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First record of parasitoids *Megastigmus* sp. and *Quadrastichus mendeli* as potential biological control agents of eucalyptus gall inducers in Tanzania

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Abstract. *Leptocybe invasa* and *Ophelimus maskelli* (Hymenoptera: Eulophidae) are serious pests causing damage to eucalypt plantations in Tanzania. Biological control is considered the best alternative to other control methods, both ecologically and economically. Thus far, no parasitoid of the eucalyptus gall wasps documented or reported. Likewise, the number of parasitoid species and their parasitic capacities are unknown. Therefore, the study investigated the parasitoids of the eucalypt gall inducer from October 2021 to April 2022. Galls were collected from *E. tereticornis* and *E. grandis x E. camaldulensis* clones and reared until wasps emerged. The emerged wasps were sorted and identified at the Sokoine University of Agriculture, Tanzania, and the Insect Biosystematics Laboratory at IPB University, Indonesia. Two species of parasitoids of eucalyptus gall-inducers in Tanzania were first collected and identified as *Megastigmus* sp. (Torymidae) and *Quadrastichus mendeli* (Eulophidae). In Korogwe, North Ruvu, and Morogoro plantation areas, the percentages of parasitization for the two parasitoids combined were 29.7%, 64%, and 33.3%, respectively. A higher parasitization rate was observed from *L. invasa* galls in the *E. grandis x E. camaldulensis* clones. These parasitoids can be potential biological control agent candidates to control the pest. However, future monitoring is needed to determine their distribution, host specificity, interactions, and efficacy.

1. Introduction

In Tanzania, eucalypts are a notable fast-growing hardwood tree species used to produce wood, pulp for paper, timbers, poles, bioenergy, and other minor goods and services. Tanzania introduced eucalypts in the 1890s, intending to supplement wood supplies from natural forests [31]. Most eucalypt clones planted in East African countries (Tanzania, Kenya, and Uganda) were introduced from Mondi South Africa from 1997 to 2003, where several attacks of gall wasps have been reported [32]. Lately, the government launched a new policy governing the ministry of energy, concerned with the use of electric poles produced within the country besides imports; This decision, in conjunction with an expansion of local wood industries, urbanization, and population increase, has triggered the need for extensive plantation forestry. Despite government's efforts to bring off the demands, growing numbers of invasive insect pests and diseases have threatened the growth and productivity of several tree species, including eucalypts. The possible reason for insect pest outbreak is seen as a result of undermined impacts of climate change and increased international trade and travel in recent years [13], especially in *Eucalypt* species where the rate of invasion of new non-native pests around the world have been common [18]. [48,33] confirmed the presence of several invasive eucalypt insect pests in sub-Saharan Africa, including two species of blue gum chalcids, *Leptocybe invasa* (Fisher & La Salle) and *Ophelimus maskelli* (Ashmead) (Hymenoptera: Eulophidae) collectively known as eucalyptus gall wasps *(EGW).*

Recent invasions and spread of the blue gum chalcids *(BGC)* to several eucalypt nurseries and plantations in Africa, Asia, the Middle East, and the Mediterranean in 2000, South America [26,35,34,21], South Africa in 2007 [20], East Africa-Uganda and Kenya in 2002 and Tanzania in 2005 [33] has been recorded. Unlike *O. maskelli*, which has not been reported in Tanzania, *L. invasa* has been continuously reported as a serious problem by infesting a range of commercially grown eucalyptus species. Some reported hosts for this insect pest are *Eucalyptus camaldulensis, E. tereticornis, E. globulus, E. gunii, E. grandis, E. botryoides, E. saligna, E. robusta, E. bridgesiana, E. viminalis and E. tereticornis* and other eucalyptus germplasms [33]. Observed impacts caused by Australian gall chalcids on several eucalyptus species infested by *L. invasa* form visual galls which appear as typical bump-shaped galls on the petioles, branches, stems, and midribs of new and tenderly meristematic tissues of the leaves and shoots. *O. maskelli* typically generates consistently circular, flat buttons-shaped galls on juvenile leaf blades of several eucalyptus species, which frequently assemble in multiple chambers or extend from the center. Contingent upon the infestation levels, a severe influx could cause leaf abscission, deprived leaves, and photosynthesis inhibition by blocking the vascular bundle's conductivity and resulting in stunted growth, stem quality deformation, and significant economic losses [5,26,27,10,28].

