockroaches groom their antenna using their mouthparts, flies use their forelegs, while ants use their specialized structures on the forelegs together with their mouthparts (Böröczky et al. 2013). Sensilla are considered to be olfactory units of the antenna, and bear multiple cuticular pores that channel odorants to the olfactory receptor neurons (Böröczky et al. 2013).

Consequentially, the effectiveness of a pheromone relies not only on its nature; but may also depend on the physiological status (e.g., age, growth and development, reproductive organs, mating status, etc.), or environmental conditions (e.g., temperature, humidity, photoperiod, light, etc.) of the individual (Anton et al. 2007). In insects that produce sex pheromones, sexual dimorphism is commonly found, and grooming plays an important role in this regard. In addition, octopamine is shown to improve pheromone blend distinction and orientation (Anton et al. 2007). It enhances antennal detection and response to pheromones via the transepithelial potential of the olfactory sensilla (Anton et al. 2007). Moreover, serotonin enhances the detection and responses of both intrinsic and extrinsic AL neurons that have been discussed earlier in this review (Anton et al. 2007).

Antennal stimulation. Insects acquire components of pheromone blends through a multitude of specialized porous sensilla that are located inside their antennae (Teal and Tumlinson 1992). These specialized porous sensilla vary in structure (Teal and Tumlinson 1992). An individual sensillum has several neurons, and each neuron responds to a specific chemical compound, allowing for an accurate sensing of any particular pheromone bouquet (Teal and Tumlinson 1992). Additionally, there are also chemoreceptors, which are seldom present in small quantities, and equally contribute towards an accurate recognition of pheromones, detecting information by comparing chemical compound composition and qualitative and quantitative proportion of the profiles (Zhang and Aldrich 2004).

In *Ad. fasciaticollis*, hexyl butyrate and (E)-4-oxo-2-hexenal elicited strong, dose-dependent electrophysiological activities in the antennae of male adults (Zhang et al. 2015). These same chemical compounds induced strong antennal responses in

Ph. difficilis (Zhang and Aldrich 2003), while hexyl butyrate alone elicited strong antennal responses in *Phytocoris calli* Knight and *Phytocoris breviusculus* Reuter (Zhang and Aldrich 2008). Strong antennal responses elicited by butyrates also were reported in *Lygo. pabulinus*, *Lygu. lineolaris*, and *Lygu. rugulipennis* (Zhang and Aldrich 2003).

In addition to esters, other chemical compounds recorded in small quantities also have key roles in the attraction and mate recognition of mirids. For example, in females of *Apolygus lucorum* Meyer-Dür and *Taylorilygus apicalis* Fieber, the removal of (E)-2-octenyl butyrate recorded in small amounts in a full four-pheromonal blend consisting of hexyl butyrate, (E)-2-hexenyl butyrate, (E)-4-oxo-2-hexenal, and (E)-2-octenyl butyrate did not successfully attract their respective heterospecific and, instead, attracted only their respective conspecific (Yang et al. 2015).

Moreover, in *T. apicalis*, an addition of hexyl butyrate in the full four-pheromone blend also proved unsuccessful in attracting males of *A. lucorum*. Historically, the union of sensilla, neurons, and chemoreceptors that acquire pheromone blends plays a role in chemical communication, especially mate recognition (Teal and Tumlinson 1992). Antennae are critical in acquiring the minute changes in quality and quantity of the pheromone blends and are, therefore, important to consider in interpreting the behavioral outcomes of pheromonal interaction in natural and applied settings, especially for mirids such as *Eccritotarsus*.

More pheromone assessments are needed to be able to distinguish between sympatric species. One approach by which this could be achieved is through antennal stimulation. Furthermore, assessing the specificity of receptor neurons, and focusing on behaviorally and physiologically active pheromones, using a single-cell recording technique is also highly recommended, as previously suggested by Zhang and Aldrich (2008).

In insects, CHCs are the most studied chemotaxonomic characters. There is sufficient evidence that hydrocarbon configuration distinguishes among species, and points to the links between a particular species and higher taxa (Lockey 1991). Chemotaxonomy, which is a relatively new field, targets elongation and decarboxylation pathways that are used by insects to synthesize their hydrocarbons (Lockey 1991). This has been shown to generally provide critical information about the biosynthesis of hydrocarbons in insects (Lockey 1991). Thus, more recently, CHCs have been used as a tool to identify several groups of insect species.

