

**GENETIC DIVERSITY AND ECOLOGY OF LEPIDOPTERAN
STEMBORERS IN RWANDA**

PhD DISSERTATION

NTIRENGANYA ELIE

HARAMAYA UNIVERSITY, HARAMAYA

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Genetic Diversity and Ecology of Lepidopteran StemBorers in Rwanda

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Ntirenganya Elie

Haramaya University, Ethiopia

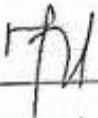
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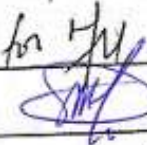
DIRECTORATE FOR POSTGRADUATE PROGRAM

As thesis research advisor, we hereby certify that we have read and evaluated this Thesis prepared, under our guidance, by: Ntirenganya Elie entitled: 'Genetic Diversity and Ecology of Lepidopteran Stemborers in Rwanda'. We recommend that it be submitted as fulfilling the Thesis requirement.

Dr. Muluken Gofishu
Major Advisor



Prof. Yoseph Assefa



Prof. Nsengimana Venuste



Dr. Sokame, Bonoukpoe Mawuko
Co- Advisor

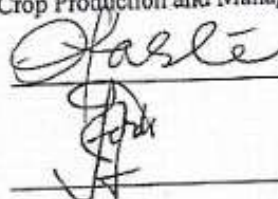
Signature

16 June 2026

Date

As members of the Board of Examiners of the PhD. Dissertation Open Defense Examination, we certify that we have read and evaluated the PhD thesis prepared by: Ntirenganya Elie and examined the candidate. We recommend that the Thesis is accepted as fulfilling the Thesis requirement for doctor of Philosophy (PhD) in Climate Smart Agriculture and Biodiversity Management (Climate Smart Crop Production and Management).

Dr. Habtamu Terefe
Chairperson



09/06/26

Dr. Ferdu Azerefegn
External Examiner



9 June 2026

Prof. Emana Getu
Internal Examiner



9 June 2026

Date

DEDICATION

This work is lovingly dedicated to my beloved wife, **Mrs Uwera Joyce**, whose unwavering support, patience, and encouragement have been my anchor throughout this journey.

To my dear mother and her family relatives, whose sacrifices and prayers laid the foundation of my education and character.

To my brothers and sisters, whose constant belief in me strengthened my resolve.

And to all my friends, who supported and stood beside me in moments of challenge and triumph.

BIOGRAPHICAL SKETCH

Ntirenganya Elie is a Rwandan researcher specializing in agricultural entomology, biodiversity conservation, and climate-smart agriculture. He is completing his PhD at the Africa Center of Excellence for Climate Smart Agriculture and Biodiversity Conservation (ACE Climate SABC), Haramaya University (2022–2026), where his research focuses on the diversity, ecology, and wild host interactions of Lepidopteran stemborers across East African agro-ecological zones.

He holds an MSc in Agricultural Entomology and Pest Management from Yunnan Agricultural University, China (2021), and a BSc in Applied Biology from the University of Rwanda (2015). Elie has served in multiple technical and research roles, including Research Fellow at the Center of Excellence in Biodiversity and Natural Resources Management (CoEB), University of Rwanda; Research Fellow at the World Vegetable Center in Arusha, Tanzania; and Technical Expert in conservation agriculture and landscape restoration under the EU-supported KUNGAHARA Project in Rwanda.

His professional work spans biodiversity conservation, IPM, climate change adaptation, sustainable agriculture, carbon markets, environmental policy, and green economy frameworks. He has contributed to national processes such as the drafting and revision of Rwanda's National Biodiversity Strategy and Action Plan (NBSAP 2025–2030) and the development of the country's Green Taxonomy. He is co-founder of Rwanda Biosolutions Organization, promoting sustainable waste management and organic agriculture.

Elie has authored and co-authored several peer-reviewed publications in international journals and has presented his research in regional and global scientific forums. His work has been recognized through multiple awards, including the DOCTAS Award for Young African Researchers (2024), the ATBC Travel Grant (2024), and the PASET-RSIF Scholarship (2022-2026). He is an active member of several scientific bodies, including the African Association of Insect Scientists and the COLOSS Honey Bee Research Association, where he serves as Rwanda's National Coordinator.

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ACRONYMES

AMOVA	Analysis of Molecular Variance
AMW	Afroalpine Mountain & Wetland mosaics
ARCOS	Albertine Rift Conservation Society
ASW	Akagera Subhumid Savanna and Wetland mosaics
BIC	Bayesian Information Criterion
CoEB	Center of Excellence in Biodiversity and Natural Resource Management
COI	Cytochrome Oxidase subunit I
CST	College of Science and Technology
ICIPE	The International Centre of Insect Physiology and Ecology
IPM	Integrated Pest Management
MEGA	Molecular Evolutionary Genetics Analysis
MFW	Montane Forests and Wetland mosaics
MINAGRI	Ministry of Agriculture
ML	Maximum Likelihood
MP	Maximum Parsimony
NISR	National Institute of Statistics of Rwanda
NJ	Neighbor Joining

NMDS	Non-metric Multidimensional Scaling
PASET-RSIF	Partnership for Applied Skills in Sciences, Engineering and Technology-Regional Scholarship and Innovation Fund
PERMANOVA	Permutation Multivariate Analysis of Variance
PERMDISP	Permutational Multivariate Analysis of Dispersion
RAB	Rwanda Agriculture and Animal Resources Board
RBIS	Rwanda Biodiversity Information System
REMA	Rwanda Environmental Management Authority
RICA	Rwanda Institute for Conservation Agriculture
SANBI	South African National Biodiversity Institute
UR	University Rwanda
WR	Wetland & Riparian mosaics

STATEMENT OF THE AUTHOR

By my signature below, I declare and affirm that this dissertation is my own work. I have followed all ethical and technical principles of scholarship in the preparation, data collection, data analysis and compilation of this dissertation. Any scholarly matter that is included in the dissertation has been given recognition through citation.

This dissertation is submitted to Haramaya University in partial fulfillment of the requirements for doctoral degree. The dissertation is deposited at the library of Haramaya University and made available to borrowers under the rules of the library. I solemnly declare that this dissertation has not been submitted to any other institution anywhere for the award of any academic degree, diploma or certificate.

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Name: **Ntirenganya Elie**

Date: 04/06/2026

School/Department: Africa Center of Excellence for Climate Smart Agriculture and Biodiversity Management

Signature: 

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LIST OF PUBLISHED OR SUBMITTED MANUSCRIPTS

This thesis/dissertation is based on the work contained in the following published papers, referred to by Roman numerals in the text:

- i. Ntirenganya E, Nsengimana V, Sokame BM, Boni SB, Assefa Y, Uwayezu J, and Goftishu M (2026). Assessing interactions between wild host plants and stemborer species in East Africa. *Journal of Tropical Ecology*. 42(e5), 1–11.
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- ii. Ntirenganya, E., Goftishu, M., Sokame, B.M. *et al.* Pest status and dynamic of sugarcane stemborer (*Eldana saccharina*) in sugarcane plantations of Nyabugogo tropical Wetland, Rwanda. *International Journal of Tropical Insect Science* (2025).
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- iii. Elie Ntirenganya*, Muluken Goftishu*, Yoseph Assefa, Bonoukpoè Mawuko Sokame, Simon Boniface Boni, Janvier Uwayezu, Hategekimana Athanas, Venuste Nsengimana (submitted on 2026) Diversity and species richness of lepidopteran stemborers and their host plants in selected vegetation mosaics of East Africa, *Journal of Evolution and Ecology*, under review.
- iv. Ntirenganya E, Nsengimana V, Sokame BM, Boni SB, Assefa Y, Uwayezu J, and Goftishu M. Genetic variation and evolutionary relationships among *Sesamia nonagrioides* lineages in ethiopian and rwandese agroecologies (Manuscript for submission)
- v. Ntirenganya E, Nsengimana V, Sokame BM, Assefa Y, Uwayezu J, and Goftishu M. Genetic diversity and phylogenetic relationships of *Eldana saccharina* Walker (Lepidoptera: Pyralidae) from different agroecological zones of Ethiopia and Rwanda (Manuscript for submission)

GENERAL ABSTRACT

This thesis examines ecology, host interactions, genetic diversity, and evolutionary patterns of Lepidopteran stemborers in the agroecological landscapes of Rwanda. It emphasizes on their impacts on cereal and sugarcane stemborers and wild species, their ecological dependence on wild host vegetation, and their responses to climate and landscape changes. Recent studies reported that stemborers have started to shift from their natural habitats and attacking new host plants that were not used to be hosts before. The research was motivated by high speed increasing landuse change by agriculture intensification and urbanization in changing climate that modify natural habitats of stemborers. In addition to this curiosity, less information on stemborer population in Rwanda is available and do not clearly show how environmental gradients, vegetation mosaics, and these changes reshape stemborers community structure and genetic composition.

The field-based ecological results revealed a substantial species richness, host-plant diversity, and habitat-driven distribution patterns. Surveys yielded 18 stemborer genera recorded from 56 host plant species. The most interesting points is that the highest abundance and diversity of stemborers were concentrated in urban and peri-urban wetlands of Kigali City, highlighting the ecological role of human-modified habitats. Poaceae hosts accounted for over 60% of all larvae collected, and common species exchangeable from wild to crop vis versa for survival, where Napier grass (*Pennisetum purpureum*) served as the primary refugia during dry.

The specific survey conducted in sugarcane plantation shows that sugarcane support mass population of *Eldana Saccharina* Walker, 1865. The population vary by season and first population start in May, a month of transition between heavy rain season (March-early May) to dry late May to August. The population reached peak in August. The infestation severity varied across sugarcane varieties with the most affected was green and red sugarcane varieties that white-powdery sugar industrial variety. Sanitation practices of old-leave removal in sugarcane plantation have shown a critical effect in reducing population dynamics of sugarcane stemborers, suggesting this practice as pesticides-free method to control stemborers in sugarcane plantation system.

For molecular ecology of key stemborer species through genetic analyses of *Sesamia nonagrioides* (Lefèbvre, 1827) and *E. saccharina*, Ethiopian population as earliest studied populations in Africa

were used as model to understand Rwandan population's genetic diversity and diververgence. Mitochondrial COI sequencing showed high genetic diversity in selected model species that were associated with wetland vegetations and these from maize and sugarcane. Among these species, wetland associated species and these associated with crops were standing alone as connected sisters that share common ancestor but evolved differently in different time. There were no shared haplotypes between population of Rwanda and model population from Ethiopia. The results suggest that over time, landscape history, wetland degradation, climate gradients, and agricultural expansion are shaping contemporary genetic differentiation, and concludes that region-specific biological control strategies, improved surveillance, and cross-habitat management are essential, as genetically distinct populations may respond differently to control measures.

Observed variations in the distribution of pest and non-pest stemborer species, together with differences in genetic structure among key model species, indicate that no single management strategy is suitable across landscapes and that continued habitat fragmentation will likely destabilize ecosystems, leading to host range expansion or local extinctions. These patterns reflect broader environmental change, where habitat fragmentation at the local scale reduces connectivity and gene flow, while climate change at regional and global scales drives shifts in species distribution, community reorganization, and biodiversity loss. Together, these processes highlight the need for multi-scale, integrative research to understand stemborers species responses to these combined effects and to support the development of context-specific, climate-smart integrated pest management strategies for sustainable agriculture.

1. GENERAL INTRODUCTION

1.1. Background and justification

Stemborers are insects that tunnel inside the plant stem and feed on the xylem and phloem tissues of the plant stem during the larval stage (Overholt et al., 2001). Typically, the massive newly hatched larvae begin by feeding on leaves before shifting to enter the stem, leaving visible holes or feeding marks on the foliage. The feeding damage results in dead shoot (dead heart) and stunted plant growth due to the disruption of plant nutrients transport (Bowden, 1976; Assefa, 2006; Rodríguez et al., 2021). The main stemborers are classified in Lepidoptera (Overholt et al., 2001) and Coleoptera insect orders particularly species from Rhynchophoridae as described in Ethiopia (Getu, E. et al., 2001).

While Coleoptera stemborers less common in crops (Getu, E. et al., 2001), Lepidoptera stemborers are the most diverse and deadly in cereal crops (Overholt et al., 2001). Most of these lepidopteran stemborers feed on cereal crops such as maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L.) (Getu, E. et al., 2001), pearl millet (*Pennisetum glaucum* L.), sugarcane (*Saccharum officinarum* L.), and many other related crops (Overholt et al., 2001). In Ethiopia, stemborers were reported to cause These pests can cause significant yield losses estimated from 20% to 50% yield loss in maize and sorghum (Emana G. & Tsedeke A. 1999). The damage may reach 80% or beyond in late-planted crops (Assefa et al., 2008; Calatayud et al., 2008; Goftishu et al., 2017). To date, more than 21 species of lepidopteran stemborers have been reported as economically important pests of cultivated crops (Maes, 1998; Kfir et al., 2002; Sokame et al., 2019). These species are mainly dominated by Noctuidae (*Busseola* and *Sesamia*), and the Crambidae (*Chilo* and *Scirpophaga*) families (Getu, E. et al., 2001; Overholt et al., 2001) and Pyralidae (*Eldana*) (Assefa et al., 2006b).

Studies conducted in Ethiopia have revealed that *Busseola fusca* (Fuller 1901) (Noctuidae), *Sesamia calamistis* (Hampson) (Noctuidae) and *Chilo partellus* (Swinhoe) (Crambidae) are the most common pests in cultivated crop (Getu, E. et al., 2001; Assefa, 2006; Assefa et al., 2010; Melaku et al., 2006; Asmare et al., 2014; Goftishu et al., 2018). *Eldana saccharina* (Walker) (Pyralidae) was less reported in cereal crop and highly associated with sugarcane plantation since species is exclusively reported in sugarcane plantation (Overholt et al., 2001; Assefa, 2008).

Initially, these stemborers species co-existed with farming fields and were not playing any economic importance (Khan et al., 1997, 2007; Le Rü et al., 2006). Previous studies in natural habitats of Ethiopia (Hofsvang, 2002; Yewhalaw D. et al, 2008; Goftishu et al., 2018; Haile & Kebede et al., 2018), Kenya (Overholt et al., 2001; Le Rü et al., 2006; Otieno et al., 2008), Tanzania (Ong'amo et al., 2018; Mutamiswa et al., 2022), Mozambique and South Africa (Moolman et al., 2013; Moolman et al., 2014; Zhou, 2016) have revealed change in species composition, diversity and host range by indicating a high diversity of stemborers in natural habitats, with descriptions of several new stemborer species. However, these studies claims that more of these species are shared from wild vegetation to cultivated crops.

Climatic conditions have seen to control the distribution of stemborers species, abundance and pest status in Africa (Ndemah et al., 2007; Calatayud et al., 2014; Wan et al., 2017; Goftishu et al., 2018; Mutamiswa et al., 2022; Morshed et al., 2023). This may due to facts that temperature and rainfall patterns significantly influence vegetation distribution (Weiskopf et al., 2020; Gutiérrez-Hernández & García, 2021), particularly Cyperaceae and related species that are adapted to wetter ecosystems (Sousa et al., 2025). Thus, may affect the spatial and temporal abundance and distributions of stemborers (Harrington et al., 2001; Jasrotia et al., 2019; Morshed et al., 2023; Régnier et al., 2023; Sokame et al., 2020). The consequences of these parameters include the expansion of host plant, phenological changes, shortening lifecycle, increase in the yearly generations, (Kanya et al., 2004; Mwalusepo et al., 2015; Sokame et al., 2019, 2020), and migration or shifting to new ecosystems (Getu et al., 2001). Further, human settlement and agricultural intensification can modify the natural habitats which affects spatio-temporal patterns of stemborers (Le Rü et al., 2006; Gounou et al., 2009; Mutamiswa et al., 2022).

Rwanda is a smallest country of East Africa with total size of 26388 km² (SANBI et al., 2022), and characterized by a diversity of agroecological regions depending on elevation, temperature, humidity, and rainfall gradients (Lillesø et al., 2024; MINAGRI, 2021; SANBI et al., 2022). The country has eight agroecological zones namely Kivu Lake shore, Impala, Imbo, Congo Nile watershed, Eastern Lowland, Buberuka highlands, Volcano chain and high plains (Mukuralinda et al., 2016). These agro-ecological zones are distributed following the elevation gradient ranging between 900 to 4500 m.a.s.l. from South to East, less than 1500 m in the East, between 1500 and

2000 m.a.s.l. in the Central Plateau and to more than 2500 m in the northern highlands (Mukuralinda et al., 2016; MINAGRI, 2021; Ruticumugambi et al., 2024).

Considering these diversity and richness in ecological parameters of Rwanda, there is a limited information about aspects of stemborer species, their host plants, diversity, abundance, population dynamics, spatial distribution, genetic diversity, and their response to pressing climate change and habitats fragmentation. Yet, such kind of information is key for forecasting future trends in stemborers' long-term integrated pest management (IPM) strategies. This aims to assess the diversity, ecology, host ranges, and phylogenetics of cereal stemborers across various Ethiopian and Rwandan agro-ecologies. Besides, using selected Ethiopian stemborers population, genetic diversity and phytogeography of stemborer species will be assessed to elucidate their divergence, evolution, and host plants relationship.

1.2. Research questions

- i. Does the stemborer species diversity vary among selected vegetation mosaics Rwanda?
- ii. Does the diversity of stemborer host plants differ significantly among Rwanda's various agro-ecologies?
- iii. Does the diversity and relative importance of stemborer pests in cultivated habitats vary among different agro-climatic conditions of Rwanda?
- iv. Does the genetic diversity of the dominant stemborer pests vary among different agro-ecologies of Rwanda by using Ethiopian population as model?

1.3. Objectives

1.3.1. General Objective

To assess the diversity, ecology, host ranges, and phylogenetics of cereal stemborers across various Rwandan agro-ecologies.

1.3.2. Specific objectives

- i. To assess the species composition, diversity, and richness of stemborers across selected vegetation mosaics in Rwanda.
- ii. To identify and document wild host plants harboring stemborers across diverse agro-ecological zones in Rwanda.
- iii. To assess the species diversity and relative distribution of stemborer pests in wild habitats and cultivated plants among different agro-climatic conditions of Rwanda.
- iv. To evaluate genetic diversity and phylogenetic relationships of key stemborer species from different agro-ecological zones of Rwanda by using Ethiopian population as model.

2. LITERATURE REVIEW

2.1. Stemborers biology

In Africa, cereal crops and sugarcane generate millions of income that contribute significantly to local, national, and regional economies. Cereal crops such as maize (*Zea mays* L.), sorghum (*Sorghum bicolor* L.) and pearl millet (*Pennisetum glaucum* L.) are essential food sources for humans and livestock in East Africa (Getu et al., 2001; World Data Atlas, 2025), while sugarcane (*Saccharum* spp.) is cultivated exclusively as a cash crop (Dinesh Babu et al., 2022). In Rwanda, 29,510 tons of maize were produced in 2024, while sugarcane production rose significantly from 1973 to 2022, reaching 97.8 thousand tons of production from 8,019 ha (World Data Atlas, 2025). The sugarcane supply-chain created employment for over 10,000 individuals in monocrop plantations in Rwanda (Veldman & Lankhorst, 2011). Amongst many factors limiting cereal crops and sugarcane production, insect pests are considered the most critical biotic factor. Some indigenous stemborer species considered economically important pest coevolved with wild host plants in natural vegetation and expanded diet-breadth to include cultivated crops (Ong'amo, 2009). Lepidopteran stemborer species are the most economically important pests of these crops.

The primary damage by stemborers is marked by the disruption of the host plant's vascular system due to the larval feeding by producing the tunnels within the stem, which interrupt or stop the flow of water and nutrients in the upper parts of the affected plant (Getu et al., 2001; Overholt et al., 2001). This results in:

Wilting and death of plant parts: Above-ground or above the attached plant parts may wilt or die due to the cut of or interruption of plant nutrient and water movement mechanisms;

Stunted growth: Stop or reduced nutrient and water movement in plant could results in stunted plant growth and crop yield reduction;

Lodging: Due to the entrance wholes and feeding channels, this creates the weakened stems which lead to the displacement of stem and roots of infested plants from their proper and vertical placement particularly in crops like cereals;

Entry points for diseases: After the larval channeling and entry, the entry hole remain open and become the exist of larval frass, which is a potential and rich medium for microbial and fungal diseases infestation to the crop (Kfir et al., 2002a; Overholt et al., 2001). All these factors are combined with environmental stressors to reduce the resistance of infested plant leading to crop vulnerable yield loss (Rodríguez et al., 2021; Sokame et al., 2020; Terblanche & Hoffmann, 2020). Details on attack and infestation symptoms are given in figure 2.1.