EGW management techniques have included chemical control [19], breeding and selection of resistant planting stock [48], and biological control [21,22,27]. Chemical control is, however, frequently criticized because of its inconsistent effectiveness, detrimental impacts on biodiversity, and environmental contamination. Silvicultural control is generally ad hoc and unlikely to be a feasible long-term response to a growing and diversified spectrum of destructive invasive pests in forestry. Biological control, or "biocontrol," is a technique for minimizing and eradicating the harm caused by pests or weeds by using a biocontrol agent. Agents of biocontrol typically include predators, parasitoids, herbivores, pathogens, phytophagous species, and or microorganisms (like *Bacillus thuringiensis*) [12]. These living organisms collectively called "natural enemies," are especially important for reducing the numbers of pest insects, mites, and weeds. [12] classical biological control displayed as self-sustaining and non-self sustaining. Their tactics as conservation involves the preservation of existing natural enemies by choosing cultural, mechanical, habitat manipulation, or selective chemical controls that do not harm beneficial species. Augmentation applied when native natural enemies are insufficient to control pest populations than can sometimes be increased (augmented) through the purchase and release of commercially available beneficial species, and classical biological control also called importation, is primarily used against exotic pests that have inadvertently been introduced from elsewhere. In addition, due to its ecological and financial advantages, biological control, particularly classical biocontrol, is widely accepted as the best alternative to other control methods, particularly in long-term projects like forestry, focusing on evidence of host specificity and capacity to control the targeted pests [41]. Only a small number of research in east Africa address biocontrol in forestry, namely the *Leucaena psyllid* and *Pine wooly aphids*, in contrast to other fields like agriculture [25].

Despite the fact that several successful stories of classical biological control of eucalyptus gall wasps using parasitoids that have been reported elsewhere, there is no study concerned with eucalyptus gall wasp biocontrol undertaken in Tanzania. [9,34,35] reported that in Israel, Three parasitic species of *O. maskelli, Closterocerus chamaeleon* (Eulophidae), *Stethynium ophelimi* (Mymaridae), and *Stethynium brevioviipositor* (Mymaridae), as well as four parasitic species of *L. invasa*, *Megastigmus zvimendeli, M. lawsoni* (Torymidae), and *Selitrichodes kryceri* were released. Moreover, *Selitrichodes neseri* (Hymenoptera: Eulophidae) is a parasitoid that plays a critical role in controlling the populations of *L. invasa* in epidemic areas in Australia [21,11,20]. However, given mounting evidence of attacks against non-target hosts and the ensuing harm to local biodiversity, only a few nations have adopted this strategy. Several parasitoids associated with the *L. invasa* invasive species have recently been discovered in the invaded areas, including Argentina, South Africa, Israel, Turkey, Italy, Sri Lanka, and Thailand [49].

In east Africa, specifically Tanzania, less has been done to counter the dangers posed by *L. invasa* and *O. maskelli* [32,33]. Research is needed on a variety of pest-related topics to create sustainable management plans, taking into account the pests' quick global expansion and the economic significance of eucalyptus species to both local and national economies in the tropics. Furthermore, no known or reported eucalypt gall wasp parasitoids exist in Tanzania. It is also unknown how many parasitoid species of gall wasps exist in nature and how parasitic they are. The objective of this study was to investigate and identify parasitoids as potential biological control agents for eucalypt gall wasps infesting eucalyptus germplasms in Tanzania.

2. Materials and methods

2.1. Study area, time, and sampling
This study was conducted in the coast agro-ecological zone of Tanzania, from October 2021 to April 2022, in three newly established forest plantations, namely (1) Ruvu forest plantation –altitude 75 meters a.s.l; (2) Korogwe forest plantation- altitude 312 meters a.s.l; and (3) Morogoro forest plantation - altitude 505 meters a.s.l, owned by Tanzania Forestry Services Agency (TFS). The fields of research were chosen because they have been planted with several eucalypt germplasms aged 0-6 years, a vulnerable stage for gall wasps attack. Infestation of gall wasps was previously reported to be higher in low-altitude climates (coastal) than in high-altitude zones i.e., above 1000m. Galls were collected randomly from the systematic plots previously used to eucalypt gall inducer infestation. Two *Eucalypt* species, *E. tereticornis* and the clonal hybrid *E. grandis x E. camaldulensis* (GC 584) were examined visually for the galls on the midribs, petioles, leaf blades, and stems.