Chemicals in *Eccritotarsus*

The two sympatric species of *Eccritotarsus* currently used as biocontrol agents of water hyacinth in South Africa were collected from their respective native ranges, which are separated by approximately 3,800 km in South America (Henry 2017, Mnguni 2019a, Mnguni and Heshula 2022, Paterson et al. 2016). *Eccritotarsus catarinensis* was sourced from Florianopolis (near Santa Catarina) in Brazil in 1994 (Ismail and Brooks 2018; Mnguni 2019a,b; Mnguni and Heshula 2022; Paterson et al. 2016; Taylor et al. 2011). It was released as a biological control agent of water hyacinth in 1996, following successful host-specificity tests. *Eccritotarsus eichhorniae*, on the other hand, was sourced from the upper Amazon basin (near Iquitos in

Yarapa River) in Peru in 1999 (Ismail and Brooks 2018; Mnguni 2019a,b; Mnguni and Heshula 2022; Paterson et al. 2016; Taylor et al. 2011). It was also released as a biological control agent of water hyacinth in 2007. This collection was conducted to increase the genetic material of the species to assist in overcoming any unforeseen catastrophic events in rearing, releases, and successful establishment in South Africa (Mnguni 2019a,b; Mnguni and Heshula 2021, 2022; Paterson et al. 2016). To date, postassessments indicate that the two sympatric species occur in more than 30 sites in the country (Mnguni 2019a, Mnguni and Heshula 2022).

Chemical compounds are known to maintain sexual isolation between insect populations. However, there is very little research on the chemical ecology of *Eccritotarsus*, with only two studies thus far available in published research literature (Mnguni 2019b, Mnguni and Heshula 2021). Interestingly, the genitalia, MTGs, and antennae involved in storing, emitting, and detecting chemical compounds differ in morphology between the two *Eccritotarsus* species (Henry 2017). Henry (2017) also noted that *E. eichhorniae* has a shorter antennal segment II than its counterpart *E. catarinensis*. This is hypothesized to be potentially important in insect communication and mate recognition.

Mnguni and Heshula (2021) conducted a study of the chemical profiles of the two sympatric species of *Eccritotarsus*, They hypothesized that differences in the profiles (a) might be caused by differences in the MTGs and the antennae; (b) may dictate success or failure of detecting a chemical compound; (c) may enhance or impede one species over the other in detecting single, binary, or multiple chemical compounds; (d) may tweak the functional groups of certain chemical compound and, thereby, cause sympatric species to possess several different chemical compounds; and (e) may result from a pheromone shared by sympatric species to be produced at varying concentrations or proportions.

The overarching aim of the studies conducted by Mnguni (2019b) and Mnguni and Heshula (2021) was to assess whether or not the presence or absence of chemical compounds was responsible for the interbreeding incompatibilities reported by Taylor et al. (2011) and Paterson et al. (2016), as well as the mating preferences reported by Mnguni (2019a). The behavioral and chemical differences are hypothesized to maintain the presence of prezygotic reproductive isolation mechanisms. Moreover, Henry (2017) postulated that the subtle morphological differences in the MTG, thorax, and antennae of these two species were responsible for differences in their chemical profile compositions. Nevertheless, differences in chemical compound compositions between the two sympatric species require further investigation. In addition, establishing which sex attracts the other in the two sympatric species of *Eccritotarsus* is also warranted, possibly using a Y-tube olfactometer.

Chemicals are key in species recognition as well as maintaining reproductive isolation between species. Although chemicals of the two sympatric species of *Eccritotarsus* are identical, there are subtle differences that could be of evolutionary origin. More importantly, at this stage, the similarities and differences of the roles of major chemical compounds remain unknown. Therefore, assessments of chemical compound concentrations as possible mechanisms that maintain reproductive isolation and speciation were needed in the two sympatric species of *Eccritotarsus*, as previously suggested by several researchers.

Chemicals in the two *Eccritotarsus* can differ by species and age. For example, 2-hexen-1-ol acetate was a major chemical compound in newly emerged adults, while hexadecanoic acid was a major chemical compound in newly emerged *E. eichhorniae* adults. The second major chemical compound for newly emerged adults of both species was hexadecanoic acid. Percentage of composition of these chemical compounds differed by species (Table 1).

In *E. catarinensis* and *E. eichhorniae* adults aged 6 to 8 d old and older, trans-2-hexenyl was the major chemical compound detected. Levels were higher in older adults of *E. eichhorniae* than in their respective counterparts (Table 1). Trans-2-hexenal was a chemical compound with the second highest measure in 6- to 8-d-old adults of both species, and in older adults of *E. catarinensis*. The chemical compound exhibiting the second highest measure was trans-2-hexenol in *E. eichhorniae* adults older than 6 to 8 d old; while trans-2-hexenal was the third highest major chemical compound.