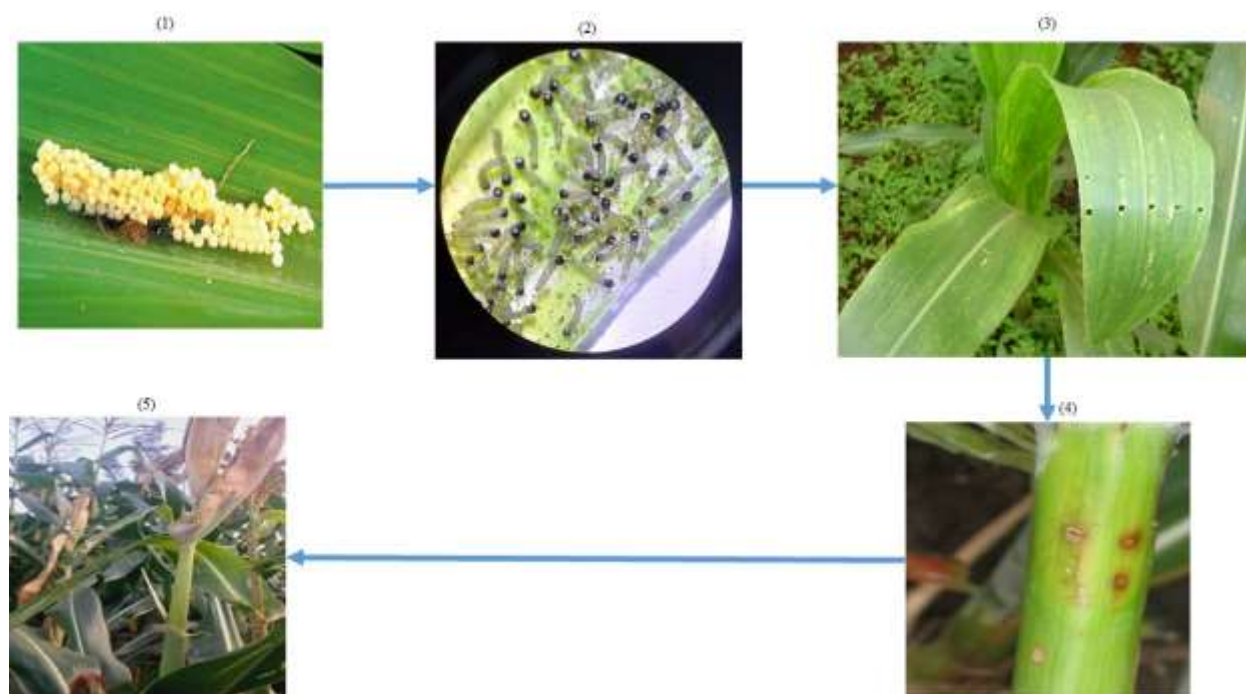


Figure 2. 1. Steps of stemborers attack on plant: (1) hundreds of eggs in single or multiple colonies are laid on suitable plant, (2) hundreds of faster running larvae emerged from the eggs and spread over the plant, (3) larvae systematically feed on young leaves or other soft parts of the plant, (4) as larvae grow, they tunnel on plant stem to feed inside, (5) plant start to get wilt or die. Source: (1) D. Cugala, Stemborer team, ICIPE; 2-5 own photos taken during the stemborers survey 2023-2024, Rwanda

Lifecycle: Like other insects, males and females' adults mate and reproduce sexually. Their reproduction is considered as Complete metamorphosis (holometabolous development) evolved from Endopterygota in super-ordinal clade of insects in infraclass of Neoptera that pass through the clearly distinctive developmental stages of eggs, larvae, pupae, and adults (Overholt et al.,

2001; Kfir et al., 2002; Nel et al., 2013;). In 2-5 days, a female oviposit in batches of 10-100 overlapping eggs, on the upper and undersides of leaves, mainly near the midribs. Some eggs are also laid on the stem. Adults live for about 2-10 days and do not normally disperse far the emergence sites and never cause any harm. In normal conditions, eggs hatch in the early morning (06:00-08:00 h) after 4-8 days of oviposition, and young larvae dispatch faster on plants to enter the leaf whorls, where they start to feed. Older larvae tunnel into stem tissue, and after feeding for 2-3 weeks, they turn to inactive form and pupate for 5-20 days. Under favorable conditions, the life cycle takes 25-50 days to complete (Fig. 2.2). Some species may have five generations in a single season during the normal conditions. In other side, when the conditions are not favorable, cold, dry, and foods scarcity conditions, larvae may enter an inactive form, resting stage of diapause in soil, stems, stubble and other crop residues, where they can survive up to 6 months before pupating when favorable conditions return during the next growing season (Yitaferu K. & Gebre Amlak A., 1994; Kruger, 2012; Tekle, 2013). However, when stemborer has host alternative nearby, the larvae may remain active in these wild hosts during harsh conditions and return to their favorite hosts during the favorable growing season (Unnithan & Reddy, 1989; Overholt et al., 2001; Xiao et al., 2010; Tanwar et al., 2024).

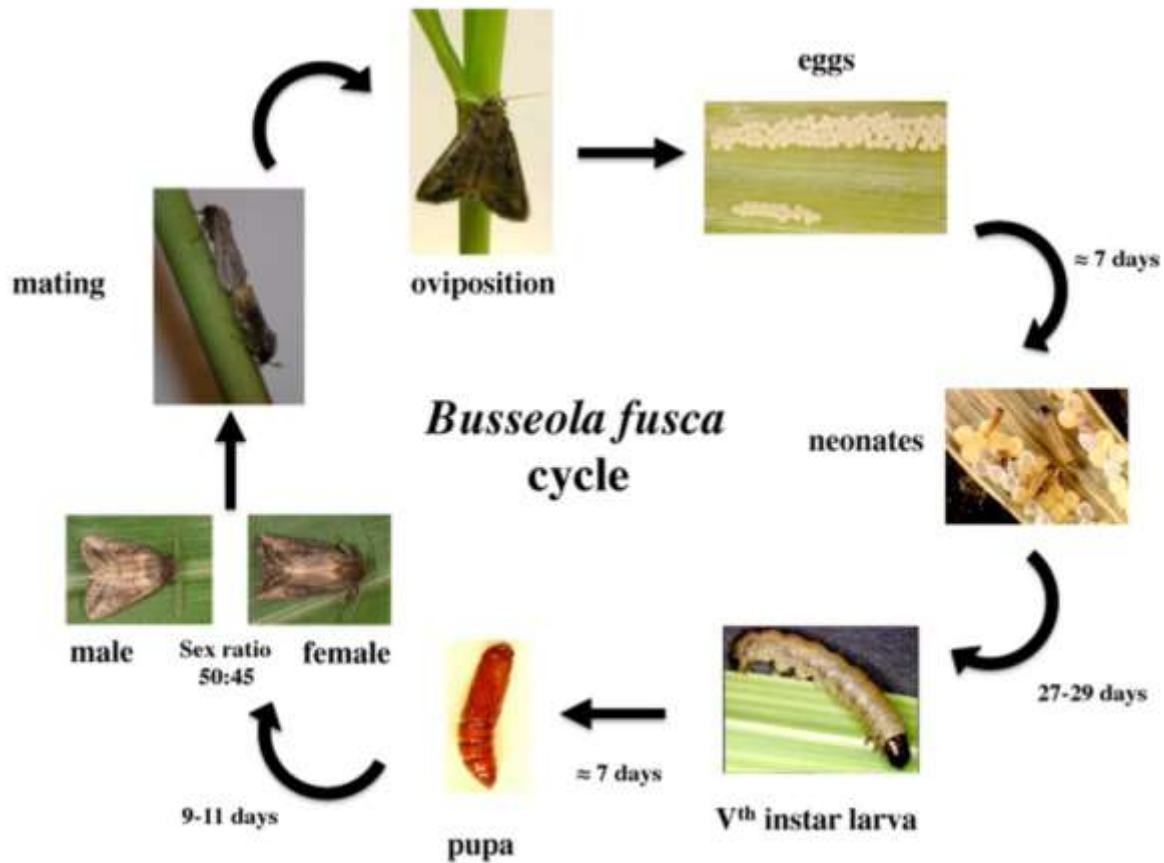


Figure 2. 2. Lifecycle of stemborers with reference on *Busseola fusca*. Sources: (Calatayud et al., 2014)

2.2. Diversity and distribution of lepidopteran stemborers in natural habitats

In Sub-Saharan region, small to medium-scale cereal fields are surrounded by uncultivated habitats that are complex and forming vegetation mosaics. Vegetation mosaics refer to landscapes composed of multiple, spatially interspersed habitat patches that differ in plant species composition, structure, and phenology (Forman, 1995; Turner et al., 2001). In agro-ecological systems, these mosaics emerge from the interaction between natural vegetation (wetlands, riparian sedges, grasslands) and human-modified habitats such cultivated fields, fallows, and farm margins. The configuration of these mosaics is determined by a combination of environmental and anthropogenic factors including topography, hydrology, soil moisture gradients, seasonal flooding, cropping systems, land-use intensity, and wetland conversion (Lillesø et al., 2024).

Vegetation mosaics play a critical role in are species composition and host plants that are believed to serve as home of beneficial insects (Getu, 2002; Assefa et al., 2010) and reservoirs of pest such as stemborer (Gounou & Schulthess, 2004; Le Rü et al., 2006; Assefa, 2015; Calatayud et al., 2016). In East Africa these vegetations are a decisive in structuring stemborer communities found in specific localities which is due to differences in plant composition, habitat heterogeneity, and land use patterns (Assefa et al., 2010; Ong'amo, 2005; Goftishu et al., 2018). African native stemborers have been documented to alternatively spend time or lifecycle in wide range of wild vegetation, particularly Poaceae, Cyperaceae, and Typhaceae and predicted the existence of more stemborers and plant hosts that have not been yet recognized (Le Rü, Ong'amo, Moyal, Muchugu, et al., 2006, 2006; Matama-Kauma et al., 2008; Moeng et al., 2018; Goftishu et al., 2018; Ong'amo et al., 2018).

Previously, surveys toward the stemborers species and their abundance have been carried out (Ingram, 1958; Walker, 1960; Bowden, 1976; Khan et al., 1997; Overholt et al., 2001; Le Rü, Ong'amo, Moyal, Ngala, et al., 2006; Moeng et al., 2018; Jendritzki et al., 2023). These surveys focused on economic importance of stemborer pests or wild habits. According to the recent stemborer surveys, 65 wild hosts in South Africa (50 species) and Mozambique (39 species) (Moolman et al., 2013; Moolman et al., 2014) were reported. Another complex survey conducted in Eritrea, Ethiopia, Kenya, Madagascar, Mozambique, Tanzania, Uganda, and Zanzibar has identified 135 species of which 44 species belong to the noctuid family, 33 crambids, 15 pyralids, 16 pyraloidea, 25 tortricidae and three cossidae in wild habitats (Le Rü, Ong'amo, Moyal, Ngala, et al., 2006). In Botswana, 12 stemborer species from *Sciomesa*, *Sesamia* and *Conicofrontia* genera are reported from 14 wild and two cultivated host plants (Moeng et al., 2018). In Kenya and Tanzania, a total of 36 noctuid species belonging to 9 genera are recorded from 49 wild plant species belonging to Poaceae, Cyperaceae and Typhaceae (Zhou et al., 2003; Kanya et al., 2004; Otieno et al., 2008).

Additional country-specific studies have been conducted in East Africa and focusing on diversity and ecology of stemborers in crop or wild habitats. In Ethiopia, earlier study has reported the recent survey revealed 44 species belonging to 14 genera under Noctuidae, Crambidae, Pyralidae and Tortricidae families. These were recorded from 34 wild host plants, of which Noctuidae were the most dominant (Goftishu et al., 2018). These stemborer species were widely distributed and

attacking plant in the families of Poaceae, Cyperaceae, and Typhaceae (Assefa et al., 2010; Goftishu et al., 2017, 2018). However, most of these species were described from small scale collections or scanty materials, obtained as adults from light traps or sometimes from single sex which could be a gap to deeply understand the ecology of the flying insects in their natural habitats.

2.3. Diversity and distribution of lepidopteran stemborers in cultivated habitats

Due to geo-climatic conditions, the presence of several lepidopteran stemborer species whose distribution and pest status varies with location is typical. According to the cultivated crops stemborer's checklist, a total of 23 species of stemborers species are reported from maize, rice, sorghum, sugarcane and wheat in Africa (Kfir et al., 2002a; Assefa, 2006; Assefa et al., 2010; Ong'amo et al., 2014). While only two species namely *Chilo partellus* (Swinhoe) and *C. sacchariphagus* (Strand) are exotic and accidentally introduced from Asia, all remaining 21 species are native to the African ecosystem (Goftishu et al., 2018; Kfir, 1992; Kfir et al., 2002a). In eastern Africa, *Chilo partellus* (Swinhoe), *Busseola fusca* (Fuller) and *Sesamia calamistis* attack several cultivated crops but not limited to maize, sorghum, pearl millet, rice (Getu et al., 2001; Melaku et al., 2006; Goftishu et al., 2017), while *Eldana saccharina* is commonly a sugarcane pest (Assefa, 2006a; Lucid Keys, 2007; Overholt et al., 2001). These species distributions vary with altitude where *B. fusca* dominates the high-altitude whereas *C. partellus* dominates low-altitude areas (Gebre Amlak, 1985; Getu E, 2002). Details on recent status of stemborers is provided in table 2.1.

Table 2. 1. Current status of stem borer species distribution in East and South Africa

Country	Family	Genus	Species	Reference
Ethiopia	Noctuidae	9	31	
	Tortricidae	-	7	
	Pyralidae	-	4	(Gebre Amlak, 1988; Getu E, 2001; Assefa, 2006; Goftishu et al., 2018)
	Crambidae	-	2	
Eritrea	Noctuidae	-	5	
	Tortricidae	-	4	
	Pyralidae	-	0	
	Crambidae	-	2	(Le Rü et al., 2006,)
Kenya	Noctuidae	-	32	
	Tortricidae	-	18	
	Pyralidae	-	15	(Ong’amo et al., 2012; Le Rü et al., 2006)
	Crambidae	-	25	
	Cossidae	-	2	
Madagascar	Noctuidae	-	3	
	Tortricidae	-	1	(Le Rü et al., 2006)

Table 2. 2. continued

	Pyralidae	-	6	
	Crambidae	-	3	
Mozambique	Noctuidae	-	11	
	Tortricidae	-	3	
	Pyralidae	-	3	
	Crambidae	-	6	(Le Rü et al., 2006)
Uganda	Noctuidae	-	20	
	Tortricidae	-	2	
	Pyralidae	-	3	
	Crambidae	-	10	(Le Rü et al., 2006; Matama-Kauma et al., 2008)
	Cossidae	-	2	
Tanzania	Noctuidae	-	14	
	Tortricidae	-	3	
	Pyralidae	-	1	
	Crambidae	-	4	(Le Rü et al., 2006; Ong'amo et al., 2018)
Zanzibar	Noctuidae	-	3	(Le Rü et al., 2006)

Table 2. 3. continued

	Tortricidae	-	1	
	Pyralidae	-	1	
	Crambidae	-	2	
South Africa	Noctuidae	-	27	
	Tortricidae	-	5	
	Pyralidae	-	6	
	Crambidae	-	10	
	Cossidae	-	1	(Assefa et al., 2009; Moolman et al., 2014)

2.3. Economic importance of cereal stemborers

The larvae of stemborers are cloaked inside the host plant's stems and feed on the internal cavity of the plant, making them very difficult to control. Generally, the estimation of yield losses due to stemborer vary from 10 to 50% rarely to 88% if no action is taken (Getu E. et al., 2001; Melaku et al., 2006; Yewhalaw et al., 2008; Midega et al., 2015; Goftishu et al., 2017). In Sub-Saharan Africa, the most economically important pest species include four to seven noctuids (Getu et al., 2001), two pyralids (Assefa et al., 2009), and twelve crambids. The family Noctuidae includes *Busseola fusca* (Fuller) and six *Sesamia* species including *Sesamia calamistis* (Hampson), *Sesamia botanephaga* (Tams and Bowden), *Sesamia nonagrioides* (Lefebvre) and *Sesamia cretica* (Laderer). *Maliarpha separatella* (Ragonot) and *Eldana saccharina* (Walker) are the only members of the family Pyralidae considered as pests. Twelve Crambidae species, seven of which belong to the genus *Chilo* (Polaszek and Khan, 1998) including *C. partellus* (Swinhoe) and *C.*

aleniellus (Strand), *C. orichalcociliellus* (Strand), *C. zacconius* (Bleszynski), *C. diffusilineus* (de Joannis), *C. sacchariphagus* (Bojer) and *C. ignefusalis* (Hampson) are also important insect pests.

In East Africa, the Noctuids, *B. fusca*, *S. calamists* (Hampson), the Crambid *C. partellus* (Swinhoe) and *C. orichalcociliellus* (Strand) are the major economic pests which cause high yield loss of maize and sorghum production (Assefa et al., 2010; Goftishu et al., 2017; Kfir et al., 2002a; Khan et al., 1997; Midega et al., 2015). In Ethiopia, *B. fusca* and *C. partellus* are both the common species and the most predominant Lepidopteran stemborers of maize and sorghum (Asmare et al., 2014; Assefa, 2006; Getu E. et al., 2001; Goftishu et al., 2018; Overholt et al., 2001). It is reported that *B. fusca* is predominating at the high altitudes (1160- 2600 m.a.s.l.), high rainfall, and in cool areas whereas *C. partellus* is at low altitudes (less than 1700 m.a.s.l.), low rainfall and warm areas of Ethiopia (Assefa et al., 2015a; Goftishu et al., 2017, 2018; Tefera, 2004). Comparing with other yield loss reports, maize, wheat, and sorghum yield losses vary from 5% to 39% (Melaku et al., 2006) or 10% to 50% (Goftishu et al., 2017), and may even reach 100% at the period of pest outbreak (Gebre-Amlak, 1988; Gebre-Amlak et al., 1989).

In Rwanda, less is known about stemborer species, their ecologies, and their response to current habitat fragmentation and climate change. Only limited information on stemborers is available without clarification on target species (CABI, 2017; Silvestri et al., 2019). But due to closely similar climate and environmental conditions as many regions of East Africa, it is predicted that Rwanda could share some of the common economic importance with neighboring countries like Kenya, Ethiopia, Tanzania, and Uganda (Lillesø et al., 2024; White, 1983). However, due to its unique landscape, Rwanda harbors diversity of microclimates that may reflect differences in some species of having unique species (Lillesø et al., 2024; SANBI et al., 2022). Therefore, it is very important to conduct studies on the ecology and host ranges of stemborers in the Rwandan ecosystem and understand their relationship to regional population for better IPM strategies.

2.4. Lepidopteran stemborers response to biotic and abiotic factors

Knowledge of the seasonal variation, altitudinal gradient, and associated abiotic parameters are essential to ensure timely preparedness to tackle pest outbreak and minimize crop damage (Getu E, 2002; Sokame et al., 2020; Morshed et al., 2023; Mutamiswa et al., 2023). Climate and Weather-based forewarning models have been used to tackle stemborer pests of cereal crops

(Prasannakumar et al., 2015; Sokame et al., 2020). Assessment of impacts of rainfall, humidity, and temperature reported correlation between these parameters and stemborers population (Sokame et al., 2020; Jasrotia et al., 2019; Morshed et al., 2023). Minimum temperature, rainfall, and relative humidity are considered to be important factors that strongly influence stemborer's population dynamics at different scales (Rehman *et al.*, 2002).

Further, biotic factors significantly influence stemborer pests' population dynamics through their interactions with natural enemies, host plants, competition, and symbiotic relationships (Getu E. 2001; Getu E. et al., 2007). These factors have potential to either suppress or promote stemborer populations (Getu E. et al., 2003; Assefa et al., 2010; Borges Filho et al., 2019). Studies demonstrated that natural enemies are associated with stemborers in natural environment, where parasitoids such as *Cotesia* and predators like bugs, spiders, ants, and birds can successfully keep stemborers population under thresholds level (Getu et al., 2001; Tefera, 2004; Assefa et al., 2010; Borges Filho et al., 2019). Also, entomopathogenic fungi (e.g. *Beauveria bassiana*), bacteria (e.g. *Bacillus thuringiensis*), nematodes (e.g. *Steinernema* spp.) have shown similar effects (Maniania et al., 2011; Otim et al., 2022; Tefera et al., 2016; Kamga et al., 2022). Additionally, some reports demonstrated the role of crop health and farming practices play the key role in regulating the stemborers population (ICIPE, 2005; Khan et al., 2007; Kebede et al., 2018; Niassy et al., 2022).