2.2. Galls rearing in the laboratory
Galled branches were collected and placed in a plastic container 9cm x 9cm x 11cm filled with water to retain freshness, labeled based on date, name of diagnosed gall inducer, and location. Galls were then transferred to an X-Large Plastic Insect rearing Container 279mm x 159mm x 102 until the emergence of wasps (gall inducers and parasitoids). The emerging wasps were collected daily and placed in 1.5 mL microtubes filled with alcohol 70%.

2.3. Parasitoid identification and descriptions
Based on morphological characteristics, emerged wasps were largely classified and recognized to the genus level at Sokoine University of Agriculture (SUA), Morogoro-Tanzania, in the Entomology laboratory. Some parasitoid specimens were sent to Indonesia, for further identification and descriptions at IPB University Bogor in Insect Biosystematics Laboratory using keys by [7], and Generic keys that allow identification to *Quadrastichus* given by [14], as Cecidotetrastichus; 1991, [24], and [40]. [15] Provides a comprehensive list of synonyms for *Quadrastichus*, and comparisons with other Chalcidoidea descriptions as shown by [21,26,31,11]. Stereomicroscope Olympus SZ51, compound microscope CX21FS1, Leica M205C with digital camera Leica DFC450, and LAS V.4.4.0 application (Build: 454) attached to a computer were used for identification and photography.

2.4. Calculation of the parasitism percentages
The parasitization percentage for each parasitoid species emerged from galls collected in various geographical populations was determined using the formula below:

$$P = (EP/EGP) \times 100\%$$

Where, *(EP)* is the number of emerged parasitoids divided by the sum of the numbers of emerged gall-formers and emerged parasitoids *(EGP)* [21,50,17].

3. Results

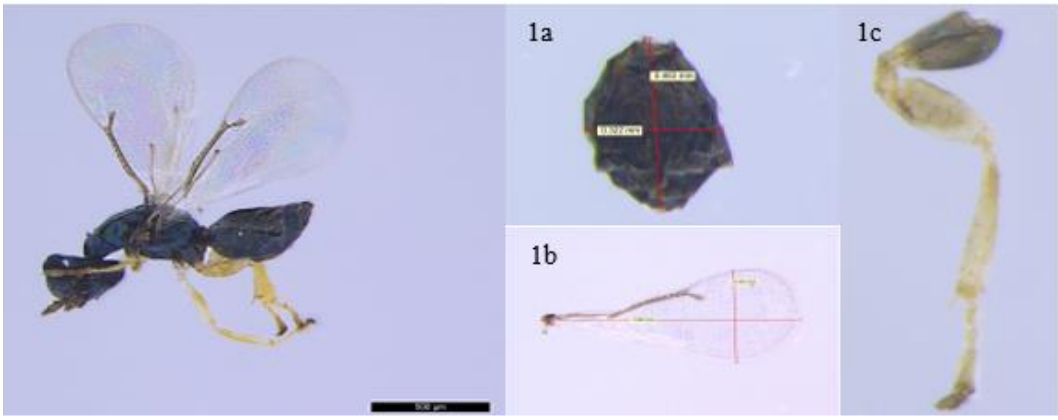
3.1. Eucalyptus gall inducers and parasitoids in Tanzania

From eucalyptus germplasms (*E. tereticornis* & GC584), two eucalyptus-gall-inducers *L. invasa* and *O. maskelli* were observed and two species of parasitoids, namely *Megastigmus* sp. and *Quadrastichus mendeli,* were emerged from the galls and being first recorded in Tanzania. *Megastigmus* sp. emerged from *L. invasa* galls in a clone hybrid of GC584 with a parasitization rate of 29.7% and 35%, in Korogwe and North Ruvu forest plantations, respectively. Likewise, the parasitization rate of *Megastigmus* sp. in *L. invasa* galls from *E. tereticornis* was 21% in Morogoro and 29% in the North Ruvu forest. *Quadrastichus mendeli* was only recorded in *L. invasa* and *O. maskelli* galls from *E. tereticornis* on Morogoro plantation with a parasitization rate of 12.3%. Linearly, the higher the infestation rate, the more parasitoids emerged.