Evidently, there are slight inconsistencies in the chemical compounds of the two sympatric species of *Eccritotarsus*. As argued and demonstrated in Mnguni and Heshula (2021), the slight differences in pheromones in *Eccritotarsus* spp. play a key role in maintaining their interbreeding incompatibilities that were extensively reported in Taylor et al. (2011), Paterson et al. (2016), Ismail and Brooks (2018), Mnguni (2019a,b), and Mnguni and Heshula (2022). The functions of the chemical compound reported here and in Mnguni and Heshula (2021) remain unexplored. Identifying which sex attracts the other in these two sympatric species of *Eccritotarsus* continues to be important going forward. However, notwithstanding that more studies investigating the chemical compounds of the mirids are needed, there is enough evidence to suggest that chemical compounds played a meaningful role in maintaining the reproductive isolation and speciation in the two mirids.

It was also hypothesized that chemical compounds, together with highly specific receptors, possibly had an important role to play in speciation and maintaining reproductive isolation in *Eccritotarsus*. It is worth investigating changes in the functional groups of synthesized chemical compounds, and testing them in the sympatric species of *Eccritotarsus*. In many other mirids, it has been demonstrated that changing functional groups may result in some species reacting differently to a particular chemical compound, or the entire pheromonal blend (Millar et al. 1997). Furthermore, an individual, binary, and multiple-combination assessment of the physiological stimulation played by the chemical compounds reported here and in Mnguni and Heshula (2021) would be very useful. Lastly, assessing whether or not the chemical compounds serve as major or minor sex pheromones also could prove important. The findings would have implications for mate recognition and attraction.

Discussion

In Hemiptera, chemical analyses have been studied in the Reduviidae, Scutelleridae, Pentatomidae, Coreidae, and Miridae. In Miridae, females and males produce, store, and emit chemical compounds that have different quantities, qualities, and concentrations or proportions. In addition, it is common knowledge that insects emit a bouquet of irritating defensive chemicals that have a strong smell

Table 1. The major pheromones from a solid-phase microextraction of *Eccritotarsus* spp. (Hemiptera: Miridae), based on newly emerged adults, 6–8-d-old, and older adults. *Eccritotarsus* spp. mature in 2 d (Ismail and Brooks 2018).

Rank	Pheromone	Retention Time (min)*	Quality**	Area Percent**
E. catarinensis newly emerged adults				
1	2-Hexen-1-ol acetate	11	93	50
2	Hexadecanoic acid	28	99	15
3	Cyclohexasiloxane	11	94	7
E. eichhorniae newly emerged adults				
1	Hexadecanoic acid	28	94	14
2	Hexadecanoic acid	25	25	12
3	Tetradecanoic acid	28	92	7
E. catarinensis 6–8 d old				
1	Trans-2-hexenyl acetate	11	94	54
2	Trans-2-hexenal	9	97	22
3	Trans-2-hexenol	12	91	13
E. eichhorniae 6–8 d old				
1	Trans-2-hexenyl acetate	12	94	78
2	Trans-2-hexenal	9	97	7
3	Trans-2-hexen-1-ol	12	93	6
E. catarinensis older adults				
1	Trans-2-hexenyl acetate	11	94	37
2	Trans-2-hexenal	9	98	17
3	Trans-2-hexen-1-ol	12	90	9
E. eichhorniae older adults				
1	Trans-2-hexenyl acetate	11	93	71
2	Trans-2-hexenol	12	91	7
3	Trans-2-hexenal	9	97	7

* Retention time = time difference calculated from injection to detection.

** Quality = degree of surety of the pheromone.

*** Area Percentage = calculated by dividing the area of the portion by the area of the whole original object.

when they are molested, disturbed, provoked, or attacked. The sexes often have the same chemical compound compositions, but esters and aldehydes that insects emit are postulated to have a dual functionality, which tends to depend on their concentrations or proportions (Aldrich 1988).

Hexyl butyrate has proved to be a sex pheromone in several mirids, such as *Lygu. rugulipennis* and *S. rubrovittatus* (Innocenzi et al. 2001, Yasuda et al. 2008). It is an antisex pheromone (similarly to [E]-2-hexenyl butyrate), and helps males to discourage other conspecific and heterospecific males from copulating with a female in hemipterans such as *Lygu. lineolaris*, *Lygo. pabulinus,* and *Ph. difficilis* (Groot et al. 2001, Zhang et al. 2007, Zhang and Aldrich 2008). It attracts scavengers in several *Lygus* species. (Ho and Millar 2002). Another example of a chemical compound with a dual function is (E)-2-hexenal, which (a) serves as a defensive pheromone in ants, beetles, cockroaches, and true bugs; (b) acts as an alarm pheromone in *Cimex lectularius* L.; and (c) acts as an aggregation or repellent pheromone in *Nezara viridula* L. (Zhang et al. 2014).