Abiotic and biotic factors are being gradually influenced by anthropogenic activities in combination with climate change effects, which may accelerate host range expansion, distribution, more generations per year in tropical and subtropical region (Mwalusepo et al., 2015). Recent studies such as one conducted from Chongming Island of China, reveals that global warming will promote the occurrence of striped stemborers (*C. suppressalis*) in rice but opposite future projection impact was reported in pink stemborers (*S. inferens*) of millet (Wan et al., 2017). Also, the recent prediction of climate change and Lepidopteran stemborers' habitat fragmentation in Africa, shows that East Africa is the most vulnerable region to the stemborers threats (Mwalusepo et al., 2015, 2018). The study results indicate a worsening of the impact of the two stemborers (*C. partellus* and *B. fusca*) pests on maize production along the East African agro-ecologies (Getu E. et al., 2001; Mutamiswa et al., 2023; Mwalusepo et al., 2015; Sokame et al., 2020). Changes already are reported about species shift (Deutsch et al., 2018; Grünig et al., 2020; Ma et al., 202; Brown et al., 2022).

It is believed that, before the agriculture intensification practices of maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* (L.) Moench) in Africa, the African native cereal stemborers like *B. fusca* (Fuller) (Lepidoptera: Noctuidae), *C. orichalcociliellus* (Strand) (Lepidoptera: Crambidae), *E. saccharina* (Walker) (Lepidoptera: Pyralidae) and *S. calamistis* (Hampson) (Lepidoptera: Noctuidae) were wild non-economic importance species (Van Den Berg et al., 1991; Le Rü et al., 2006;). For example, *B. fusca* is assumed to have evolved from wild sorghum and millets, later on with habitats fragmentation and climate change, they have expanded their habitats to cultivated graminous crops (Harris & Nwanze, 1992; Getu E. et al., 2001; Le Rü et al., 2006; Van Den Berg et al., 1991). This mechanism of host range expansion of stemborer species will continue and affect yield production and economic loss due to yield reduction and management efforts (Potts et al., 2016; Lehmann et al., 2020; Schneider et al., 2022; Uhler et al., 2021). Besides, these critical changes may accelerate target species management failure due to pest resistance and species variations.

2.5. Stemborers population dynamics

Stemborer population occurs in large numbers in cultivated crop during cropping seasons, but can survive in wild hosts and crop residues during dry seasons (Borges Filho et al., 2019; Gebre-Amlak, 1988; Mutamiswa et al., 2023; Toussaint et al., 2012). Alternative hosts in the vicinity of the crop fields and crop residues enhance the survival of stemborers during the non-cropping season, and considered responsible for pest attacks on crops in the following cropping season. Thus, the persistence and buildup of stemborer pest populations in the cropping fields early in the season was blamed on the movement of individuals from crop stubbles and wild hosts growing in the uncultivated fragments (Getu et al., 2001; Overholt et al., 2001).

In contrast, studies from East and Southern Africa have questioned about the contribution of wild host plants as reservoirs and source of stemborer infestation in cultivated crops (Assefa, 2006; Getu E. et al., 2001; Moolman et al., 2014; Ong'amo et al., 2014; Goftishu et al., 2018; Moeng et al., 2018). If well managed, wild vegetation adjacent to cultivated crops can provide refugia to parasitoids and other natural enemies during the cropping and non-cropping season (Getu E. et al., 2001; Assefa, 2010; Melaku et al., 2006). Therefore, it is expected to contribute to the reduction of stemborer pest population and degree of infestations in cultivated plants, by creating habitats

tha support parasitoids and other natural enemies of stemborers (Khan et al., 1997; Midega et al., 2015). Similarly, oviposition preference studies showed that certain grasses are highly attractive to ovipositing moths, but survival of the immature stages are generally low (Van Den Berg et al., 1991; Van Den Berg & Ebenebe, 2001; Haile & Hofsvang, 2002), which support their contribution to the regulation of stemborers population.

Due to certain physiological characteristics, most of wild grasses and sedges may host specific stemborers species, which may increase in number when attached cultivated plants (Gounou et al., 2009; Kanya et al., 2004; Otieno et al., 2008; Ong'amo et al., 2014; Goftishu et al., 2018). There are several examples used wild hosts as trap plants and reduced degree of stemborers infestation (Khan *et al.*, 1997b; Ndemah *et al.*, 2002; Yewhalaw *et al.*, 2008) by intercropping or push and pull strategies (ICIPE, 2005; Khan et al., 2011; Khan et al., 2007). For example, *B. fusca* and *C. partellus* moths highly preferred to oviposit on *Pennisetum purpureum* than maize but larval survival was drastically reduced on *P. purpureum* (Khan et al., 1997, 2007; Glas et al., 2007; Calatayud et al., 2008). Kruess et al. (2000) reported that farm field's surroundings play a critical role in beneficial and economically important insects exchanges, that positively or negatively affect yield. Overall, these authors concluded that wild hosts surrounding cereal fields play an insignificant contribution for population carry-over and infestation of stemborers in the subsequent crops. But, any modification in this tritrophic interaction network of cultivated crop - wild vegetation - and stemborer population may lead to stemborers outbreak in cropping systems.

2.6. Taxonomic studies and evolution of stemborers

2.6.1. Taxonomy of stemborers

The main characteristics used in the ordinal, sub-ordinal, and family-level classifications of the Lepidopteran species are based on: the mouthparts, ranging from having a proboscis or degenerated mouthparts; the venation and shape of the wings, the venation of the forewings and hind wings homoneurous (identical forewings and hind wings or heteroneurous (forewings and hind wings different), aculeate (more or less covered with specialized bristles called microsetae) or nonaculeate, and type of coupling (jugum or frenulum); the anatomy of the female reproductive system; the exarate or obtect condition of the pupa; and the larval structure and pattern of the

primary setae (Tams & Bowden, 1953; Bowden, 1976; Gofitshu et al., 2019; Le Rü et al., 2022 Hévin et al., 2024).

The nature of veins of the wings is mostly important for establishing both differences and relationships in the classification of Lepidoptera. However, venation patterns are considered in terms of the evolution of these patterns from primitive to advanced conditions within individual phyletic lines (Schachat. et al., 2016) The most primitive groups tend to have the maximum number of veins and branches in each wing. More advanced groups lack some veins or branches in the forewing and have a minimum hind-wing venation considerably, which challenge identification and classification of stemborers (Royal et al., 2017). Keys to the adults stemborers African stemborers rely on old references (Tams & Bowden, 1953), with updated keys recently developed (Le Rü et al., 2022; Hévin et al., 2024). Despite efforts made, more confusion to most of all taxonomic group not solved.

Chaetotaxy of the various segments of the body were complemented by additional feature of the arrangement of the crochets on the abdominal prolegs, chaetotaxy of the head capsule for the identification of the genera to species levels (Williams, 1953; Meijerman & Ulenberg, 1996; Overholt et al., 2001). The design and arrangement as well as the starting point of stemborer on plant host stem were also noted as guidance to the stemborer identification and classification at the field level (Rothschild, 1967; Meijerman & Ulenberg, 1996; Overholt et al., 2001).

Due to climate change and anthropological pressure, natural habitats are changing and more insects' taxa have developed variations, speciation, and species similarities (Elie et al., 2021; Halsch et al., 2021; Lawlor et al., 2024), which is considered as big challenge in taxonomic studies, particularly these focusing on morphological markers (Lucid Keys, 2007; Moyal et al., 2011). According to the digitalized revised Global Lepidoptera Index database based on the Global Lepidoptera Names Index (LepIndex), there is estimation of 15,578 genera and 327,530 species names of Lepidoptera (moths and butterflies), of which more than 140,000 species are synonyms, misspellings and misidentifications (Beccaloni et al., 2022). Some revisions of lepidopteran families have provided failures in identifying closely related or similar species of Lepidoptera, especially stemborers.

Some examples are members of Sesamiina (Goftishu et al., 2019; Hévin et al., 2024; Le Rü et al., 2022; Tams & Bowden, 1953), others of Pyralida family like *Chilo* Zincken (Lepidoptera: Pyralida), was described as new species of *C. argyrolepis* (Hmps) at the same time, synonymized with *C. orichalcociliellus* (Strand) (Bleszynski, 1970); Similar situation in *C. venosatus* Wlk synonymized with *C. sacchariphagus* (Bojer) (Bleszynski, 1970). Commonly known as alligator weed stemborer (*Alternanthera philoxeroides*) is synonymized with *Arcola malloi* and *Vogtia malloi* is synonymized with *Macrorrhinia endonephele* (Hampson) (Wells & Minter, 2021). In East Africa, Lepidopteran taxa synonymies were reported with some examples such as *Malgassesia pauliani* (Viette, 1955) synonymized with *Rodolphia hombergi* (Le Cerf, 1911a), *Malgassesia biedermaani* (Viette, 1982) synonymized with *Malgassesia rufescens* (Le Cerf, 1922), genus *Epitarsipus* (Le Cerf, 1922) synonymized with *Malgassesia* (Le Cerf, 1922) (Bartsch, 2018).

Misidentification has not only occurred at species level, but has reached even at genus level. An updated checklist of the Lepidopteran stemborers from Sesiini (Lepidoptera: Sesiidae) of Southern Africa, the list provides genus *Cicinnoscelis* (Holland, 1893) as a synonymy with *Alonina* (Walker, 1856) and *Felderiola* (Naumann, 1971) as a synonymy of *Monopetalotaxis* (Wallengren, 1859) (Bartsch, 2013). However, with the modern technologies, application of genetic studies has played critical benefits in studying insects' taxonomy, ecology, and biodiversity, and environmental components (Franck et al., 2017; Liu et al., 2020; Schleifer & Ludwig, 1994; Young et al., 2019), with long-term data storage such gene banks for global access.

2.6.2. Genetic diversity studies of stemborers

The genetic structure of cereal stemborer populations is shaped by both historical geological processes and contemporary ecological drivers (Anderson and Weir, 2022; Joshi et al., 2024). Geological barriers, landscape heterogeneity, and long-term environmental isolation have historically restricted dispersal, promoting genetic divergence across regions (Wiens, 2024). Phylogeographic studies across Sub-Saharan Africa and the Mediterranean demonstrate clear spatial structuring in major taxa, including *Sessamia* species (Goftishu et al., 2016, 2019), *Eldana saccharina* (Assefa, 2006), and more other stemborer species (Moyal et al., 2011a), reflecting historical isolation and evolutionary differentiation (Moyal et al., 2011a). For instance, the regional

evolutionary history of *B. fusca* highlights distinct genetic lineages associated with ecological zones, while studies on *S. nonagrioides* reveal divergence between Palearctic and African populations (Moyal et al., 2011b). Despite efforts made, there is no clear information on genetic relationship between wild habitat and crops stemborers species to understand evolution time, genetic diversity, and what control their new host selection to predict the future target hosts for precision IPM in current pressing climate change and habitats fragmentation.

2.6.2.1. Cause of insects' selection and evolution

Starvation and desertification are the main factors that cause the insect's rapid response such as selection, adaptation, migration, and rapid evolution (Mundinger et al., 2025; Péliissié et al., 2018). The recent study has revised the cause of insect pests' rapid evolution in agro-ecosystem. The study suggested that some populations of insect pests in agro-ecosystem may change their genome and acquire invasive character (Sun et al., 2023). However, genome is not known to rapidly change over the short period of time (McCartney et al., 2021; Sun et al., 2023).

Therefore, this may be possible by sympatric speciation through different discussed arguments:

(1) Population genomic changes: More studies conducted on population genomics show that genetic changes may occur from ancestral population or pest co-evolution with their host or environmental changes. Conducting experimental evolution by assays or by modeling the responses to selection of specific traits cannot indicate rapid pest insurgence. Identifying the factors that contribute to the pest population outbreak correlation with climatic and rain distribution in relation to host plant development is important for integrated pest management and species conservation. Further research into lepidopteran stemborers genomics highlighted significant variations within and across species, genera, and families (Goftishu et al., 2019; Zhang & Bu, 2022; Elameen et al., 2024).

(2) Anthropogenic activities: Anthropogenic activities contribute to pest outbreak created by agricultural intensification, infrastructures expansion, and all activities that contribute to insects' habitats reduction and ecosystem deterioration. Studies shown that the agro-ecosystem and crop rotation may affect changes in pest life cycle to adapt to available cultural practices. In this context, more insects' pest species can produce various genotypes that rapidly synchronize their lifestyles

to the local conditions and by epigenetic factors that help them in adapting to their host plant phenology (Pélissié et al., 2018). Studies have been conducted on lepidopteran stemborers responses to anthropogenic activities and role of cultural practices in regulating the population of these insects in their ecologies. These studies revealed the speciation, host range expansion, shortening the lifecycle time, and more associated resistant strategies have been developed by these insects in response to changes (Forbes et al., 2017; Wiens, 2024; Huang et al., 2025).

2.6.2.2. Overview of insects' genome and genome composition

Insect genomes are like usual animal cells composition. The nuclear complement of the genome consists of chromosomes with DNA around histone proteins, that makes a coiled, compact, linear structure. Genes in chromosomal regions can make functional transcript. Except the non-coding regions of DNA (introns) coding alignment of genes (exons) are active in the development of genome (Hox Box). Different forms of the same gene are called alleles. Genes can have two or more possible alleles that can determine individual traits. Heterochromatin has specific histone modifications, such as H3K9me2, H3K9me3, and H3K27me3 and it can form attachments between chromosomes and the nuclear envelope. Chromatin regions marked with H3K27me3 (refer to glossary) can mediate intra-and inter-chromosomal interactions in the nucleus. Heterochromatin play several functions, from gene regulation to the protection of chromosome integrity. Some of these functions may attributed to the dense DNA, which makes it limit the accessibility to protein factors that usually bind DNA or its associated factors.

Genome methylation can be silenced by DNA sequences that might be dangerous to the organism. Heterochromatin with an important role in DNA methylation is associated with cytochrome residue (Li et al., 2019; Xie et al., 2025). Each nucleotide or genes with four amino-acids C-G and A-T contain active alignments. Alignments are related to chromatin with attachment of methyl to CG and cNG. Genome function may change by asymmetric methylation of nucleotide (Thomas et al., 2020). Environmental stress by methylation regulates gene functions. Loss of DNA methylation or transposon activation through transposable elements (TE) may change genome structure. It reduces the motor element that can influence evolutionary mechanism in the organism. Adaptation to environmental conditions through genetic drift and selection for resistant forms while declining the source population produce new trait. Neutral genetic markers and adaptive

traits through microsatellites create the resistance to stress (Nuñez et al., 2021). Meta – analyses in response to environmental stress may result in fluctuating asymmetry and variations in traits of many insect pests (Thomas et al., 2020 ; McCartney et al., 2021; Gupta & Nair, 2025).

To date, more than 5000 species of insects have been sequenced genomes that about 155 of them are annotated and published in different platforms. Stemborer studies on their genetic and evolution conducted (Assefa et al., 2015a; Franck et al., 2017; Gofitshu et al., 2019; Thomas et al., 2020; Sproul et al., 2023; Yi et al., 2024). Advances with molecular genomic techniques provide the effective genetic traits of insect pest and their natural enemies. Population genomics and adaptive evolution play a critical role for biological control.

Various studies concluded that Population genomics provides insights into a long-term biological, the ecological success and sustainability of this pest management tactic, and non-target effects on native species, populations and ecosystems management (Sethuraman et al., 2020). Despite the essential and functional role of genome sequencing to study the biological composition and evolutionary histories resulting in data accuracy and availability, scientists criticise the high cost of the full genome operations (Schwarze et al., 2020). As consequence, more research have been conducted and proposed that partial DNA sequences of the cytochrome c oxidase I (COI) mitochondrial gene might serve as DNA barcodes for identifying and differentiating between species, such as birds, fish, insects, among others (Lunt et al., 1996; Zhang & Bu, 2022), and analysing their historical evolutionary relationship (Regier et al., 2009; Gofitshu et al., 2016b, 2019; Thomas et al., 2020).

2.6.2.3. Stemborers characterization with Cytochrome Oxidase subunit I (COI)

Identification of field-collected stemborer larvae is not possible, instead, requires rearing of the larvae to adult stage. The rearing practice is usually complicated to unsuccessful because of insect deaths during rearing, or larvae may take a very long time to develop to an adult if larvae diapause happened due to climatic conditions or feed deficiency during rearing (Kfir et al., 2002a). Additionally, lack of expertise in traditional morphological taxonomy makes molecular systematics a best taxonomic alternative.

Mitochondrial DNA (mtDNA) become essential in identifying microinvertebrates because of the high mutation rate, maternal inheritance, and abundance in cells. The COI (cytochrome oxidase I) gene serves as a DNA barcode due to its properties of clear genetic distinctions between species, lacks recombination, ensuring lineage stability, and its high copy number that allows for efficient PCR amplification, even from degraded samples. These features make mtDNA a reliable marker for distinguishing closely related insect species, contributing in biodiversity studies, pest management, and conservation efforts (Johnson et al., 2014; Kumar et al., 2019; Harper, 2023). These studies have confirmed mtDNA COI gene as a highly conservative part with low variability in most regions. Such fact in mitochondrial gene, make the COI gene useful for evolutionary studies (Li et al., 2019; Xie et al., 2025).

Studies on lepidopteran stemborers using COI gene revealed genetic variation in different species, and more evolutionary relationships from families to species levels, providing species specification in different ecosystems (Assefa, 2006; Gofitshu et al., 2016a, 2019; Le Rü et al., 2022). This makes this method accurate and potential for new surveys that combine stemborers genetic diversity, phylogeography, and host expansion or shifting behavior.

3. DIVERSITY AND SPECIES RICHNESS OF LEPIDOPTERAN STEMBORERS AND THEIR HOST PLANTS IN SELECTED VEGETATION MOSAICS OF RWANDA

Abstract

Lepidopteran stemborers are among the most important insect pests of maize, sorghum and sugarcane in sub-Saharan Africa. Except for *Chilo partellus*, the other stemborer pests are indigenous to Africa and are assumed to have coevolved with some native grasses and sedges in the natural habitat. In addition to pest species, natural habitats harbour diverse non-economic stemborer species, some of which are new to science. However, with the growing threats of pressing climate change and natural habitats fragmentation, some non-economic stemborer species may switch or expand their host ranges to include cultivated crops and evolve as “new” pests. Besides host switch, some of the unknown species currently limited to natural habitats may disappear. In this context, we carried out a survey in Rwanda, in main ecological vegetation mosaics, of Afroalpine mountain mosaics within the Guineo-Congolese region in northern, Akagera sub-humid savanna and wetland mosaics in eastern, Montane forest mosaics in southern (transitional) and urban wetland and its riparian mosaics in Kigali, the capital city within the Sudanian - Guinea - Congo transition zone were surveyed. At each selected location, a purposive (biased) sampling was used to detect stemborer infestations. These habitats included (i) the edges of the cultivated field and adjacent uncultivated zones, (ii) open patches along the forest access roads and pathways, (iii) riparian zones along urban areas, rivers and streams, (iv) the edge of lakes and (v) swamp ecosystems. The survey reached a total of 6,098 larvae belonging to 18 genera representing five families were recovered from 56 wild host plant species with uneven distribution. The abundance of larvae and host plants was highest in the urban wetland and its riparian vegetation (47.1% of larvae; 47 host plants) and lowest in mountain Afroalpine vegetation (10.3% of larvae; seven host plants). The Poaceae family hosted 62.5% of all stemborers, with *Pennisetum purpureum* being the most affected host, accounting for 29.53% (N = 1,801). Two major economically important pests in sub-Saharan Africa dominated collections: *Busseola fusca* (47.11%, N = 2,873) and *Chilo partellus* counting for 17.61%, (N = 1,074). Additionally, five new

host plant species and a new stem-feeding polyphagy in the genus *Thaumatotibia* are reported for new attention and alarming the need of urgent IPM strategies.

Keywords: Ecological mosaics, Lepidoptera, Network, Rwanda, Stem feeders, Wild habitat

1. Introduction

Current estimations indicate that more than 150 species of lepidopteran stemborer have been described globally (Kfir et al., 2002; Borges Filho et al., 2019;), with an uneven distribution across regions (Sallam, 2006; Moolman et al., 2014;). In East Africa surveys encompassing Eritrea, Ethiopia, Kenya, Madagascar, Mozambique, Tanzania, Uganda, and Zanzibar reported more than 135 species (Le Rü et al., 2006b). Additional surveys reported 44 species in Ethiopia (Goftishu et al., 2018), 20 species in the Democratic Republic of Congo (Kankonda et al., 2017), 50 species in South Africa, and 39 species in Mozambique (Moolman et al., 2014). West Africa is less represented, with 32 species recorded in Cameroon (Ong'amo et al., 2014). These species are distributed primarily in three dominant lepidopteran families, namely Noctuidae, Crambidae and Pyralidae (Getu E. et al., 2002; Le Rü et al., 2006; Moyal et al., 2010), with some representatives from Tortricidae (Moyal et al., 2010; Ong'amo et al., 2016) and Thyrididae families (Moolman et al., 2014).