3.2. Descriptions of Eucalyptus gall inducer

The eucalyptus gall wasp *(EGW)* has five developmental stages corresponding to the growth of galls, including three larval instars, a non-feeding pupal stage, and the adult, which emerges by drilling a circular hole in the gall wall just below the leaf's plane [35]. However, not documented between male and female wasps, which emerges first.

Leptocybe invasa (Figure 1a-c) was observed causing severe injury to young foliage in both nurseries and fields of eucalypt plantations, inducing galls mainly on growing shoots of *E.tereticornis* and GC 584. The length of the female wasps ranged from 1.1-1.44 mm with a body and head size larger than the males. Morphological identifications showed that the body length of male ranged from 0.78-1.23 mm; mesosoma brown with metallic shine (blue or dark green); metasoma brown with a slight metallic hint on the dorsal portion. Prothorax is short, well-developed mesothorax; the scutellum is divided into three separated zones by sublateral lines. Wings hyaline with yellow veins completely covered with setae and slight wing venation. Postmarginal vein is shorter than the stigmal vein. Submarginal vein generally with 3–4 dorsal setae. Scutellum with submedian and sublateral lines. A similar observation were done by [45].



Figures 1. Habitus *Leptocybe invasa*; a. Metasoma; b. Wing; and c. Foreleg

Due to its wide host range, polyvoltinism, overlapping generations, concealed lifestyle (galls), and reproductive strategies, *L. invasa* has a very high potential for invasion. From a small number of individuals, it can spread swiftly and proliferate exponentially. Because no male was seen to arise throughout the investigation, *L. invasa* was assumed to have a thelytokous reproduction method. Following [37], who noted that the most common lineage had a female-biased sex ratio, while a biparental lineage also can occur [8]. The ovaries of females, on average, contain 160 eggs/female and lay eggs immediately as they emerge, however, the number of eggs and oviposition collates with temperature [39]. However, a rise in temperature from 20 to 29°C is directly correlated with an increase in the number of eggs a female produces. Moreover, the pest was estimated to have two overlapping generations per year. Oviposition is mostly occurs near the rainy season. However, other studies indicated that in the majority of the nations where it is found, *L. invasa* produces two or three overlapping generations annually, however this is dependent on the ecological circumstances [26]. On average, it takes 138 days for *EGW* to develop from an egg to an adult in the wild [16]. Various studies have found that the typical development time in the greenhouse varies greatly: 132 days [26], 60 days [22], or 46 days [39], with varied duration in each development stage.

The largest group of gall-inducer wasps in the family Eulophidae and several genera of Tetrastichinae is composed of *Ophelimus maskelli* (Figure 2) [23]. Just one seta on the submarginal vein sets *O. maskelli* apart from its congeners. *Ophelimus* species were observed in all study sites, mostly on *E. tereticornis*. The galls ranged in size from tiny blister gall, with a single individual inside and some were symmetric and spreading evenly on both sides of the leaf blade, soft to the touch, and green or a green-reddish, depending on the age of the gall. It was challenging to identify the gall inducer based solely on gall morphology, though [21,2]. [10] estimates that females of 0.83 to 1.07 mm in length lay about 100 eggs.

Figures 2. Habitus *Ophelimus maskelli* (BiCEP, 2022)

3.3. Parasitoids descriptions

The first parasitoid that emerged was *Megastigmus* sp. (Figure 3a-e). Under the microscope, important features to identify adult *Megastigmus* sp. were the body color, which has dominant yellow color with brown spots and has long ovipositor almost the same size as the length of the body. The adult also had a dark brown stigma, yellow veins, and a wing with hyaline dots. The face is smooth, and there are fine longitudinal striae on the head. Antennas had transverse flagellum segments that were clavate and the same length. *Megastigmus* Dalman 1820 (Hymenoptera: Chalcidoidea: Torymidae) was first described as subgenus Torymus. [7], singled out megastigmus in the Subfamily Megastigminae and presented the diagnostic characteristics of the genus, saying that the genus has roughly 98 species spread globally. From the investigated areas, Gall inducers, *L. invasa*, and *Ophelimus sp*. gave rise to *Megastigmus* sp.