Chemical compounds such as hexyl butyrate, (E)-2-hexenal, (E)-2-hexenyl butyrate, and (E)-4-oxo-2-hexenal are consistently reported in more than 10 mirids assessed to date (Yang et al. 2015). For this reason, it has been hypothesized that they exist in other mirids that require assessing. For an unknown reason, mirids with hexyl butyrate lack (E)-2-octenyl butyrate, and this notion should be investigated. The lack of (E)-2-octenyl butyrate is concerning considering that (E)-2-hexenyl butyrate and (E)-4-oxo-2-hexenal successfully attracted males in *A. spinolae* (Yang et al., 2014), while a binary blend of the chemical compounds was inhibitory.

Furthermore, the three most common chemical compounds, hexyl butyrate, (E)-2-hexenyl butyrate, and (E)-4-oxo-2-hexenal, have also been reported in several *Lygus* species, including *Lygus elisus* van Duzee, *Lygu. hesperus*, and *Lygu. lineolaris* (Byers et al. 2013). In addition, butyl butyrate and hexyl butyrate were reported in *Pi. perplexus*, whereas hexyl hexyrate and (E)-2-hexenyl hexyrate were recorded in *B. angulatus* (Knight et al. 1984). In Zhang and Aldrich (2004), (E)-4-oxo-2-hexenal was shown to be a kairomone in predators of several mirid species, despite being a sex attractant in *Lygu. rugulipennis* (Innocenzi et al. 2005). This extensively and unequivocally demonstrates the complexity of chemical compounds in general.

In Yang et al. (2015), a combination of hexyl butyrate, (E)-2-hexenyl butyrate, and (E)-4-oxo-2-hexenal was produced in *A. lucorum*, *A. spinolae*, *Orthops campestris* L., *S. rubrovittatus*, and *T. apicalis*. The primary chemical compound in all species was hexyl butyrate, except for *A. spinolae*, whose primary chemical compound was (E)-2-hexenyl butyrate. In Byers et al. (2013), (E)-4-oxo-2-hexenal is noted as a sex pheromone in all three species (*Lygu. elisus, Lygu. hesperus*, and *Lygu. lineolaris*); while (E)-2-hexenyl butyrate was prominent for *Lygu. elisus* and *Lygu. lineolaris*, and hexyl butyrate was prominent for *Lygu. hesperus*. In agitated insects, hexyl butyrate, (E)-2-hexenyl butyrate, and (E)-4-oxo-2-hexenal were recorded in Byers et al. (2013). For this reason, they were deemed to be alarm pheromones. This further showcased the inconsistencies of chemical communication in insects.

In *Phytocoris relativus* Fallén, hexyl acetate, hexyl butyrate, and octyl acetate were recorded in both sexes (Millar et al. 1997). According to MacLeod et al.

(1975), trans-2-hexenyl acetate is a major chemical compound in *Lethocerus indicus* Lepeletier & Serville; while trans-2-octenyl acetate is reported to be a major chemical compound in *Musgraewia sulciventris* Stål, and trans-2-decenyl acetate is reported to be a major chemical compound in *Biprorulus bibax* Breddin. In *Ph. calli*, major chemical compounds were (E)-2-hexenyl acetate, octyl acetate, and (E)-2-octenyl acetate (Zhang and Aldrich 2008).

To date, 10 out of 16 species have the combination of hexyl butyrate, (E)-2-hexenyl butyrate, and (E)-4-oxo-2-hexenal as sex pheromones. In Miridae, sex pheromones released by females are reported in *Phytocoris californicus* Knight, *Ph. relativus*, *Ph. difficilis*, *Ph. breviusculus*, *Ph. calli*, *Creontiades dilutus* Stål, *Trigonotylus caelestialium* Kirkaldy, and *S. rubrovittatus*. In the previously listed bugs, conspecific males are also reported to be successfully attracted to synthetic lures. The male attraction to conspecific virgin females has also been recorded in several *Lygus* and closely related species such as *Lygocoris communis* Knight, *Lygus desertinus* Knight, *Lygus shulli* Knight, *Lygu. rugulipennis*, *Lygo. pabulinus*, *Lygu. lineolaris*, and *Lygu. hesperus*.

In true bugs (Hemiptera: Heteroptera: Miridae), research has extensively shown that males are generally more responsive to insect-produced pheromones, while females are commonly more responsive to plant-produced kairomones (Groot et al. 1999). Generally, researchers have rigorously demonstrated that traps that have been baited with binary blends catch significantly more males. In such cases, the size and structure of the organs dedicated to process olfactory information in the central nervous system could be responsible for such responses. Furthermore, chemotaxonomy is notable as a field that uses hydrocarbons as a tool to identify insect species. Therefore, more studies investigating chemical profiles of Miridae and other closely related groups of insects are warranted.

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