Stemborers exhibit a broad host range that extends beyond cultivated cereals to numerous wild vegetation (Assefa et al., 2010), which serve as alternative hosts (Overholt et al., 2001). Surveys across East Africa reported over 75 wild host plants (Le Rü et al., 2006), in southern Africa reported over 65 (Moolman et al., 2014), while only 22 wild host plant species were listed in West Africa, particularly in Cameroon (Ong'amo et al., 2014). These studies reported wild host plants of in Poaceae, Cyperaceae and Typhaceae families (Goftishu et al., 2018; Le Rü et al., 2006b, 2006a). The widely distributed genera of host plants are from the family of Poaceae particularly *Pennisetum*, *Panicum*, *Sorghum*, and *Phragmites* (Haile and Hofsvang, 2002; Moolman et al., 2014), compared with genera of the Cyperaceae and Typhaceae families (Moeng et al., 2018; Ong'amo, 2009; Ong'amo et al., 2016).

East Africa supports highly diverse vegetation characterized by complex, interwoven ecosystems that form dynamic ecological mosaics (Lillesø et al., 2024). These vegetation patterns are primarily structured by gradients in altitude, rainfall, soil conditions (edaphic factors), and human land-use activities (White, 1983; Lillesø et al., 2024), leading to restructuring the stemborers communities (Maicher et al., 2018; Morshed et al., 2023; Ong'amo et al., 2014).

Vegetation mosaics in East Africa play a decisive role in the structure of stemborer communities due to differences in vegetation composition, habitat heterogeneity, and land use patterns

(Ong'amo, 2005; Goftishu et al., 2018). Recent surveys conducted in Ethiopia and Kenya showed that generalist oligophagous species such as *B. fusca* (Assefa et al., 2015a) and *Chilo partellus* are the most dominant stemborers colonizing multiple vegetation mosaics (Goftishu et al., 2018; Ong'amo et al., 2018). This reflects their adaptability to various crops (Getu E. et al., 2001), grasses, fodder, and wetland vegetation (Overholt et al., 2001). In addition, *Sesamia* species remain largely confined to humid or semi-aquatic habitats (Overholt et al., 2001) while sugarcane growing zones are dominated by *E. saccharina* (Assefa, 2006; Assefa et al., 2010; Ntirenganya et al., 2025). However, recent changes in land use and vegetation fragmentation activities have pushed a range of stemborer expansions and habitat shifts to new ecological zones and host plants. These trends are reported in *B. fusca* and *C. partellus* in Ethiopia, for example (Getu et al., 2001; Goftishu et al., 2018), Kenya (Kanya et al., 2004; Ong'amo, 2009; Mutyambai et al., 2022) and in other regions (Le Rü et al., 2006b; Moolman et al., 2014; Kankonda et al., 2017; Ong'amo et al., 2018).

Considering the ecological regions and vegetation mosaics, Rwanda's natural vegetation is a regional mosaic comprising Guineo-Congolese in the western and northern parts, Sudanian forests in the eastern parts, and Guineo-Congolese - Sudanian transition zones in the central parts and Kigali City (White, 1983; SANBI et al., 2022; Lillesø et al., 2024). These ecological zones are marked with different vegetation and habitat heterogeneity, where Sudanian forests are characterized by a mixture of shrub-savanna and grassland ecosystems dominated by tall perennial grasses comprising *Andropogon*, *Hyparrhenia*, and *Brachiaria* (SANBI et al., 2022; Lillesø et al., 2024). These grasses form dense herbaceous layers supporting grazing animals (MINAGRI, 2021; SANBI et al., 2022). In contrast, Guineo-Congolian vegetation mosaics feature a blend of semi-evergreen forests and open grasslands dominated by *Hyparrhenia*, *Imperata* and *Pennisetum* (White 1983; MINAGRI 2021; SANBI et al. 2022). The Guineo-Congolese - Sudan transition zones in central parts of the country are marked by mixed vegetation made up of grasslands, bushlands, forests and rich water bodies (Lillesø et al., 2024; Morpurgo et al., 2024).

Despite this ecological diversity and landscape differentiations, few studies on stemborers population have been conducted (Silvestri et al., 2019) and a newly small-scale stemborer survey in sugarcane plantation (Ntirenganya et al., 2025). Other studies were limited to ants (Nsengimana et al., 2021, 2023, 2025), odonates (Uyizeye et al., 2021), butterflies (Uwizelimana et al., 2022), and general survey of arthropods in eastern part of Rwanda (Ruticumugambi et al., 2024). Studies on

stemborers in relation to ecological regions and vegetation mosaics in Rwanda are still in the infancy stage, where study (Le Ru et al., 2006) has reported *Poeonama* species recorded in Kigali City's wetland but without more information of species adaptation to local host range and habitat changes. To address these knowledge and data availability gaps, this study attempts to (i) describe the diversity of stemborers and wild host plants in selected vegetation mosaics Rwanda and (ii) to describe the ecological interactions of lepidopteran stemborers and their associated wild host plants to predict the potential effect of habitat fragmentation on the evolution of stemborer species.

2. Materials and Methods

2.1. Study site description

The study was carried out in four administrative regions representing the major vegetation mosaics and bioclimatic gradients of Rwanda (Fig. 3.1), following the African phytogeographical classification (White, 1983), East African vegetation (Lillesø et al., 2024), and the national classification of ecological zones of Rwanda (MINAGRI, 2021; SANBI et al., 2022). Selected sites capture transitions between the Guineo–Congolese vegetation mosaic of the humid western highlands, the complex Sudanian woodland savanna of the eastern plains, and the transition zones Sudanian-Guineo-Congolese.

In the eastern plains, the Kayonza district, located at 1 ° 45'–2 ° 05' S; 30 ° 25'–30 ° 50' E; 1,200–1,500 m altitude was selected. It lies within the Sudanese regional mosaics, dominated by *Acacia-Combretum* forests, bushland and low-altitude wetlands in a subhumid to semi-arid climate (Lillesø et al., 2024; MINAGRI, 2021; Ruticumugambi et al., 2024). In the northern part of Rwanda, the study was carried out in the Burera district (1°21'–1°34' S; 29°44'–29°54' E; >2,000 m altitude) in Afroalpine mountain and wetland mosaics (AMW), within the Guineo–Congolese regional center. This zone features montane grasslands dominated by *Pennisetum*, bamboo (*Yushania alpina*), and high-altitude wetlands dominated by Typhaceae and Cyperaceae around Burera and Ruhondo lakes (Lillesø et al., 2024; MINAGRI, 2021; Nsengimana et al., 2025).

In the central part, Kigali City (1 ° 57 ' - 1 ° 59 ' S; 30 ° 03 ' -30 ° 09 ' E; 1300-1,800 m above sea level) was selected as an urban wetland and river mosaics (WR) within the transition zone Sudanian - Guinesseo - Congo. Its landscape comprises urbanized hill–valley systems interspersed with Nyabarongo and Nyandungu wetlands, supporting riparian forests and secondary woodland–savanna mosaics with more modified greeneries (REMA, 2012; ARCOS, 2021; SANBI et al., 2022). In southern Rwanda, the Kamonyi district (2°00'–2°15' S; 29 ° 45' - 29 ° 55' E; 1,500–2,000 m above sea level), dominated by Montane Forest and Wetland (MFM) mosaics, was selected. It is in a continuous transition zone with a large part lying within the Guineo–Congolese forest mosaic of the southern highlands. The mosaic structure in this zone is defined by canopy trees interspersed with herb-rich wetlands, resulting in high biodiversity and complex community (Iiyama et al., 2018; Mukuralinda et al., 2016; SANBI et al., 2022).

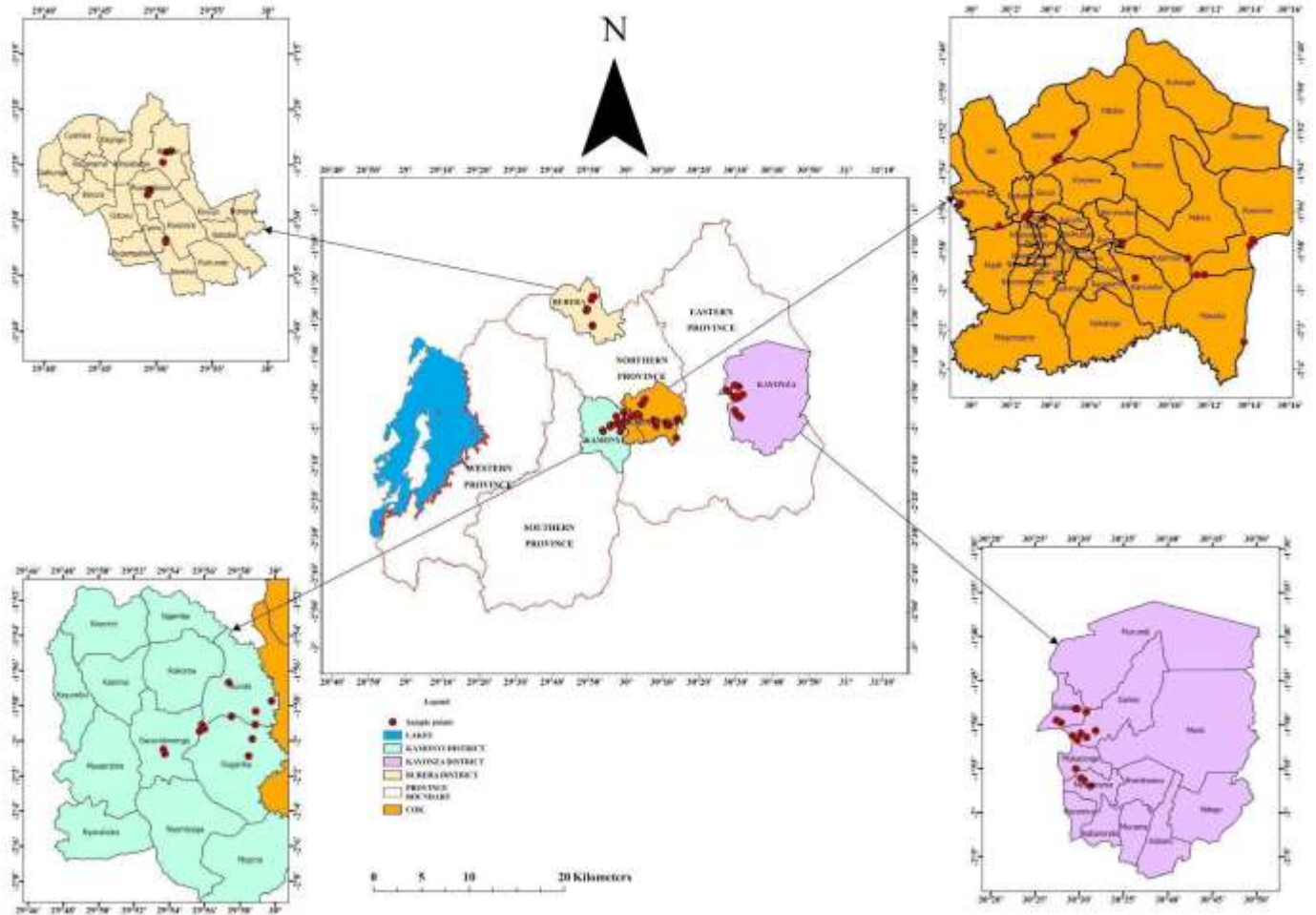


Figure 3. 1. Geographical coverage of the study area with administrative boundaries that represent vegetation mosaics.

Sampled localities within the vegetation mosaics were strategically selected based on ecological composition, particularly nature of vegetation, weather conditions, potential stemborer host plant availability as detailed in Table. 3.1. Source of data: (SANBI et al., 2022).

Table 3. 1. Details on physico-ecological characteristics and mosaic vegetation types of study areas in Rwanda

Province	District	Elevation (m)	Temperature (°C)	Rainfall (mm year ⁻¹)	Mosaics vegetation type
Northern	Burera	> 2000	19.4	1200 - 2200	Afroalpine mountain and wetland

Southern	Kamonyi	1500 – 2000	21.3	1000 – 1400	Montane forest and wetland
Eastern	Kayonza	< 1500	23.29	800 – 1000	Akagera sub-humid savanna and wetland
Kigali City	Nyarugenge -Gasabo	1000 – 2000	21.9	900 – 1200	Wetland and riparian zones to the wetland

Source: (MINAGRI, 2021; SANBI et al., 2022; Lillesø et al., 2024)

2.2.Study design

A purposive sampling method was used to increase the chance of finding stemborers. At each selected location, habitats harboring wild host plants from stemborers were systematically surveyed for stemborer infestations. These habitats included (i) the edges of the cultivated field and adjacent uncultivated zones, (ii) open patches along the forest access roads and pathways, (iii) riparian zones along urban areas, rivers and streams, (iv) the edge of lakes and (v) swamp ecosystems. Within each habitat, plant species belonging to Poaceae, Cyperaceae, and Typhaceae were sampled (Fig. 3.2). They were preferred based on previous records of their vulnerability to be infested by stemborers (Goftishu et al., 2018; Le Rü et al., 2006b, 2006a), but closer families were checked. Additionally, morphologically similar or closely related vegetation belonging to Cannaceae, Marantaceae, Juncaceae and Zingberaceae were also sampled. In particular, all plants that had leaf scarring, dead and desiccated shoots, frass deposits, and entry/exit holes were particularly targeted for stemborer sampling.

2.3.Data collection

Data were continuously collected during dry, short, and long rain seasons in selected areas. Each observed plant was carefully examined for visible signs of stemborer infestation. The infested plants were excised and dissected to recover the stemborer larvae and occasionally pupae, with eggs sometimes found on the surface of the plant. The recovered larvae were transferred to artificial diets in plastic vials (7.5 × 2.5 cm) closed with cotton wool and maintained in the laboratory until pupation (Devi and Kaur, 2015; Overholt et al., 2001). The recovered pupae and the egg colonies were kept separately in plastic vials (15 × 7 cm) closed with cottonwool until emergence (Fig. 3.2).



Figure 3. 2. Stemborers collection in different vegetation mosaics and infestation checking; (1) wetland and riparian mosaics, (2) grassland mosaics, (3) Kigali urban introduced aesthetic and social gathering space (Mu Marangi, Nyamirambo) with introduced hornemental vegetation, (4) examining of suspected plants, (5) rearing of recovered stemborers, (6) emerged adult moth

2.4. Identification of the host plant and the stemborers

The families, genera, and species of host plants were identified in the field by direct observation and identification keys. When immediate identification was not applicable, representative specimens were collected and examined by plant taxonomists and botanists at the University of Rwanda, the Centre of Excellence in Biodiversity and Natural Resource Management (CoEB), and the Rwanda Institute for Conservation Agriculture (RICA) to confirm the name of the plant species.

The identification of stemborers at the species level was performed using microscopic observations to identify morphological characteristics following the guide for stemborer identification keys developed by the International Center of Insect Physiology and Ecology (ICIPE) (Overholt et al., 2001), the recently published stemborer identification keys (Assefa et al., 2017; Le Rü et al., 2014;

Meijerman and Ulenberg, 1996; Moyal et al., 2011), and the Lucid Central insects identification platform (<https://www.lucidcentral.org>). In addition, a stemborers taxonomic expert from ICIPE was consulted to validate the names of species. Few stemborer species were only identified at the genus or family level. The voucher specimens are deposited in the Zoology Laboratory, Biology Department, College of Science and Technology, University of Rwanda, under the stemborers collection (access code: KG, KM, KY, B 2023 & 2024). The letters represent the district or city where the specimens were collected, and the numbers indicate the year of collection. Samples from Ethiopia were deposited at Laboratory of Entomology, College of Agriculture and Environmental Sciences, Haramaya University.

2.5.Data Analysis

The analysis of stemborer species was carried out by comparing abundance and diversity between the ecological zones studied and between host plants. The abundance was calculated based on counting the number of individual heads rather than the mean. Furthermore, species diversity was calculated using Shannon diversity (H') (Shannon, 1948) and dominance (D) (Simpson, 1949) indices. Additionally, the network specialization index (H'_2), generality (HL), linkage diversity (Ld) and vulnerability (LL) were calculated to assess the degree of interaction specialization and the average number of host plant species. These indices were selected for their robustness to sampling effort, network size variability, and their ability to capture key structural properties of ecological networks (Delmas et al., 2019). The specialization index describes the level of specialization or degree of selectiveness that increases from zero to one in the entire network, similarly to specificity index increases (Almeida-Neto and Ulrich, 2011).

The network was constructed using the algorithm in R packages: igraph, bipartite, and network analysis (R Core Team, 2025).

3. Results

3.1. Stemborers abundance and diversity among ecological zones

A total of 6,098 stemborer individuals belonging to 18 species, 11 genera, and five families were identified, namely Crambidae, Noctuidae, Pyralidae, and Tortricidae. Noctuidae was the dominant family with 11 species from five genera comprising *Acrapex*, *Busseola*, *Pirateolea*, *Poeonoma*, and *Sesamia*. The Crambidae family was represented by one genus and two species (*Chilo partellus* and *Chilo suppressalis*), Tortricidae was represented by two genera and two species (*Bactra* sp. and *Thaumatotibia* sp.), while Corossidae (*Carelis* sp.) and Pyralidae (*Eldana saccharina*) were represented by one genus and one species, respectively.

The highest number of species was recorded in urban wetlands and river mosaics with 47.1% (N = 2536) and the Akagera subhumid savanna and its wetlands with 27% (N = 1651) of the total specimens. The remaining was distributed in montane forests and their wetlands with 21% (N = 1282) and the Afroalpine mountain and its wetlands with 10.3% (N = 629) (Tab. 3. 3). According to individual species preferences of ecological habitats, diversity indices showed that urban wetlands and riparian mosaics harbor the highest diversity ($H' = 2.02$, $D' = 0.837$), indicating a more even distribution of species, while Afroalpine mountain mosaics have the lowest diversity ($H' = 0.64$, $D' = 0.263$), suggesting a smaller number of species in the area compared to other surveyed areas.

Table 3. 2. Abundance of stemborers and their distribution across ecological vegetation mosaics of Rwanda.

Stemborers				Abundance per mosaics type				
Family	No	Species	Author (s)	WR	ASW	AMW	MFW	Total

Noctuidae	1	<i>Acrapex</i>	(Hampson, 1894)	6	0	0	3	9
	2	<i>Busseola fusca</i>	(Fuller, 1901)	649	975	538	711	2873
	3	<i>Busseola</i>	(Thurau, 1904)	0	0	0	9	9
	4	<i>Pirateolea piscator</i>	(Fletcher, 1961)	61	7	0	5	73
	5	<i>Pirateolea</i>	(Moyal et al., 2010)	0	3	0	0	3
	6	<i>Poeonoma serrata</i>	(Hampson, 1910)	44	2	5	15	66
	7	<i>Poeonoma</i>	(Tams & Bowden, 1953)	0	0	2	0	2
	8	<i>Sesamia calamistis</i>	(Hampson 1910)	168	37	0	47	252
	9	<i>Sesamia nonagrioides</i>	(Lefèbvre, 1827)	339	129	15	105	588

	10	<i>Sesamia</i>	(Guenée, 1852)	17	0	0	6	23
	11	<i>Speia</i>	(Stoll, 1790)	4	0	0	0	4
Crambidae	12	<i>Chilo partellus</i>	(Swinhoe, 1885)	567	282	31	194	1074
	13	<i>Chilo suppressalis</i>	(Walker, 1863)	148	91	0	41	280
Corossidae	14	<i>Carelis.</i>	(Bowden, 1956)	3	0	0	1	4
Pylaridae	15	<i>Eldana saccharina</i>	(Walker, 1865)	21	4	0	4	29
Tortricidae	16	<i>Bactra</i>	(Stephens, 1834)	210	32	25	59	326
	17	<i>Thaumatotibia</i>	(Zacher, 1915)	299	89	13	82	483
Total				2536	1651	629	1282	6098

Species richness (S)	14	11	7	14
Shannon Index (H')	2.02	1.35	0.64	1.54
Simpson Index (D)	0.837	0.609	0.263	0.654

Note: AMW: Afroalpine mountain and wetland; MFW: Montane forest and wetland; ASHW: Akagera sub-humid savanna and wetland; WR: Wetland and riparian zones to the wetland

3.2. The abundance and distribution of stemborer species among wild host plants

Among economically important species, *B. fusca*, *C. partellus*, *S. calamistis*, and *S. nonagrioides* were the most dominant (Tab. 3.2). They sum up more than 78% of the total collected species. *B. fusca* itself counted 47.1% (N = 2873), followed by *C. partellus* with 17.6% (N = 1074) and *C. suppressalis* with 4.5% (N = 280). The *Thaumatotibia* genus was found in small grasses and small *Cyperus* located in wetlands with 7.8% (N = 483) compared to *Bactra* sp. with 5.3% (N = 326). In less economically important species, *Pirateolea* was represented with 1.2% (N = 73), *Poeonoma* with 1.08% (N = 68), and *E. saccharina* was less represented with 0.47% (N = 29). Rare species were limited to very few individuals, where *Acrapex* sp. was nine, *Carelis* sp., and *Speia* sp. were only four individuals each (Tab. 3.2).