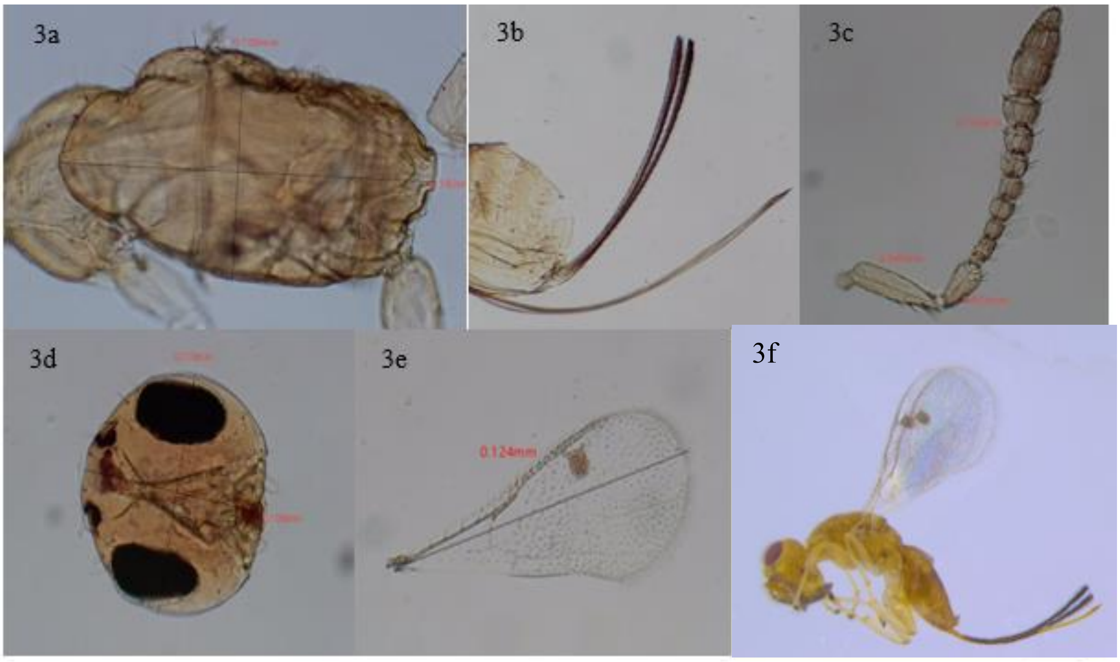


Figure 3. Habitus *Megastigmus* sp; a. Scutellum; b. Metasoma with ovipositor; c. Antennae; d. Head; e. Doted stigmal wing; f. adult of *Megastigmus* sp.

Quadrastichus mendeli Kim & La salle (Hymenoptera: Eulophidae), an Australian-native parasitoid, was the second parasitoid to emerge (Figure 4). This parasitoid was recognized using morphological traits mentioned in [21]. Small bodies measuring 1.15–1.35 mm in length were among the specific traits seen. Antennae have a big anellus, three funicular segments that are longer than they are wide, and are light brown (or testaceous). The body is predominantly yellow with dark brown patterns, and the gaster is longer than the head and mesosoma combined. When viewed from the dorsal side, the pronotum measures around 0.3 times the length of the mid-lobe of the mesoscutum. Submarginal vein on the fore wing with 1 seta, slightly basal to the center. Absence of a hyaline break in the stigmal vein and parastigma. A setae-free costal cell. The postmarginal vein is elementary. Many species of *Quadrastichus* are associated with galls that emerged from both *L. invasa* and *O. maskelli* galls only in *E. tereticornis* planted on Morogoro plantation.