Among the 56 identified wild host plants, the most infested species was *P. purpureum* Schumacher (family: Poaceae) with 29.53% (N = 1801) followed by *Carex cephalosphora* (Family: Cyperaceae) with 10% (N = 611), *Panicum* (Family: Poaceae) with 5.3% (N = 327) stemborers. *Rottboellia cochinchinensis* and *Sorghum halepense* (L.) Pers. The species were less affected with 3.7% (N = 228) and 3.6% (N = 225), respectively (Tab.3.3).

Table 3. 4. Stemborers species host preference and abundances per wild host plant in Rwanda

Host plants		Number of stemborer by genera												Statistics			
No	Species name	Niche	Ac	Bu	Ca	Ch	Ed	Se	Po	Pi	Bt	Th	Sp	Ng	Ia	H'	D
Poaceae																	
1	<i>Arundo donax</i>	G	1	0	0	1	0	0	0	0	0	0	0	2	15	0.39	0.23
2	<i>Chrysopogon zizanioides</i>	G	0	1	0	1	0	1	0	0	0	0	0	3	78	0.95	0.56
3	<i>Coix lacryma-jobi</i>	G	0	0	0	1	0	0	0	0	0	0	0	1	8	0.00	0.00
4	<i>Cenchrus purpureus</i>	G	0	0	0	1	0	1	0	1	0	0	0	3	39	0.81	0.47
5	<i>Cymbopogon citratus</i>	G	0	0	0	1	0	0	1	0	1	0	0	3	11	0.86	0.51
6	<i>Echinochloa crus-galli</i>	G	0	0	0	2	0	2	0	0	0	0	0	4	32	0.51	0.27
7	<i>Echinochloa frumentacea</i>	G	0	1	0	1	0	2	0	0	0	0	0	4	67	1.57	0.75
8	<i>Echinochloa pyramidalis</i>	Aq	0	1	0	2	0	3	1	1	0	0	0	8	119	1.28	0.62
9	<i>Eleusine coracana</i>	Dl	0	0	0	2	0	0	0	0	1	1	0	4	64	1.62	0.76
10	<i>Hyparrhenia hirta</i>	Dl	0	0	0	1	0	0	0	1	1	1	0	4	23	1.30	0.66

Table 3.3. continued

11	<i>Hyparrhenia papillides</i>	<i>Dl</i>	0	0	0	1	0	0	0	0	0	1	0	2	38	1.02	0.62
12	<i>Hyperthelia dissoluta</i>	<i>Dl</i>	0	0	0	1	0	0	0	0	1	0	0	2	41	0.77	0.50
13	<i>Imperata cylindrica</i>	<i>Ae</i>	1	0	0	2	0	0	0	0	0	0	0	3	42	0.79	0.45
14	<i>Lolium</i>	<i>G</i>	0	0	0	1	0	0	0	1	2	0	0	4	23	0.84	0.50
15	<i>Miscanthus Variegatus</i>	<i>Ae</i>	0	0	0	1	0	0	0	0	0	0	0	1	7	0.00	0.00
16	<i>Panicum maximum</i>	<i>H</i>	0	1	1	2	0	2	0	1	0	0	0	7	245	1.36	0.64
17	<i>Panicum ripens</i>	<i>H</i>	0	0	0	1	0	2	1	0	0	0	0	4	82	1.32	0.71
18	<i>Paspalum</i>	<i>G</i>	0	0	0	2	0	0	0	0	2	0	0	4	99	0.89	0.52
19	<i>Pennisetum clandestinum</i>	<i>G</i>	0	1	0	1	0	0	0	0	0	0	0	2	86	0.89	0.52
20	<i>Pennisetum macrourum</i>	<i>G</i>	0	0	0	1	0	1	1	1	0	0	0	4	34	1.02	0.55
21	<i>Pennisetum purpureum</i>	<i>G</i>	0	1	0	1	0	1	2	0	0	0	0	4	1801	0.31	0.17
22	<i>Pennisetum polystachion</i>	<i>G</i>	0	1	0	1	0	0	0	0	0	0	0	2	87	0.05	0.01
23	<i>Pennisetum thunbergii</i>	<i>G</i>	0	1	0	1	0	1	1	0	0	0	0	4	63	1.36	0.68

Table 3.3. continued

24	<i>Phragmites</i>	<i>H, Ae</i>	0	1	0	1	1	2	0	1	0	0	0	6	199	1.48	0.73
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25																	
	<i>Rottboellia cochinchinensis</i>	<i>G</i>	0	1	0	1	0	2	0	0	0	0	0	4	228	1.02	0.62
26	<i>Rottboellia Exaltata</i>	<i>H</i>	0	0	0	0	0	0	0	0	1	0	0	1	16	1.08	0.54
27	<i>Saccharum officinarum</i>	<i>G</i>	0	0	0	0	13	0	0	0	0	0	0	1	13	0.00	0.00
28																	
	<i>Setaria megaphylla</i>	<i>H</i>	0	0	0	1	0	0	0	0	2	0	0	3	44	0.69	0.50
29	<i>Setaria sphacelata</i>	<i>H</i>	0	0	0	1	0	0	0	0	1	0	0	2	67	0.63	0.44
30																	
	<i>Sorghum arundinaceum</i>	<i>G</i>	0	1	0	1	0	1	1	1	0	0	0	5	46	1.36	0.66
31	<i>Sorghum bicolor</i>	<i>G</i>	0	1	0	1	0	0	0	0	0	0	0	2	127	0.30	0.16
32	<i>Sorghum halepense</i> .	<i>G</i>	0	1	0	1	0	0	1	0	0	0	0	3	225	0.57	0.30
33	<i>Tripsacum</i>	<i>G</i>	0	1	0	0	0	0	0	0	0	0	0	1	75	0.18	0.08
34	<i>Zizania latifolia</i>	<i>Aq</i>	0	0	0	2	0	0	1	0	2	0	0	5	142	0.44	0.22
35	<i>Zea mays</i>	<i>G</i>	0	1	0	1	0	1	0	0	0	0	0	3	176	1.16	0.64

Table 3.3. continued

Cyperaceae

36	<i>Carex cephalosphora</i>	H	0	0	0	0	0	1	0	0	1	0	0	2	611	0.69	0.50
37	<i>Carex chlorosaccus</i>	H	0	0	0	0	0	1	0	0	1	0	0	2	68	0.78	0.44
38	<i>Cyperus articulatus</i>	H, Aq	0	0	0	0	0	1	0	0	1	1	0	3	142	1.04	0.59
39	<i>Cyperus difformis</i>	H, Aq	0	0	0	0	0	2	0	0	1	1	0	4	162	0.94	0.56
40	<i>Cyperus distans</i>	H, Aq	0	0	0	1	0	1	0	1	0	1	0	4	126	1.16	0.65
41	<i>Cyperus dives</i>	H, Aq	0	0	0	0	0	1	0	0	0	1	0	2	112	0.66	0.47
42	<i>Cyperus exaltatus</i>	H, Aq	0	0	0	0	0	1	0	1	0	1	0	3	88	0.91	0.55
43	<i>Cyperus latifolius</i>	H, Aq	0	0	0	0	0	1	0	1	0	1	0	3	37	1.06	0.64
44	<i>Cyperus odoratus</i>	H, Aq	0	0	0	0	0	0	0	0	0	1	0	1	27	0.00	0.00
45	<i>Cyperus prolifer</i>	H, Aq	0	0	0	0	0	0	0	0	0	1	0	1	6	0.00	0.00
46	<i>Cyperus strigosus</i>	H, Aq	0	0	0	0	0	1	0	1	0	1	0	3	21	0.91	0.56
47	<i>Schoenoplectus</i>	H, Aq	0	0	0	1	0	1	0	0	0	1	0	3	72	1.01	0.61
48	<i>Scleria gaertneri</i>	H, Aq	0	0	0	0	0	1	0	0	0	1	0	2	15	0.64	0.44

Typhaceae

50	<i>Typha domingensis</i>	Aq	0	0	0	0	0	1	0	0	0	1	1	2	32	0.60	0.32
51	<i>Typha latifolia</i>	Aq	0	0	0	0	0	1	0	1	0	1	0	3	79	1.20	0.61

Juncaceae

52	<i>Juncus effusus</i>	<i>H, Aq</i>	0	0	0	1	0	1	0	0	1	0	0	3	37	1.32	0.72
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Marantaceae

53	<i>Thalia dealbata</i>	<i>Ae</i>	0	1	0	0	0	0	0	0	0	0	0	1	5	0.00	0.00
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Cannaceae

54	<i>Canna indica</i>	<i>Ae</i>	0	0	0	0	0	1	0	0	0	0	0	1	6	0.00	0.00
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Zingiberaceae

55	<i>Etlingera</i>	<i>Ae</i>	0	1	0	0	0	0	0	0	0	0	0	1	7	0.00	0.00
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56	<i>Alpinia</i>	<i>Ae</i>	0	0	0	0	1	0	0	0	0	0	0	1	3	0.00	0.00
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Note: Ac: *Acrapex*, Bu: *Busseola*, Ca: *Carelis*, Ch: *Chilo*, Ed: *Eldana*, Se: *Sesamia*, Pi: *Pirateolea*, Po: *Poeonoma*, Th: *Thaumatotibia*, Bt: *Bactra*, Sp: *Speia*, Ng: Number of genera per host plant, Ia: Individual abundance of stemborers per host plant, H': Shannon index and D: Simpson index; Niche: Ae: Aesthetic, Aq: Aquatic, Dl: Dryland, G: Glassland, H: humidland

3.3. Host plant-stemborer interaction network

A complete dataset of the analysis of the plant-stemborer interaction network comprising 56 plant species and 18 stemborer species specifically shows that 64.2% (N = 36) of the host plant species belong to Poaceae, while 23.6% (N = 13) of the plant species belong to Cyperaceae. The host plant families Typhaceae and Zingiberaceae were represented by two individuals only for each family. The remaining families were represented by a single species (Tab. 3.3). The analysis of network indices showed a high linkage density ($L_d = 5.86$), indicating multiple associations between species at trophic levels. The specialization index ($H'_2 = 0.63$) reflects moderate niche partitioning, while the analysis of stemborer species connections showed high generality ($HL = 9.57$). Furthermore, host plants showed moderate vulnerability ($LL = 2.15$) to being attacked by an average of two stemborer species (Fig. 3.3)

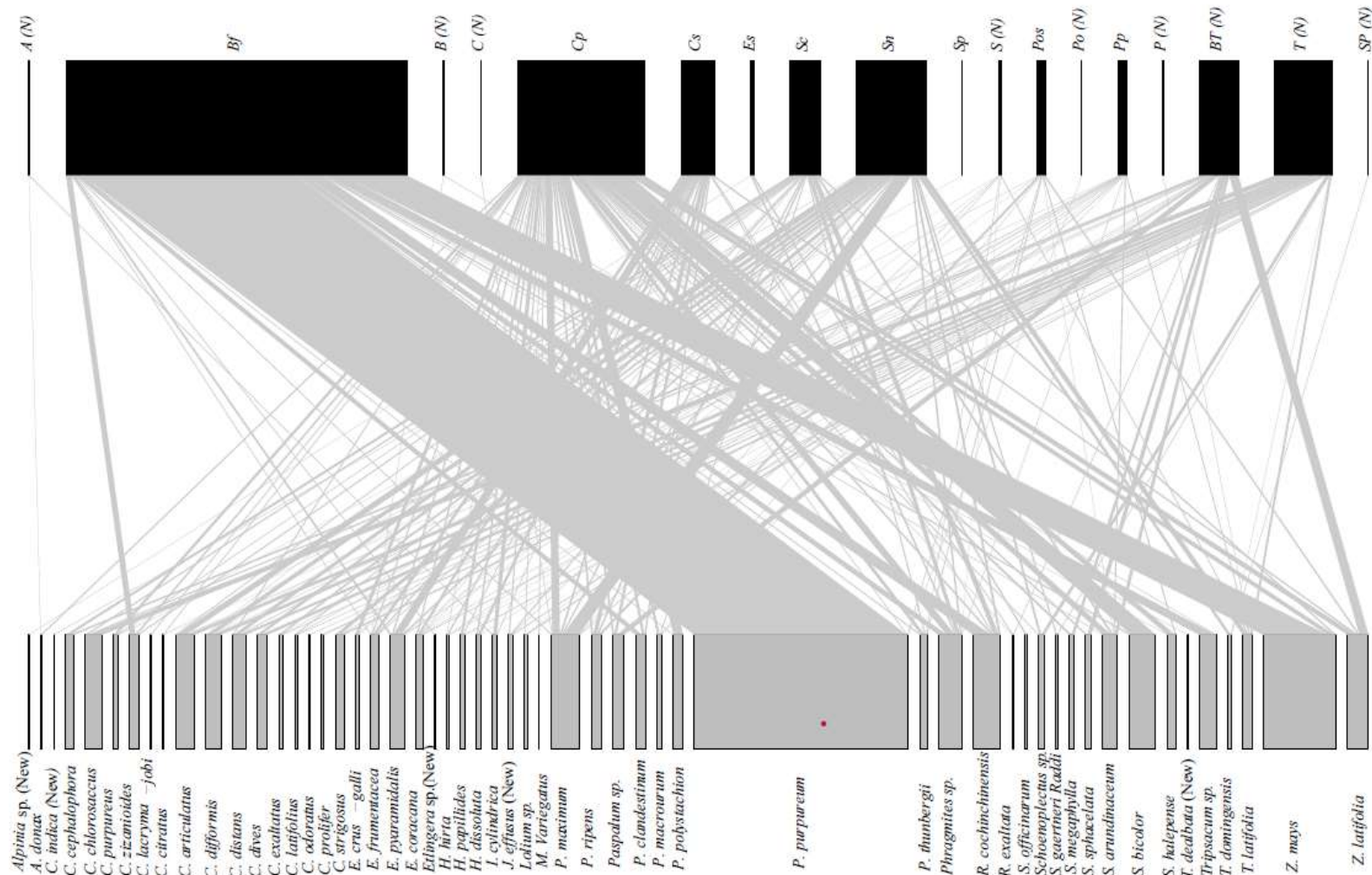


Figure 3. 3. Stemborer–host plant interaction network of complete data set showing connections between stemborer species (top) and host plants (bottom). The abbreviations of the stemborers and host plants refer to Table 3.2 for stemborers, while those of the plant refer to Table 3.3 of the species or genera names.

The analysis of sub-data sets on host plant distribution in surveyed areas shows that urban wetland and its riparian mosaics counted 83.9% (N = 47), followed by montane forest and its wetlands, with 58.9% (N = 33) of the total host plants recorded. The subhumid savanna of Akagera and its wetlands represented 55.3% (N = 31), while the Afroalpine mountain and its wetland mosaics had the lowest abundance of host plants with 12.5% (N = 7). In addition, five host plants of stemborers, namely *Canna indica* (Cannaceae), *Thalia dealbata* (Marantaceae), *Juncus effusus* (Juncaceae), and two species of *Etlingera* sp. and *Alpinia* sp. (Zingiberaceae) were reported as host plants for lepidopteran stemborers for the first time, in addition to rarely stemborer host plants known as *Schoenoplectus* and *Scleria gaertneri* from the Cyperaceae family.

The analysis of the ecological interaction network of stemborer-host plants in four ecological vegetation mosaics revealed a different structure of the interaction network (Fig.3.6). Urban wetland and its riparian mosaics showed the highest density of linkage ($L_d = 6.38$) and generality ($H_L = 10.15$), along with the lowest specialization ($H'_2 = 0.58$). In contrast, the Afroalpine mountain and its wetlands exhibited the lowest link density ($L_d = 1.46$) and generality ($H_L = 1.82$), but the highest specialization ($H'_2 = 0.95$). Intermediate values were observed for the montane forest and its vegetation mosaic of wetlands ($L_d = 3.15$; $H'_2 = 0.75$; $H_L = 4.69$) and the subhumid savanna of Akagera sub-humid savanna and its wetlands ($L_d = 3.00$; $H'_2 = 0.74$; $H_L = 4.48$). The vulnerability analysis of the ecological mosaics to stemborers showed a similar trend, where urban wetlands and riparian zones were the most exposed ($LL = 2.60$) while the Afroalpine mountain was the least exposed to stemborer attacks ($LL = 1.11$) (Fig. 3.4).

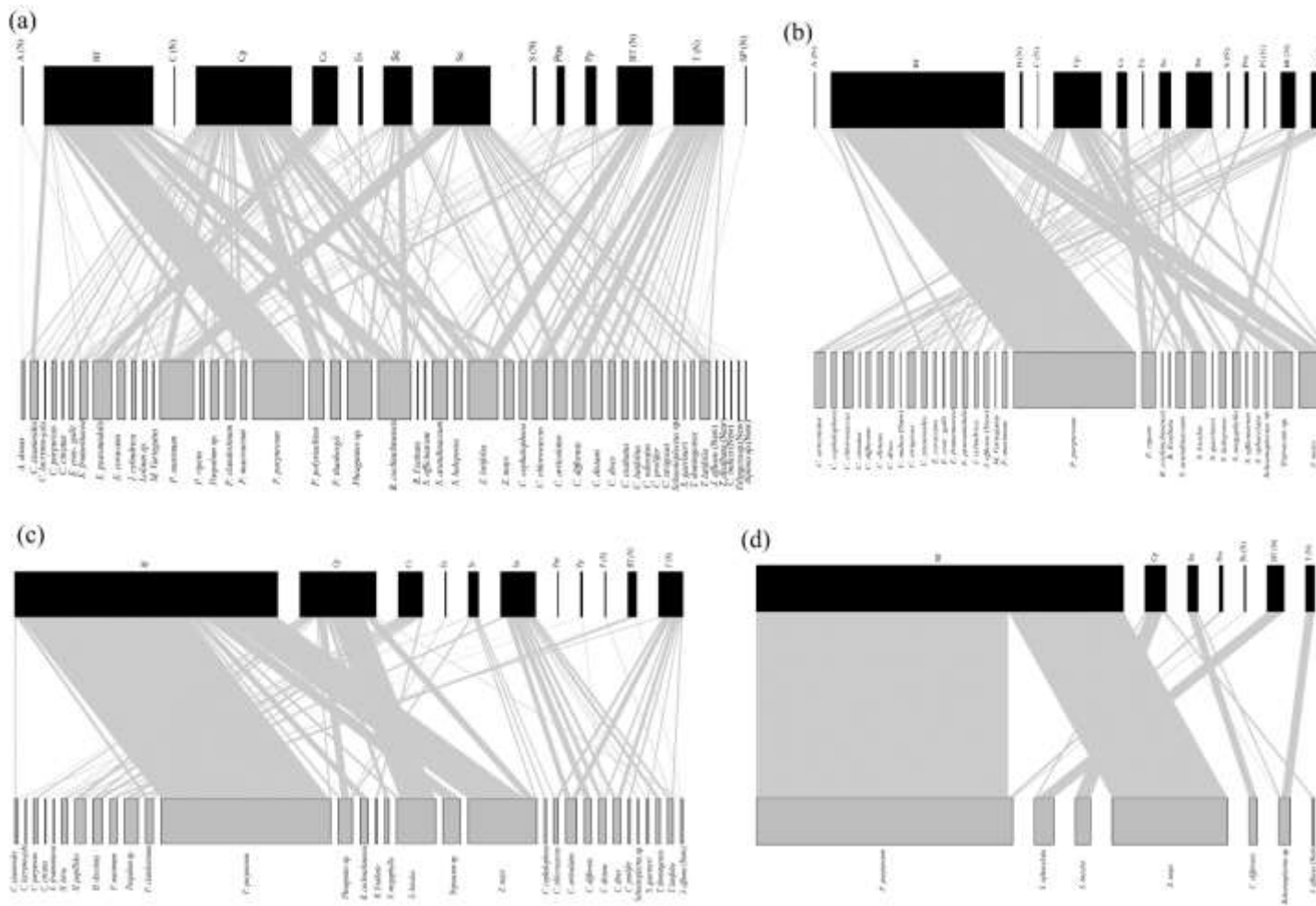


Figure 3. 4. Stemborer–host plant interaction network of subdataset. Stemborer species (top) and host plants (bottom). The abbreviations of the stemborers and host plants refer to Table 3.3 for the stemborers. (a) Kigali wetlands & riparians, (b) Montane & Forest of Southern Midland, (c) Akagera Subhumid of Eastern, (d) Afroalpine of Northern highland. *Note that high resolution figures (a, b, c, d) are appendix 2.*

4. Discussion

The results of this study report 18 stemborers species representing 5 families which are distributed in 56 wild host plants. Higher abundance was in Wetland and riparians zone of Kigali City. This area forms the transition region between the Sudanese woodland savanna and the Guineo-Congolese vegetation mosaics, which supported higher number of host plants species, followed by the Akagera subhumid savanna and wetland of Eastern lowland zone of Rwanda. Given that the first zone overlaps with Kigali city, while the second covers the eastern parts of Rwanda, extending to the East African Akagera region in Tanzania and Uganda (Lillesø et al., 2024; SANBI et al., 2022; White, 1983), the observed diversity and high abundance of stemborers in these areas may likely be due to the high richness in wetter and wetlands microhabitats, often referred as the best hotpots of wild vegetation that can serve as alternative host plants of stemborers (Overholt et al., 2001; Le Rü et al., 2006b; Gofitshu et al., 2018). Similarly, a high diversity of noctuid stemborers was reported in humid and grassland mosaics in the Ethiopia (Gofitshu et al., 2018), South Africa and Mozambique (Moolman et al., 2014), and in West Africa (Hailemichael et al., 2009; Ong'amo et al., 2014).

The results of study report the presence of rare species sampled areas, dominating in Eastern lowland of Rwanda. These species varied in number and species composition within genera. Foreexample, a single individual from *Carelis* and two individuals from *Acrapex* were reported. Similar findings on few numbers of rare species in natural habitat were reported during stemborer surveys in eastern (Nye, 1960; Le Rü et al., 2006b, 2006a; Gofitshu et al., 2018), southern (Moolman et al., 2014; Assefa et al., 2017; Moeng et al., 2018) and western (Hailemichael et al., 2009; Ndemah et al., 2007; Ong'amo et al., 2014) regions of Africa. This may not necessarily indicate sampling artifacts, since high sampling efforts increase the incidence of rare species (Le Rü et al., 2014). Other studies in eastern and southern Africa reported a high rate of rare and endemic noctuid stemborers such as *Acrapex*, *Bactra*, *Carelis*, *Pirateolea* (Moyal et al., 2011; Le Rü et al., 2014; Moeng et al., 2018; Gofitshu et al., 2018) and *Speia* genera (Moolman et al., 2014).

This study documented the widespread occurrence of two major economically important stemborer species, i.e. *B. fusca* and *C. partellus* across surveyed zones. Beyond its distribution, *B. fusca* was the only species that colonized the high altitude of the Afroalpine mountain zone of northern part

of Rwanda. *Chilo partellus* dominated eastern lowland and coexisted with *Sesamia* species at the central mid altitude zones, which is the transition zones between the low altitude of the Akagera subhumid savanna and the high altitude of the Afroalpine mountain zones. The dominance of these species by altitudinal gradient were previously reported in Kenya and Tanzania (Mwalusepo et al., 2018; Ong'amo et al., 2006).

Ethiopia This align with previous studies that reported *B. fusca* as the dominant species in high altitudinal zones, while *C. partellus* dominated low altitudinal zones (Mwalusepo et al., 2018; Mutamiswa et al., 2022). Nevertheless, on this different distribution, the study alarms the ecological shifting in *B. fusca* and *C. partellus* which could be critical to the current pressing climate change and fragmented habitats. In Ethiopia, researchers predict that this shifting will continue and this will affect agriculture productivity (Getu E. et al., 2002). We recommend future research toward factors that control these shifts for sustainable adaptive IPM.

The study findings revealed an unusual pattern, with a remarkably high abundance of *Thaumatotibia* individuals infesting sedges (*Carex* and *Cyperus*, family Cyperaceae), a smaller presence in grasses (*Eleusine*, *Hyparrhenia*, and *Hyperthelia*, family Poaceae) and only a few detected in cattails (*Typha*, Typhaceae) in Rwanda. This is particularly striking given that all previously known members of *Thaumatotibia*, such as *T. leucotreta* (false codling moth), are globally recognized as fruit borers, primarily infesting citrus, stone fruits, and macadamia nuts (European Food Safety Authority (EFSA) et al., 2020).

To date, there have been no documented reports of species belonging to the genus *Thaumatotibia* that behave as stemborers and feed on plant stems. This unexpected finding raises important ecological, taxonomic, or host expansion questions and suggests a behavioral shift or the presence of an undescribed or misclassified species within the genus. Given the importance of quarantine and the polyphagous nature of *Thaumatotibia* species of *Thaumatotibia* like *T. leucotreta* (European Food Safety Authority (EFSA) et al., 2020), more research is warranted to confirm the stem feeding behavior of *Thaumatotibia* for quick integrated management action.

New host plants from introduced and adapted as home and entertainment spaces flowers were found hosting stemborers.

These host plants are *Etlingera* sp. and *Alpinia* sp Zingiberaceae, *Canna indica* (Cannaceae) and *Thalia dealbata* (Marantaceae) and initially adopted for horticultural and aesthetic purposes in urban gardens periurban landscapes.

Such urban-driven vegetation structures can create microhabitats that may assist arthropods like stemborer persistence or serve as refuge (Bowler et al., 2025; Morpurgo et al., 2024). Such shifts may reflect the capacity of insects to exploit novel resources in fragmented urban landscapes as previously reported by Planchuelo et al., (2020), Tartaglia and Aronson (2024). This finding raises critical questions about the ecological roles of these new host plants, whether they serve as true hosts or temporary refuges in the network structure of stemborer-host plants in urban ecosystems, and underscores the need for further investigation. Suggesting that extending ecological assessments based on stemborer response to current urban eco-tourism and urban greening initiatives is important for more accurate information to for early warning and planning for horticulture and gardens IPM.

This study employed an active field-based approach, focusing on the detection of larval damage symptoms at irregular intervals across wild habitats to assess stemborer diversity within different vegetation mosaics. This method facilitated a rapid and efficient inventory of stemborer species associated with wild host plants. However, it presents inherent limitations for rigorous quantitative comparisons among regions, as some infested plants may remain undetected. In addition, the likelihood of missing certain stemborer species cannot be excluded when sampling does not coincide with their larval or pupal stages. To minimize these constraints, surveys were conducted across multiple seasons to maximize species detection within similar ecological niches. Comparable integrative sampling approaches have been applied in previous studies on stemborer diversity in East Africa

5. Conclusions

This study documented 18 Lepidopteran stemborer species associated with 56 host plants in multiple agroecological mosaic zones in Rwanda and six species associated with 13 host plants in Ethiopia. In both countries, revealed a wide spread of *Busseola fusca* (Fuller) (Noctuidae) which is the most economically important stemborer. The species proved it high complex interaction with wild habitats. While most species were recorded in wild grasses and sedges, several species were found in fodders that are adopted around cultivated land boundaries for soil stability and livestock feed, challenging management strategies. Given ongoing habitat fragmentation by agricultural and urban expansions, there is a plausible risk of host range shifts and the emergence of new pests from species currently confined to natural habitats that may be associated with our findings of the polyphagous fruits-feeder genus *Thaumatotibia* that is newly feeding on stems and four new plant families. Cannaceae (*Canna indica*), Marantaceae (*Thalia dealbata*), Juncaceae (*Juncus effusus*) and Zingiberaceae (*Etlingera* sp. and *Alpinia* sp.) previously unreported on a global list of host plants of stemborers. These findings underscore the need for continuous monitoring and integrated adaptive pest management strategies that account for both natural and cultivated ecosystems.

4. SPECIES DIVERSITY AND RELATIVE IMPORTANCE OF STEMBORER PESTS IN CULTIVATED PLANTS AMONG DIFFERENT AGRO-CLIMATIC CONDITIONS OF RWANDA

4.1. Assessing interactions between crop-wild host plants and stemborer species in Rwanda

Abstract

Lepidopteran stemborers are potential pests of cereal crops particularly maize and sorghum in East and southern Africa. Except for *Chilo partellus* (Swinhoe), the other important lepidopteran stemborers like *Busseola fusca* (Fuller), and *Sesamia calamistis* Hampson, are indigenous to the Africa and native to wild habitats. While some species remain in wild habitats, more species became pest and expended to cereal crops. However, understanding their host range and their population dynamics in uncultivated habitats and their switch to cultivated crops remained receiving a little attention. To respond to the gap, a survey species diversity, host alternative, and their seasonal switch to cultivated crops were studies during both rainy (maize growing) and dry seasons of 2023–2024, targeting infested maize fields and surrounding wild vegetation. A total of 2,691 stemborer individuals were recovered from nine host plants, with 1474 (54.8%) from wild and fodder vegetation and 1217 (45.2%) from maize plantations. Species richness was highest in mid-altitude zone, while overall abundance peaked at low altitudes. *Busseola fusca* was the most abundant in high-altitude zone, *Chilo partellus* in the low altitude, and *Sesamia* spp. were concentrated in the mid-altitudes. Seasonal variation significantly influenced population dynamic, with the highest abundance (1,251; 46.4%) recorded during the dry season. Notably, *Pennisetum purpureum* (Napier grass) hosted 1,156 (42.9%) of all specimens, highlighting its role as a key refugium during maize off seasons. These findings underscore the ecological importance of wild vegetation in sustaining stemborer populations and suggest that wild vegetation, altitudinal and seasonal factors must be considered in designing integrated lepidopteran stemborers pest management strategies.

Key words: Agroecology, Lepidoptera, Cultivated crops, Wild vegetation, Host-switching

4.1.1. Introduction

The ecological and agricultural significance of lepidopteran stemborers lies not merely in their presence but also in the extent of their impacts on crop productivity across diverse environments (Assefa et al., 2010; Goftishu et al., 2017; Ntirenganya et al., 2025). They tunnel and cloaked inside the host plant's stems and feed on the internal cavity of the plant (Overholt et al., 2001; Assefa, 2006; Goftishu et al., 2017;), making them very difficult to control (Ntirenganya et al., 2025). More effort has been devoted to understanding stemborers' ecological habitats and their relationships with host plants (Midega et al. 2015; Goftishu et al. 2018; Ntirenganya et al. 2025). However, the interactions between crop and wild stemborer hosts remain poorly understood, limiting our ability to develop integrated management strategies that effectively address both the pests and the full range of their host plants (Ong'amo et al., 2018).

In the past, assessments of stemborer species and their host plants were conducted (Overholt et al., 2001; Moolman et al., 2014;). From these studies, about 150 lepidopteran stemborer species (families Crambidae, Noctuidae, Pyralidae, and Tortricidae) were reported (Le Rü et al., 2006a), of which more than 21 species have major effects on agricultural yield, attacking maize, sorghum, wheat, rice, sugarcane, and related cereal crops across tropical and subtropical regions (Overholt et al., 2001; Sokame et al., 2019). The remaining species occur primarily in wild plants under the Poaceae, Cyperaceae, Typhaceae and related vegetation families, where they play important ecological roles as native herbivores. These vegetation serve as reservoirs for pest (Tefera, 2004; Le Rü et al., 2006b).

In Sub-Saharan Africa, Noctuids *Buseola fusca* (Fuller 1901) and *Sesamia calamistis* (Hampson 1910), crambids *Chilo partellus* (Swinhoe 1885), *Chilo orichalcociliellus* (Strand), and the pyralid *Eldana saccharina* (Walker 1865) are key stemborers widely distributed (Assefa, 2006; Ntirenganya et al., 2025). With the exception of *C. partellus*, which is native to Asia (Kfir, 1992; Kfir et al., 2002 Asmare et al., 2014), these species are native to Africa (Kfir, 1992; Khan et al., 1997; Maes, 1998). *Sesamia calamistis* Hampson, 1910, *Sesamia nonagrioides* Lefèbvre, 1827, and other *Sesamia* species are widely distributed across sub-Saharan Africa, including West, Central, East, and Southern Africa, and extending to Indian Ocean islands (Madagascar, Réunion) predominantly in humid to sub-humid agroecosystems (Kanya et al., 2004; Le Rü et al., 2006b,

2006a; Moolman et al., 2014). Studies of dynamics of these species in their habitats reported the influence of altitude, temperature, humidity, and cropping systems, with seasonal fluctuations affecting their community structure (Gounou et al., 2009; Régnier et al., 2023; Ntirenganya et al., 2025).

Phylogeographical studies have indicated that African maize stemborer, *Busseola fusca*, is natively adapted to higher altitudes commonly above 1500 m (Asmare *et al.* 2014; Calatayud *et al.* 2014). With climate change and variation, this species was also observed expanding its range to mid and low altitudes of less than 1500 m in East Africa (Calatayud *et al.* 2014; Goftishu *et al.* 2017; Mutamiswa *et al.* 2017). Similarly, recent surveys conducted in Ethiopia indicated that *Chilo partellus*, naturally adapted to low altitude with warmer temperature has widened its agroecological distribution to high altitude of 2084 m.a.s.l (Tamiru, A. et al., 2008; Asmare et al., 2014; Goftishu et al., 2018). In other sides, a reasonable number of *Sesamia calamistis* was continuously reported across all altitudes in Ethiopia (Goftishu et al., 2018, 2017; Melaku et al., 2006; Tefera, 2004; Wale et al., 2007), with a relative increase in mid-altitude of most parts of East Africa (Le Rü et al., 2006b, 2006a; Kaçar et al., 2023).

Despite decades of research on lepidopteran stemborers, critical gaps persist in our understanding of their ecology, particularly regarding interactions with host plants, species-specific seasonal dynamics, and adaptive responses to climatic variability (Ong'amo, 2005). Ecological role of wild vegetation surrounding farmlands remains contested (Ntirenganya et al., 2026). Some studies suggested the low stemborers abundance in wild habitats (Kankonda et al., 2017; Le Rü et al., 2006b, 2006a; Ong'amo, 2005), while others reported active crop–wild vegetation exchanges that challenge traditional assumptions about stemborer habitat specialization and pest emergence (Khan et al., 2007; Midega et al., 2015; Assefa et al., 2010;Goftishu et al., 2018).

Emerging evidence also suggests that non-economic stemborer species may evolve into significant pests under changing land use and climatic conditions (Gounou et al., 2009; Mutamiswa et al., 2022; Régnier et al., 2023). These dynamics underscore the need for localized ecological assessments, particularly in understudied regions such as Rwanda, where information on stemborer community structure and agroecological functioning is limited.

Addressing these knowledge gaps is essential for developing climate-resilient and ecologically informed pest management strategies.

This study aims to address critical gaps in the ecology of lepidopteran stemborers in Rwanda by (i) conducting the first assessment of stemborer species diversity and abundance across different altitudinal zones, (ii) evaluating seasonal variation in infestation patterns, and (iii) determining the role of wild host plants persistence of stemborers populations. We hypothesize that higher altitudes support fewer stemborers due to cold microclimates , while lower altitudes may exhibit higher overall richness driven by warmer temperatures and continuous cropping systems (Mwalusepo et al., 2018; Mutamiswa et al., 2022;). Mid-altitude zone with moderate temperatures may facilitate co-existence of multiple stemborer species (Mwalusepo et al., 2018, 2015). Seasonal variation is expected to influence stemborers community dynamics by switching from maize to alternative host plants during the dry season when maize is is not in the field (Van Den Berg et al., 1991; Khan et al., 2007; Ntiri et al., 2019;). Conversely, maize plantations are expected to show peak infestation levels during the long rainy season, with the availability of preferred hosts, and favorable humidity that support rapid larval development. Wild host plants such as *Pennisetum purpureum* (Napier grass) could serve as persistent reservoirs that sustain stemborer populations across seasons (Ong'amo, 2005; Moolman et al., 2014;). The findings contribute to improved resilience in smallholder farming systems while supporting broader goals of biodiversity conservation and sustainable agriculture intensification.

4.1.2. Materials and Methods

4.1.2.1. Study zones characteristics

The study was conducted (2023-2024) across three ecologically distinct zones of Rwanda: Eastern, Southern, and Northern provinces, each representing unique biogeographical zones and unique vegetation types (MINAGRI, 2021; SANBI et al., 2022). Kayonza district (1°51'04" S, 30°39'04" E; ~1324 m a.s.l.) represented eastern province. This district lies within the low altitudinal zone of Akagera sub-humid savanna region, part of the eastern savannah corridor, characterized by *Cyperus papyrus*, hippo grass (*Vossia cuspidata*), and swamp forest vegetation along floodplains and wetland valleys (Ruticumugambi et al., 2024; SANBI et al., 2022).

Kamonyi district (2°00'15" S, 29°54'19" E; ~1661 m a.s.l.) represented southern province. The district is located in mid altitudinal zone in montane forest region extending to the Albertine Rift, dominated by upper montane forests, and grasses (Lillesø et al., 2024; SANBI et al., 2022). Burera district (1°28'26" S, 29°50'05" E; ~2182 m a.s.l.) represented northern province. The district is located in high altitude zone encompassing Afroalpine mountain ecosystems with extensive wetlands dominated by Poaceae (*Miscanthidium* and *Pennisetum*), Typhaceae, and Cyperaceae families (Nsengimana et al., 2025; SANBI et al., 2022) (Fig. 4.1).

Although the sampled areas of southern and central regions of Rwanda exhibit overlapping altitude ranges with parts of the eastern region due to similar geomorphological processes, they are classified into distinct altitudinal zones based on differences in elevation gradients, vegetation structure, and microclimatic conditions (Lillesø et al., 2024; MINAGRI, 2021; SANBI et al., 2022), which are key ecological factors influencing stemborers community structure (Mutamiswa et al., 2022; Mwalusepo et al., 2018; Tamiru et al., 2007). Details on sampled areas are given in Table 4. 1.

Table 4. 1. Geographical description of sampled sites and their respective altitudes and the nature of the landscape in Rwanda

Location	GPS-coordinates	Altitude (m a.s.l)	Landscape
1. Burera-Cyeru-Ndongezi	S1°32'0"; E29°50'6"	2,010.9	Highland
2. Burera-Cyeru-Butare	S1°30'26"; E29°49'44"	2,019	Highland
3. Burera-Cyeru-Ruyange	S1°30'0"; E29°49'0"	2,080	Highland
4. Burera-Nemba-Kavumu	S1°35'30"; E29°51'39"	2,097	Highland
5. Burera-Nemba-Rubona	S1°34'40"; E29°53'7"	2,020	Highland
6. Burera-Nemba-Rushara	S1°33'53"; E29°50'53"	2,030	Highland
7. Burera-Rusarabuye-Kabona	S1°26'17"; E29°49'43"	2,274	Highland
8. Burera-Rusarabuye-Ndago	S1°27'44"; E29°48'27"	2,018	Highland
9. Burera-Rusarabuye-Ruhanga	S1°27'20"; E29°49'43"	2,234	Highland
10. Kamonyi-Musambira-Buhoro	S1°27'20"; E29°49'43"	1,699	Midland
11. Kamonyi-Musambira-Kivumu	S2°2'29"; E29°48'47"	1,713	Midland
12. Kamonyi-Musambira-Cyambwe	S2°3'8"; E29°51'26"	1,581	Midland
13. Kamonyi-Runda-Gihara	S1°56'15"; E29°58'10"	1,684	Midland
14. Kamonyi-Runda-Muganza	S1°57'9"; E29°58'14"	1,567	Midland
15. Kamonyi-Runda-Ruyenzi	S1°57'46"; E29°59'20"	1,508	Midland

Table 4.1. continued

16. Kamonyi-Rugalika-Kigese	S2°0'38"; E29°57'53"	1,501	Midland
17. Kamonyi-Rugalika-Nyarubuye	S2°2'14"; E29°57'20"	1,624	Midland
18. Kamonyi-Rugalika-Nyarubuye	S2°2'14"; E29°57'20"	1,624	Midland
19. Kayonza-Gahini-Kahi	S1°45'35"; E30°37'8"	1,435	Lowland
20. Kayonza-Gahini-Kagarama	S1°51'45"; E30°29'48"	1,456.8	Lowland
21. Kayonza-Gahini-Jambo Beach	S1°50'54"; E30°28'43"	1,485.2	Lowland
22. Kayonza-Kabare-Mubumba	S2°4'12"; E30°39'52"	1,497	Lowland
23. Kayonza-Kabare-Gashiru	S2°4'59"; E30°41'34"	1,314	Lowland
24. Kayonza-Kabare-Gituku	S2°6'44"; E30°37'34"	1,482	Lowland
25. Kayonza-Murundi-Buhabwa	S1°45'17"; E30°29'37"	1,428	Lowland
26. Kayonza-Murundi-Karambi	S1°42'26"; E30°28'17"	1,385	Lowland
27. Kayonza-Murundi-Ngugu	S1°40'34"; E30°28'42"	1,355	Lowland

Data were organized from MINAGRI (2021) and SANBI et al. (2022)

Each farm was subdivided into five 3 m × 3 m sampling quadrants, and all maize stems within these quadrants were systematically examined for the presence of stemborer infestation. Wild vegetation was surveyed in natural habitats along the margins of maize farms, with sampling sites located within a maximum distance of 50 m from each sampled cultivated field.