Figure 4. Habitus adult parasitoid *Quadrastichus mendeli*

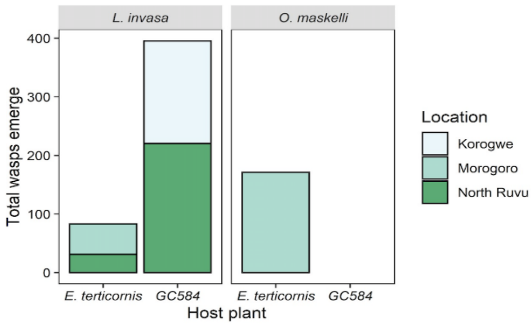


Figure 5. Total number of wasps that emerged in each location

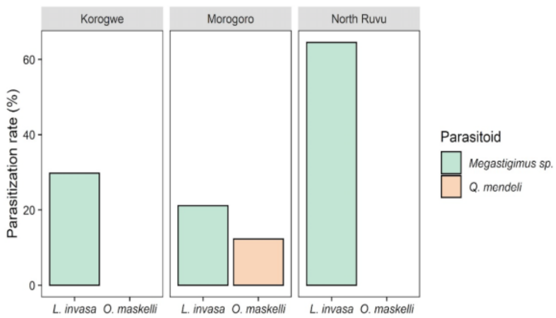


Figure 6. The Overall parasitization percentages of parasitoids

4. Discussion

This study provides a possible recital on how the novel eucalyptus gall wasp pests and their parasitoids were introduced to Tanzania and descriptions of two *Eucalyptus* spp. as hosts for *L. invasa* and *O. maskelli* and two potential parasitoids for biological control. Despite the gall wasps, *L. invasa* and *O. maskelli* (Hymenoptera Eulophidae) have become established in the Mediterranean Basin during the last 15 years causing serious damage in eucalypt plantations on large areas. Biological control is the only sustainable approach set forward to control these pests. In Australia, the six parasitoids of both galleries seem restricted to galls in *Eucalypts*. The parasitoids, of the Eulophidae, Torymidae, and Mymaridae, were released in Israel. Most of gall-inducing wasps in Australia, that attack *Eucalypt* species, are members of the family Eulophidae, which also contains inquilines and parasitoids of gall-inducing wasps [25]. About 100 Chalcidoidea species have been connected to eucalypt galls thus far [31]. However, *L. invasa* and *O. maskelli,* are the most prevalent and inflict the most significant harm globally. Several writers [26,35,50,33] have provided detailed descriptions of the severe harm produced by *L. invasa* and *O. maskelli*. [37], reported the presence of *Q. mendeli* in South Africa parasitizing *L. invasa*. Therefore, it is possible to obtain similar parasitoids in Tanzania. During this survey, a higher infestation was observed in *Eucalyptus tereticornis* and clone hybrids of GC584 planted in Morogoro and North Ruvu plantations. The high infestation was found at North Ruvu Plantation, with low altitude and moderately high temperature compared to other study sites. Consistently with [26,33], who noted that the ideal temperature is essential for the development and proliferation of new leaves in eucalypts and simultaneously enhancing metabolic activities within the wasp body. This information supports the findings of various researchers who have shown that various *Eucalyptus* species and their hybrid clones provide appropriate hosts for gall-inducing wasps [26,44].