Sampling was guided by a targeted selection of vegetation families known to host stemborers or closely related taxa. This purposive sampling strategy, rather than purely random selection, was adopted based on recommendations from previous studies that reported low stemborer population densities in wild vegetation (Goftishu et al., 2018; Kanya et al., 2004; Otieno et al., 2008). The method aimed to increase the likelihood of detecting stemborers by focusing on vegetation with higher ecological relevance to the pest.

4.1.2.3. Data Collection and rearing of Stemborers

At each locality, both cultivated maize and farm margin's wild vegetation were carefully checked for stemborer infestation symptoms such as wilting leaves, dry leaves, dead hearts, holes bored on stems, and fresh frass (Goftishu et al., 2018; Moeng et al., 2018). For wild vegetation, special consideration was given to species belonging to the Poaceae, Cyperaceae, and Typhaceae families with known records of host stemborers in cultivated and wild ecosystems (Goftishu et al., 2018; Otieno et al., 2008). Infested plants were cut, dissected, and carefully checked for the presence of stemborers. Recovered larvae were transferred to artificial diets in plastic vials (7.5 × 2.5 cm) closed with cotton and kept in the laboratory until pupation (Devi and Kaur, 2015; Goftishu et al., 2018; Overholt et al., 2001). Recovered pupae and egg colonies were kept separately in plastic vials (15 × 7 cm) closed with cottonwool until emergence (Moeng et al., 2018).

4.1.2.4. Sample processing and laboratory identification

Identification of stemborers to genus and species levels was done using microscopic observations, following established morphological keys. Primary identification followed the guide for stemborers identification developed by the International Centre of Insect Physiology and Ecology (ICIPE) (Overholt et al., 2001), the recently published *Sesamia* species identification key (Le Rü et al., 2022), and the insect identification platform of Lucid Central (<https://www.lucidcentral.org>).

To ensure taxonomic accuracy, identifications were further validated in consultation with stemborer specialists from ICIPE. Voucher specimens were deposited at the zoology laboratory, biology department, College of Science and Technology, University of Rwanda, under the stemborers collection (access code: WS2023 and WS2024).

4.1.2.5. Statistical data analysis

Stemborer communities were analyzed by comparing their abundance and diversity across three altitudinal zones (low, mid, and high), seasonal conditions (dry season, long rainy season, and short rainy season), and host plant species. Abundance was quantified by counting the number of individual adult heads rather than using mean estimates per agroecology. For egg colonies, each colony was considered as a single individual after adult emergence, as all individuals within a colony originated from the same oviposition event. To compare diversity across altitudes and seasons, multiple diversity indices Shannon diversity (Shannon, 1948), Simpson (Simpson, 1949) were calculated and created rarefaction curves at 95% confidence intervals using bootstrap methods in iNEXT package (Morris et al., 2014). Chi-square tests were used for testing independence or differences in proportions while non-parametric Kruskal-Wallis test was used when assumptions of normality were as alternative tests. To test the influence of altitude and season on species assemblages, we performed permutation multivariate analysis of variance (PERMANOVA) and Non-metric Multidimensional Scaling (NMDS) to demonstrate and visualize compositional differences, providing complementary insights into community structure beyond richness alone (Hsieh et al., 2016). To test whether seasonal variation reflected stemborer movement between maize and alternative hosts, we analyzed community dispersion by using marginally significant permutation dispersion (PERMDISP, $p = 0.055$) (Anderson, 2017). All statistical data analysis were performed in R Statistics software version 4.4.0 at significance of 0.05 for all tests (R Core Team, 2025).

4.1.3. Results

4.1.3.1. General abundance of stemborers

A total of 2,756 stemborer individuals were collected during the study period. Of these, 2,691 (97,6%) were successfully identified to species or genus levels, while 65 individuals were classified only to the family level.

These unidentified individuals were included in the analyses restricted to developmental stages. Across developmental stages, the larvae were overwhelmingly abundant (n = 2,604; 94.5%), followed by pupae (n = 131; 4.7%) and egg colonies (n = 21; 0.8%) (Fig. 4. 2). Species level abundance patterns showed that *B. fusca* was the most abundant species (n = 1068; 39.68%), followed by *C.o partellus* (n = 919; 34.15%), *S. calamistis* (n = 617; 22.92%), *S. nonagrioides* (n = 77; 2.8%), and the least represented was *E. saccharina* (n = 9; 0.45%).

Abundance varied across altitudinal zones. Higher abundance (n = 1,076; 39.2%) was recorded in low altitude, followed by mid-altitude (n = 942; 34.1%), and high altitudinal (n = 738; 26.7%) zones. Seasonal variations also had a notable effect: the dry season exhibited the highest abundance (n = 1,302; 47.2%), compared to the long rainy season (n = 956; 34.7%) and the short rainy season (n = 498; 18.1%). The significant chi-square test ($\chi^2 = 67.52$, df = 4, p < 0.001) confirms a strong seasonal pattern in stemborer developmental stages distribution. Larvae dominated samples during the long rainy season, whereas pupae were mainly sampled during the dry season.

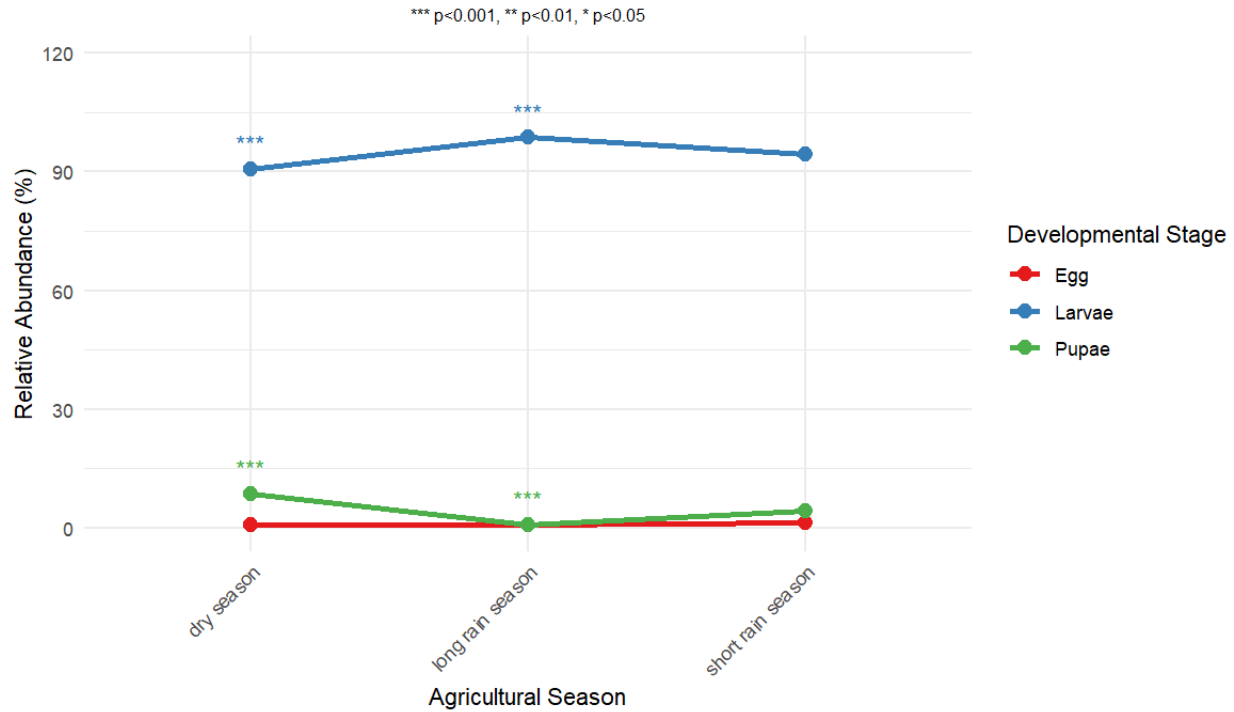


Figure 4. 2. Relative abundance and distributions of stemborers developmental stages where *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ show the level of significances

4.1.3.2. Stemborers abundance across altitudinal gradients

Analysis of the effect of altitude on stemborer species revealed that *B. fusca* was the most abundant species at high-altitude ($n = 726$; 26.97%), compared to the mid-altitude ($n = 214$; 7.95%) and a low altitude ($n = 128$; 4.75%) zones. In contrast, *C. partellus* species showed an opposite pattern with highest abundance at low altitude ($n = 705$; 26.2%), followed by mid-altitude ($n = 211$; 7.84%), and only three individuals were recorded at higher altitude zones. *Sesamia* species was the most numerous in mid-altitude ($n = 474$; 17.6%) compared to low altitude ($n = 220$; 8.17%). As expected for a species that is not strongly associated with maize cultivation, *E. saccharina* was rarely encountered, represented by eight individuals at mid-altitude and a single individual at low altitude (Tab. 4. 2). Kruskal-Wallis test analysis revealed a significant difference in species distribution across the altitudinal gradients ($\chi^2 = 185.99$, $df = 10$, $p < 0.05$).

Table 4. 2. Distribution and abundance of stemborers across different altitudinal gradients in Rwanda, (0=Absence) where abbreviations represent genera of stemborers

S/N	Species	Low altitude			Mid-altitude			High altitude			p-value
		Dry season	Short Rain season	Long rainy season	Dry season	Short rainy season	Long rainy season	Dry season	Short rainy season	Long rainy season	
1	<i>Busseola fusca</i>	69	18	41	86	38	90	462	97	167	0.045
2	<i>Chilo partellus</i>	330	158	217	55	74	82	3	0	0	0.003
3	<i>Sesamia calamistis</i>	76	28	87	114	73	239	0	0	0	0.008
4	<i>Sesamia nonagrioides</i>	9	2	18	37	0	11	0	0	0	0.007
5	<i>Eldana saccharina</i>	1	0	0	8	0	0	0	0	0	0.028

4.1.3.3. Stemborers abundances across seasons

Seasonal patterns showed a clear and significant influence on stemborer abundance. Overall, dry season showed a higher number of identified stemborers individuals (n = 1251; 46.48%), followed by the long rainy season (n = 951; 35.4%) and the short rainy season (n = 498; 18.5%). Species-level responses further highlighted distinct ecological preferences. *B. fusca* peaked with 617 (49.3%) individual species during the dry season. *C. partellus* maintains relatively stable populations across dry season (n = 388; 31%) and long rain season (n = 298; 31.3%) but declines during the short rainy season (n = 153; 16%). *S. calamistis* was abundant in long rainy season (n = 329; 34.6%). In contrast, *E. saccharina* and *S. nonagrioides* remain consistently low across seasons with limited association to maize plantation (Fig. 4. 3) and Table 4.2. Statistically, Kruskal-Wallis test of species abundance across agricultural seasons shows that all species exhibit a significant population variation over seasons where p-values of statistical analysis of individual species response to seasonal variation are provided in table 4. 2.

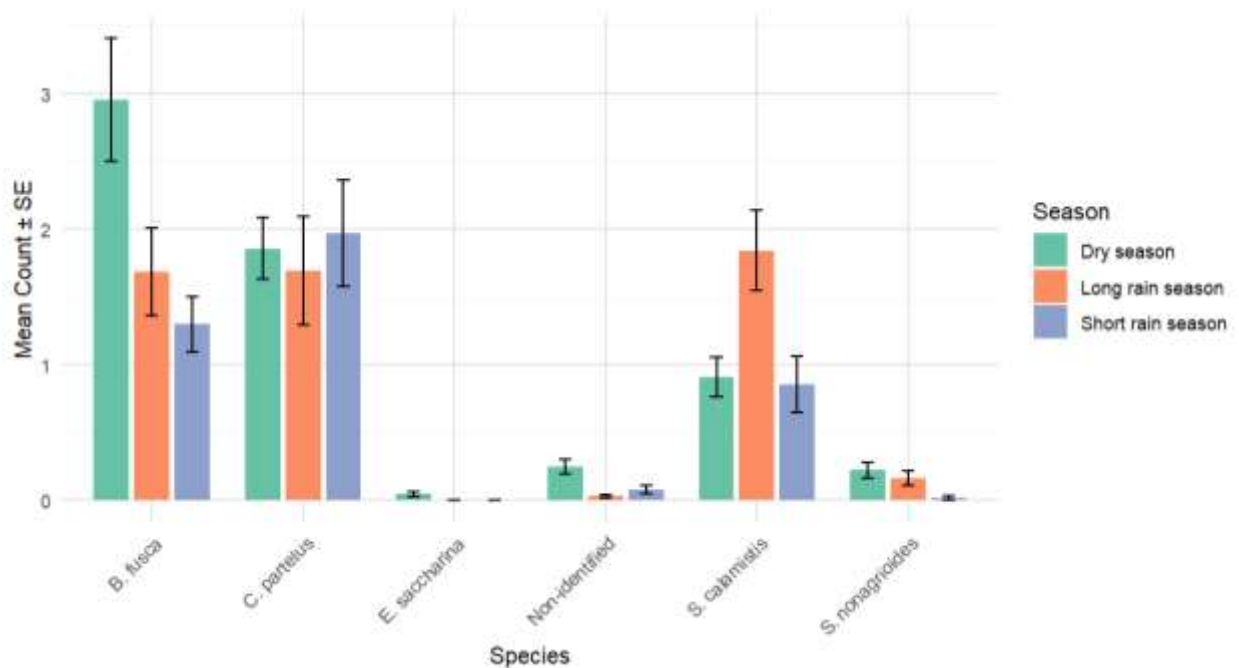


Figure 4. 3. Comparative analysis of seasonal population dynamics of stemborers (B=*Busseola*, C=*Chilo*, E=*Eldana*, S=*Sesamia*)

4.1.3.4. Stemborers abundances across host plants

Stemborer species abundance analysis in relation to the host plants revealed nine plants serving as alternative hosts, of which seven species belong to Poaceae family, while the Cyperaceae and Typhaceae were represented by a single species (Tab. 4. 3). In a total of 2691 stemborer individuals recorded, maize and Napier grass emerged as the predominant host plants (maize – 1217 individuals, 45.2%; Napier grass – 1156 individuals, 42.9%). The remaining 318 individuals (11.9%) were distributed among other wild host plants. Statistical analysis revealed a significant difference in species distribution across host plant types ($\chi^2 = 194.28$, $df = 14$, $p < 0.05$). Notably, an accelerated increase in stemborer abundance on wild vegetation was observed during periods when maize was absent, particularly in high and low altitudes zones (Tab. 4. 3).

Table 4. 3. Distribution and abundance of Stemborers by host plant across different altitudinal gradients and seasonal variations in Rwanda

No	Plant		Low altitude	Medium altitude			High altitude					
	Family	Scientific name	Common name	Dry season	Short rainy season	Long rainy season	Dry season	Short rainy season	Long rainy season	Dry season	Short rainy season	Long rainy season
1	Poaceae	<i>Zea mays</i> L. (C)	Maize	0	149	286	0	169	394	0	52	167
2	Poaceae	<i>Pennisetum purpureum</i> (W, F)	Napier grass	379	50	23	181	14	3	460	46	0
3	Poaceae	<i>Tripsacum mandersonii</i> (W, F)	Guatemala grass	29	0	2	41	0	0	3	0	0
4	Poaceae	<i>Chrysopogon zizanioides</i> (W, F)	vetiver	39	5	21	0	0	0	0	0	0
5	Typhaceae	<i>Typha</i> (W)	cattail	13	0	0	21	2	0	3	0	0
6	Cyperaceae	<i>Cyperus</i> . (W)	Cyperus	26	0	0	17	0	0	0	0	0
7	Poaceae	<i>Echinochloa pyramidalis</i> (W)	Grass	9	0	0	0	20	0	0	0	0
8	Poaceae	<i>Panicum</i> (W)	Grass	3	2	0	31	0	0	0	0	0
9	Poaceae	<i>Sorghum halepense</i> (W)	Johnson grass	11	0	0	20	0	0	0	0	0

C=Cultivated crop; F=Fodder; W=Wild; 0=Absence

4.1.3.5. Stemborers diversity and richness

Rarefaction curves have reached asymptotes and sample coverage exceeded 95% across all assemblages, indicating adequate sampling effort to capture the majority of stemborer species present. Sample-based rarefaction confirmed a significant difference in species richness among altitudinal gradients (Fig. 4. 4a) and indicated seasonal variation as potential regulator of stemborer community structure (Fig. 4. 4b). Diversity metrics further supported these patterns. The mid-altitude zone exhibited the highest Shannon Diversity Index ($H' = 1.239$), Simpson Index ($D = 0.675$), and Pielou's Evenness ($J' = 0.770$). In comparison, the low-altitude zone showed moderate diversity ($H' = 0.944$) and evenness ($J' = 0.587$). The high-altitude zone had extremely low diversity ($H' = 0.029$) and evenness ($J' = 0.041$). Shannon diversity declined significantly with altitude (Spearman's $\rho = -0.41$, $p < 0.001$).

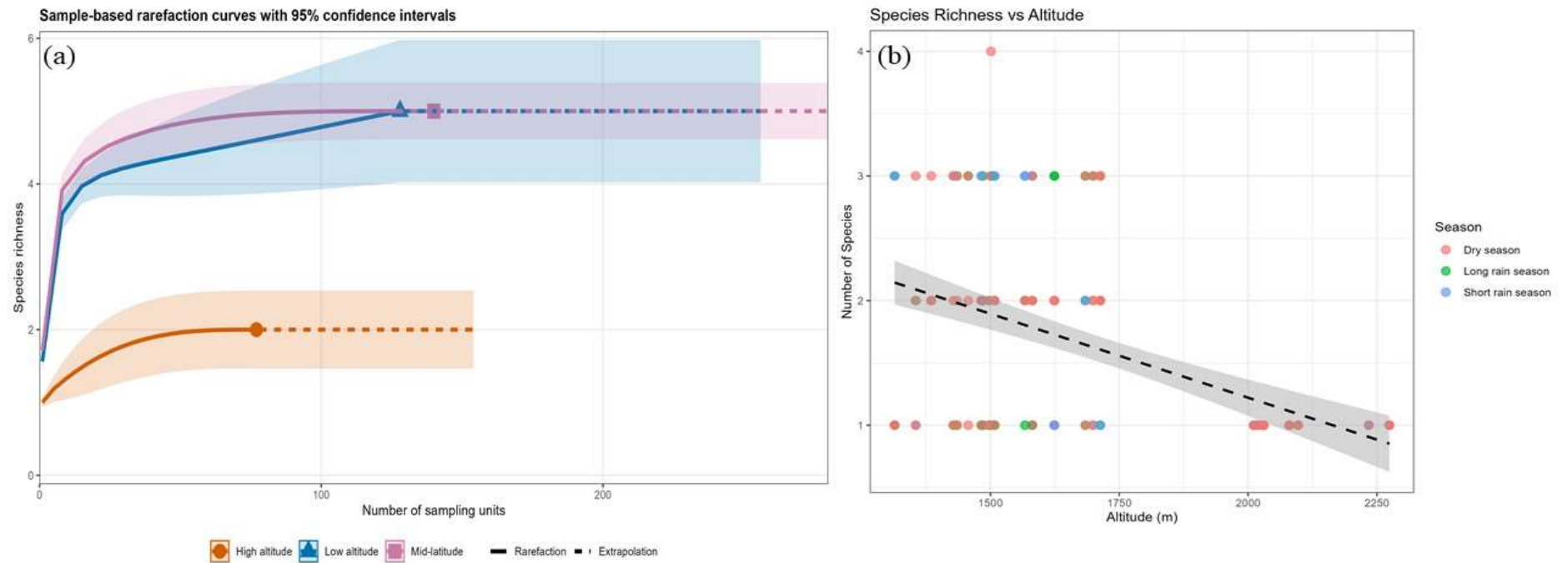


Figure 4. 4. Rarefaction and species richness. (a) Sample-based rarefaction curve with extrapolation (dotted line segments) at 95% confidence intervals (shaded areas); (b) Altitudinal species richness in response to seasonal variation

4.1.3.6. Altitude vs. season effects on stemborer communities

Results revealed altitude as the primary driver of community structure, explaining 16.5% of the observed variation in stemborers population ($R^2 = 0.1649$, $F = 35.73$, $p < 0.001$). Season also has a smaller but significant effect, accounting for 2.8% of stemborers community variation ($R^2 = 0.0282$, $F = 2.61$, $p = 0.002$). The analysis of altitude-season combined effect exhibited 21.2% of the community variation ($R^2 = 0.1876$, $F = 13.78$, $p < 0.001$) while the remaining 78.8% were attributed to unmeasured factors. Further, there was negligible shared variance between altitude and season (negligible shared variance: -0.2%).