Q. mendeli emerged from both *L. invasa* and *O. maskelli* galls, solitary ectoparasites that mature outside of the host body. The behavior and biology of *Q. mendeli* showed it is uniparental in which males are unknown, and its reproductive mode is thylotoky, which demonstrates that its population can expand quicker than a sexual one, with a developing duration of about 30 days at 28.1 °C [21], but faster in 25 °C [34]. The average developmental time of *Q. mendeli*, from egg to adult stage, was 27.06 ± 1.19 days [37]. According to [21,27], demonstrated that *Q. mendeli* parasitizes *L. invasa* larva and increases the probability that *Q. mendeli* could also develop on other gall wasp species. Indeed, it is unknown whether the larvae of the host wasp are killed immediately or shortly after parasitization [21]. The parasitization rate of *Q. mendeli* was 12% in Morogoro, similar to Mexico records (3-10.9 %) [1], and China (2.96%–19.53%) [50]. But lower than the results from field monitoring in Italy which shown a parasitism rate of 30.2 to 50.5% [29], Argentina 4.3 to 79.8% [3] in Argentina. Distinctively laboratory studies in Israel have shown a parasitism rate between 7.9% to 84%, [21], 58.6-79.3% [43], 81.7 to 94.0%, [42]. According to [29], parasitization percentages of *Q. mendeli* showed a short life cycle compared with that of *L. invasa*, therefore, this thought to offer potential benefit if used as a biological control agent, despite the fact that, parasitism depends on the life stage attacked, biotic and abiotic factors [37]. Moreover, no parasitoids emerged from *O. maskelli* galls; the same situation was also observed by [2]. A possible reason could be an inappropriate climatic condition for reproduction and parasitoid development. Overall, the findings are consistent with the theory that, the lack of parasitism in *Ophelimus* sp. maybe attributed by seasonal mismatch between the phenology of the parasitoids and that of the gall wasps. Therefore, if a parasitoid and its potential host are not active simultaneously or in the same season, they may exist in the same region but not interact. Finally, based on host-parasitoid systems, parasitism may escape, whether totally or partially [47].

Megastigmus are effectively regulating a larva of *L. invasa* because they are biparental, laying a single egg inside the gall with larva or pupa and developing as a solitary ectoparasitoid. However, [34] revealed that some species are ineffective in suppressing gall inducers. According to some experts, the parasitoids *Eurytoma sp.* and *Megastigmus* sp. naturally control the species of *Ophelimus*. [11] examined the Australian *Megastigmus* species related to *Eucalypt* species and discovered that 30 out of 35 analyzed species were connected to galls on different plant sections. *Megastigmus* sp. showed a high parasitization rate in all three-study locations. However highest parasitization was recorded in the clone hybrid of GC 584, with percentages of 35% in North Ruvu, 29.7% in Korogwe from *L. invasa* galls 21% and 29% in *L. invasa* galls from *E. tereticornis* planted in Morogoro and North Ruvu plantation. The parasitization rate of *Megastigmus* sp. was higher compared to *Megastigmus viginii* 24.93% by [50] in China, lower than that reported in Indonesia at 42.85% [5], and [46] in Sri Lanka, reported Megastigmus to be the most abundant and mean percent parasitization of 67.00 ± 8.00 in Sri Lanka. *Megastigmus* sp. can control gall inducers *L. invasa* and *O. maskelli*. The existence of a suitable host is a significant factor in determining the success of parasitism. Additional elements, including the size of the host and rivalry between parasitoids, can also impact on the parasitism's effectiveness. However, identification of megastigmus based on morphological characteristics is problematic e.g. assigning these species to the Palaearctic parasitic due to changes in body pigmentation [36]. [50], Reported that emphasis is given to the Torymidae genus megastigmus Dalman, distributed globally, and associated with all current known invasive *Leptocybe* spp. The knowledge of the global movement of associates of *Leptocybe* spp. is expected to provide a model for understanding parasitoid - host and host plant relationships.

The ideal way to deal with the damages posed by *L. invasa* and *O. maskelli* is through developing several biocontrol tactics such as conservation, argumentation and classical biological management, as recommended by [27], which is strongly backed by the fact that both gallers are fairly rare in their native location. The parasitization of the parasitoid showed linearity with the rate of gall wasp infestations. Perhaps all wasps belong to the same order and family and have the same development stages. Regarding the rate of parasitization, Tanzania can exploit the potential of naturally available parasitoids before planning for mass rearing through the use of sustainable control strategies, i.e, reducing the of pesticides which have proven to impact both parasitoids and pests.

5. Conclusion

Parasitoids species*, Megastigmus sp*. and *Quadrastichus mendeli,* were first recorded in Tanzania and can be used as potential candidates for fortuitous biological control for gall inducers, specifically chalcid wasps, *Leptocybe invasa* and *Ophelimus maskelli*. However, *Megastigmus* sp. showed the highest percentages of parasitization than *Q, mendeli* in all three study locations. Further research is required for monitoring throughout infested areas, and to ascertain the parasitoid's prevalence, distribution, host species range, host specificity, and efficacy in reducing the pest population in Tanzania.. Moreover, biology and molecular identifications remain puzzling.

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