4.1.3.7. Stemborers community structure and seasonal variation

The NMDS test indicated reliable representation of community dissimilarity patterns (Stress: 0.065, $R^2 = 0.212$, $F = 9.53$, $p < 0.001$). Environmental vector fitting assessment identified altitude as the strongest ecological driver shaping stemborers community patterns, explaining 51% of ordination variance ($p < 0.001$) (Fi. 5a). In addition, the permutational multivariate analysis of dispersion (PERMDISP) revealed marginally significant differences in stemborer groups dispersion among seasons ($F = 2.83$, $p = 0.055$), with short rainy season, showing significantly lower dispersion (mean distance to centroid = 0.522) compared to dry season (0.577) and long rainy season (0.584) (Fig. 5b). Furthermore, the assessment of host plant use has indicated seasonal host-switching. During the dry season, stemborers population were available in all 8 alternative host plant species with the highest (Shannon = 1.30) in terms of host plant differences per season. The rainy season, however, was characterized by high maize abundance (49 – 59% of samples), associated with reduced alternative host plant diversity (Shannon = 0.97 – 1.20).

Results have also indicated that host plant composition differed significantly among seasons ($\chi^2 = 246.74$, $df = 16$, $p < 0.001$), with *P. purpureum* (Napier grass) serving as the primary dry season refugium sustaining 1,020 stemborers across 105 samples (61% of dry season samples). Within seasons, the Bray-Curtis showed higher similarity during short rainy (0.271) compared to the long rainy (0.191). *B. fusca* was higher in dry season's alternative hosts (49.4%) while *C. partellus* showed higher abundance during short rainy season maize plantation (47.5%).

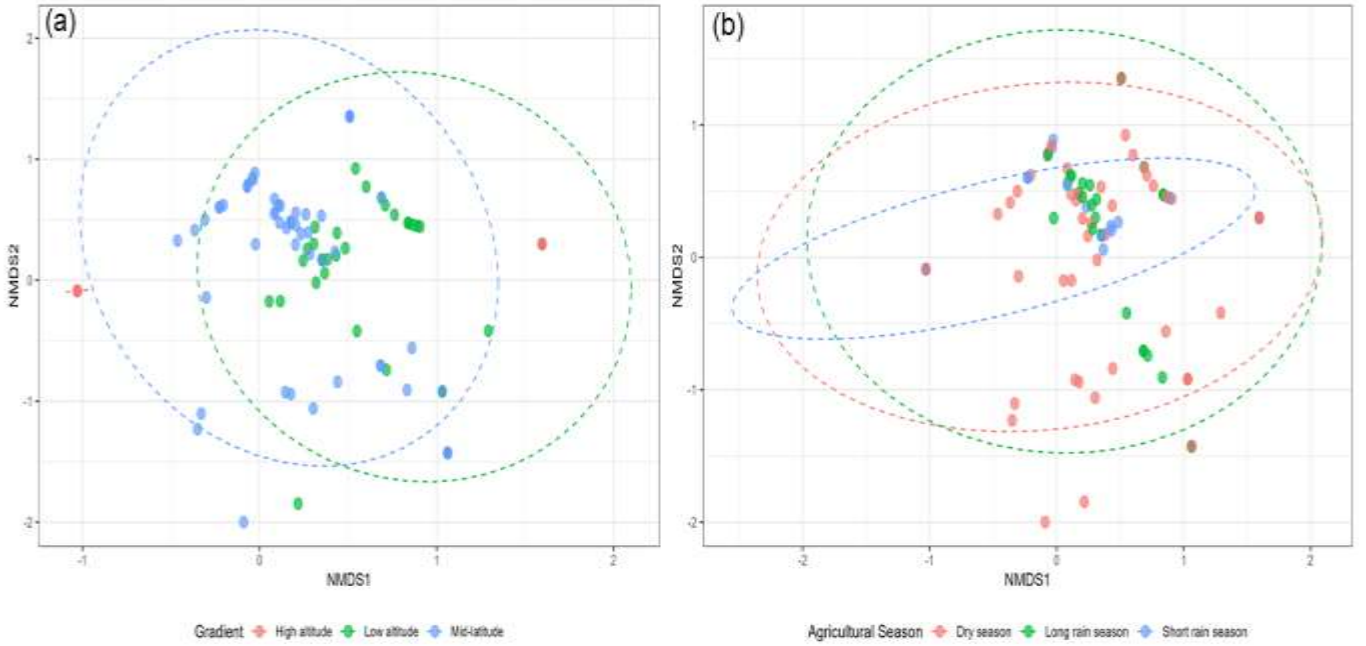


Figure 4. 5. Altitudinal and seasonal structures of stemborer communities. (a) Altitudinal structures of stemborer communities ($R^2 = 0.21$, $p < 0.001$), (b) seasonal structures of stemborer communities ($F=2.83$, $p = 0.055$, $\alpha = 0.10$)

4.1.4. Discussion

In this study, five key stemborer species were identified, namely *B. fusca*, *S. calamistis*, *S. nonagrioides* (Noctuidae), *C. partellus* (Crambidae), and *E. saccharina* (Pyralidae). All these species are known for their wide distribution range and ecological adaptability in East and Southern Africa (Assefa et al., 2010; Goftishu et al., 2018; Mutamiswa et al., 2022; Overholt et al., 2001). Our results showed that the larval stage was the most abundant across sites and seasons and exchanged from wild-crop habitats, indicating supporting their yearly population survival mechanism (Assefa, 2006; Goftishu et al., 2017; Overholt et al., 2001; Sokame et al., 2019). Wild vegetation played a substantial role in sustaining stemborer populations during maize off-seasons, suggesting that non-crop habitats act as reservoirs that support the pests' inter-seasonal persistence.

In our study, *B. fusca* was the most abundant species in the high-altitude zones but was also found in mid- and low-altitude areas, suggesting a potential expansion of its ecological niche. *Sesamia* spp. were concentrated in mid-altitudes, with limited presence in lowland zones, while *C. partellus* was found across both low and mid-altitudes. These patterns reflect the recent observations from Ethiopia, Kenya, Mozambique, South Africa, and the Democratic Republic of Congo, where shifts in stemborer distributions have been associated with climate change, habitats fragmentation, and resource competition (Goftishu et al., 2018; Kankonda et al., 2017; Le Rü et al., 2006a; Melaku et al., 2006; Wale et al., 2007). The strong altitudinal structuring of stemborer communities underscores the role of altitude-dependent environmental gradients in shaping stemborers pest assemblages, consistent with broader patterns as observed in tropical herbivorous insects (Tamiru et al., 2007; Zhao et al., 2023).

Contrary to earlier studies reporting higher stemborer abundance in cultivated cereals and limited presence in wild vegetation (Moolman et al., 2014; Ong'amo, 2005; Otieno et al., 2008), our data showed a greater proportion in wild vegetation. This finding challenges the conventional assumption that wild grasses serve only as minor refugia during maize off-seasons (Haile and Hofsvang, 2002; Moolman et al., 2014; Ntirenganya et al., 2025). Instead, our results aligned with emerging evidence from Ethiopia, where wild grasses have been reported to support substantial stemborer populations (Goftishu et al., 2018), especially during dry periods when maize is absent (Gounou et al., 2009). While this trend may partly reflect our purposive sampling of host-relevant

plant families, as noted in the methodology, acknowledging this bias is essential to enhance transparency and strengthens the validity of ecological interpretation by distinguishing design-driven patterns from genuine biological processes (Pili et al., 2025; Zvereva and Kozlov, 2019).

Seasonal host-switching patterns were also evident. During the rainy cropping season, maize plantations supported a significantly higher abundance of stemborers. While, the dry season, characterized by the absence of cultivated maize, was marked by a notable shift in stemborer populations toward wild vegetation, particularly *P. purpureum* (Elephant/Napier grass). *P. purpureum* emerged as the primary dry season refugium, harboring approximately 61% of the stemborer population. This seasonal host-switching behavior underscores the ecological importance of wild grasses in sustaining stemborer populations during maize off-seasons (Assefa et al., 2010; Gofishu et al., 2018; Gounou et al., 2009; Ong'amo, 2005).

The observed dominance of stemborers, particularly *Busseola fusca* in *Pennisetum purpureum* (Napier grass) compared to maize across seasons is notable and may be explained by regional differences in Napier grass application and management. In the highland of northern province of Rwanda, Napier grass is commonly maintained to full maturity and harvested after two years for non-agronomic uses such as supporting climbing beans, constructing domestic infrastructure, and producing handcraft materials (Ayuko et al., 2024; Chen et al., 2024). In the lowland of eastern province, Napier grass is frequently harvested for livestock feed and mulching (Ayuko et al., 2024; Chen et al., 2024; Tulu et al., 2023), keeping the yearly young generations in the area. These contrasting practices influence the phenological stage of the host availability to stemborers. Previous studies have shown that *B. fusca* exhibits a strong preference of younger Napier grass for oviposition (Assefa et al., 2015; Calatayud et al., 2008; Khan et al., 2007). Therefore, the predominance of *B. fusca* in low altitude of eastern Rwanda may be linked to the continuous availability of younger Napier grass due to regular harvesting, creating favorable conditions for oviposition and population buildup. Our results do not ignore other eco-socio-economic benefits of Napier grass (ICIPE, 2005; Niassy et al., 2022).

The role of *P. purpureum* as a trap crop has been well-established within the framework of push-pull technology (ICIPE, 2005; Khan et al., 2011, 2007; Niassy et al., 2020). When strategically planted around maize fields, it attracts ovipositing female moths, whose larvae suffer high

mortality due to the grass's physical and chemical defenses, including a sticky exudate that impedes development (Abedi et al., 2019; Calatayud et al., 2008; Glas et al., 2007; Haile and Hofsvang, 2002; Khan et al., 2007). This mechanism effectively reduces pest pressure on maize and contributes to environmentally sustainable and economically viable integrated pest management (IPM) strategies (Niassy et al., 2022, 2020). However, in East African and regional agro-ecosystems, fodder grasses such as *Pennisetum*, *Tripsacum*, *Vetiveria*, and *Setaria* have historically been domesticated for livestock feed and erosion control (Ayuko et al., 2024; Chen et al., 2024; Tulu et al., 2023), often without standardized spacing or integration into pest management frameworks. We propose that future research should adopt a multifunctional approach to evaluate the dual role of these grasses in IPM and soil conservation, alongside improving farmer's awareness on standard distancing/spacing between fodders and cultivated crops.

Such studies are essential for redesigning sustainable push-pull or intercropping pest management strategies tailored to mountainous and terraced landscapes like Rwanda, where landslides or erosions control and pest suppression must be co-optimized.

4.1.5. Conclusion

This study provides the first comprehensive data on stemborer species composition and distribution across Rwanda's agroecological zones. The findings confirm that *B. fusca*, *S. calamistis*, *S. nonagrioides* (Lepidoptera: Noctuidae), *C. partellus* (Lepidoptera: Crambidae), and *E. saccharina* (Lepidoptera: Pyralidae) are the most abundant stemborer species in wild plants. Even though altitudinal gradient and seasonal variation can independently influence stemborers community structure, our findings demonstrated that their combined effects exert a stronger and more ecologically meaningful influence on stemborer community structures. *Pennisetum purpureum* (Napier grass) emerged as a key alternative host, whose widespread and varied harvesting practices may contribute to stemborer persistence across different agroecological zones. Evidence of potential habitat shifts by *B. fusca* and *C. partellus*, further suggests potential niche expansion that warrants long-term monitoring under changing climatic and agricultural conditions. We recommend future research to determine the optimal spatial arrangement between crops and fodder grasses, and to assess the multifunctional benefits of these grasses such as their roles in soil stabilization, erosion and landslide mitigation, and pest suppression particularly within mountainous and terraced agroecosystems common in Rwanda. This would help for redesigning sustainable push-pull and intercropping strategies that enhance both crop productivity and ecological resilience pest management.

4.2. Pest status and dynamics of sugarcane stem borer (*Eldana saccharina*) in sugarcane plantations of Nyabugogo Tropical Wetland, Rwanda

Abstract

Lepidopteran species richness and composition are influenced by complex interactions between biotic and abiotic factors that operate on different spatial scales. This study investigated the diversity and population structure of sugarcane stemborers across three phenotypically distinct sugarcane varieties: green, red, and white-powdery (CO 421) within the Nyabugogo Tropical Wetland ecosystem. Additionally, seasonal variation and the effects of gradual removal of older leaves-an important sanitation practice on stemborer population dynamics were evaluated between May and November 2023. During the investigation, 899 stemborers individuals were recorded from 601 infested stems out of a total of 5400 examined stems. The results revealed that *Eldana saccharina* Walker (1865) (Lepidoptera: Pyralidae) was the predominant species. Infestation levels were highest during the dry season, accounting for 31.4% (July) and 23% (August). This species showed a clear preference for green sugarcane (55.5%) compared to red (37.59%) and CO 421 (6.91%) varieties. The analysis indicated a strong negative correlation between *E. saccharina* and rainfall ($r = -0.80$, $P < 0.05$) across both treated (removal of older leaves) and untreated sugarcane ($r = -0.83$, $P < 0.05$). Conversely, there was a positive correlation with temperature ($r = 0.76$, 0.82 ; $P < 0.05$). In conclusion, this study highlights the significant influence of rainfall, temperature, and the sanitation practice of removal of older leaves on population dynamics of *E. saccharina* and the overall levels of infestation in the sugarcane plantation.

Keywords: Sugarcane, varieties, stemborer, leaves-removal, *Eldana saccharina*

4.2.1. Introduction

Sugarcane (*Saccharum*, Poaceae) is a perennial crop extensively grown and domesticated across tropical and subtropical regions Worldwide. Currently, major sugarcane producers include Brazil, India, Thailand, China, Pakistan, and Mexico (IISD and SSI, 2019). In East Africa, the leading producers are Kenya, Uganda, Zambia, and Tanzania (Statista, 2023). Of which, Kenya produces 7.8 million metric tons from 92,400 ha, Uganda produces 5.4 million metric tons from 85,300 ha, Zambia produces 5.2 million metric tons from 49,200 ha, and Tanzania produces 3.5 million metric tons from 49,300 ha (World Data Atlas, 2025).

In Rwanda, sugarcane production rose significantly from 1973 to 2022, reaching 97.8 thousand tons of production from 8,019 ha (World Data Atlas, 2022). Since its introduction, sugarcane has been adopted for domestic consumption, cash profitability, and its supply-chain created employment for over 10,000 individuals in monocrop plantations (Veldman and Lankhorst, 2011). Its holds a significant commercial value by serving as domestic source of sugar and reducing the country's dependence on sugar importation (Habes, 2017). In the country, sugarcane production is dominated by red, green, and white powdery varieties. The green and red sugarcane varieties belong to Pindari cultivar recognized by their softness and sweetness and widely cultivated for direct chewing or juice production. In contrast, industrial White-powdery sugarcane cultivar is characterized by its hardness and lower juice-water content, making it a commercial variety for the sugar industry in Rwanda and East Africa.

Harvesting of sugarcane plantation does not follow a strict monthly schedule; rather, it depends on maturity ranging from first harvest to range of 15-24 months (Habes, 2017). However, farmers are able to harvest green and red sugarcane between 12-15 months for sale to local consumers (Veldman and Lankhorst, 2011). Beyond its economic significance in sugar production and consumption, sugarcane plantation play a crucial role in landscape greening, soil restoration, and carbon sequestration (Mateus et al., 2016; Xu et al., 2019; Canning et al., 2023). These positive environmental impacts have been recognized by the Rwanda Government and the international community. As results, there is a growing attention to sugarcane crop within restoration and management plans for wetlands marshlands in Rwanda, with projection suggesting an increase in sugarcane production to 133,410 tons by 2026 (World Data Atlas, 2022).

Nevertheless, the sugarcane industry remains poorly understood and undervalued by local extension services, agriculture and research institutions, and academic circles. This lack of recognition contributes to increased threats from pests to diseases in sugarcane production system.

Globally, major insect pests have been investigated in sugarcane production system (Overholt et al. 2001; Assefa 2006; Borges Filho et al., 2019), and lepidopteran stemborers identified a primary pests affecting sugarcane (Sallam, 2006). In Sub-Saharan ecosystems, the most common stemborers in sugarcane plantations include *Eldana saccharina* Walker, 1865 (Lepidoptera: Pyralidae), *Chilo partellus* Swinhoe 1885 (Lepidoptera: Crambidae), *Sesamia calamistis* Hampson 1910 (Lepidoptera: Noctuidae), *Busseola fusca* Fuller 1901 (Lepidoptera: Noctuidae), and *Busseola phaia* Bowden 1965 (Lepidoptera: Noctuidae) (Assefa, 2006; Assefa et al., 2010; Nikpay, 2016). The success of these insects in sugarcane plantations is largely attributed to their life cycle and their ability to infest a wide range of plants including maize, sorghum, and sugarcane, as well as various wild host plant (Kanya et al., 2004; Le Rü et al., 2006; Otieno et al., 2008; Goftishu et al., 2018). Female stemborers lay clusters of 100–850 eggs in batches on either side of the leaves or occasionally on the plant stems. The larvae are highly mobile and begin feeding immediately after hatching (Overholt et al., 2001). Subsequently, the larvae tend aggregate while individually boring into the plant stems and, in rare cases, into leaf sheaths. After this active feeding phase, they pupate before emerging to the adult moths (Overholt et al., 2001; Calatayud et al., 2008).

Further, the successful invasion of stemborers across various areas can be attributed to their ability to fly both short and long distances. They can travel between 8 and 16 km when carried by the wind (Urca and Ribak, 2021). In addition, larvae have the capacity to crawl from one plant to another particularly within small farms or when they have exhausted food resources in a given location (Cohen et al., 2000; Sokame et al., 2020a, b). Farming practices and the movement of infested plant material by humans also contribute to the spread of stemborers. This transmission may happen when infested plant material is moved from one field to another, during the transport to and from the market, and when infested post-harvest waste is utilized for animal feed or composting. Additionally, the use of post-harvest and crop residues of infested plants as mulch can facilitate the spread of these pests (Melaku et al., 2006; Renault et al., 2018).

Natural dispersal has also been documented, where in eggs or small larvae can be carried to new areas by wind currents (Silvestri et al., 2019; Sokame et al., 2020b).

Stemborer damage severity is shaped by species, infestation density, crop variety, growth stage, fertilization method, seasonal patterns, and location (Togola et al., 2020). Their diversity is influenced by agronomic and environmental factors such as temperature, rainfall, altitude, landscape structure, plant diversity, and farming practices (Mutiyambai et al., 2022). With current persistent rising temperatures due to climate change, stemborer populations and infestation levels in both wild and cultivated crops are expected to increase (Soltani Ong et al., 2014). Stemborers cause significant crop losses globally up to 30% in sugarcane and between 10% and 88% in maize and sorghum (Silvestri et al., 2019; Sokame et al., 2020b). Their spread from wild vegetation to cultivated fields poses a major challenge for pest management in cereal and sugarcane systems (Assefa, 2006). While biological and mechanical controls have been recommended under changing climate conditions (Lucid Keys, 2007; Assefa et al., 2010), the role of integrating these methods with field sanitation remains underexplored in maintaining stemborer populations below economic thresholds.

Despite the known impact of stemborers, knowledge about their species and the role of sanitation practices such as leaf removal in managing infestations remains limited. In particular, data on stemborer species, their population dynamics, and interactions with sugarcane varieties in sugarcane plantations are scarce. This study investigated the status of sugarcane stemborers in Nyabugogo tropical wetland in Rwanda, focusing on (i) species identification, (ii) the influence of sugarcane variety, (iii) old-leaf removal sanitation practice, and (iv) weather conditions on pest abundance. The findings offer essential baseline data to guide pesticide-free pest management strategies and inform future research in sugarcane production system in Rwanda and abroad.

4.2.2. Material and methods

4.2.2.3. Description of the area of study

Nyabugogo tropical wetland is located in Kigali city at coordinates 1° 58'S and 30°07'E with an elevation ranging from 1,331 to 2075 meters above sea level. The annual rainfall in this area varies between 992 and 1128 mm, and the average annual temperature fluctuates between 19 and 21°C across the various slopes, rambling that encompass four mountainous edges in a valley (Fig. 4. 6). Nyabugogo wetland drains the total catchment area of 1,661 km² (166,100 hectares), accounting approximately 6.31% of Rwanda's total surface area. This catchment extends from Kigali city to the south (Kamonyi District), east (Bugesera and Rwamagana Districts), and north (Rulindo District), ultimately flowing into Nyabarongo River catchment (Fig. 4. 6) (Ministry of Environment, 2018).

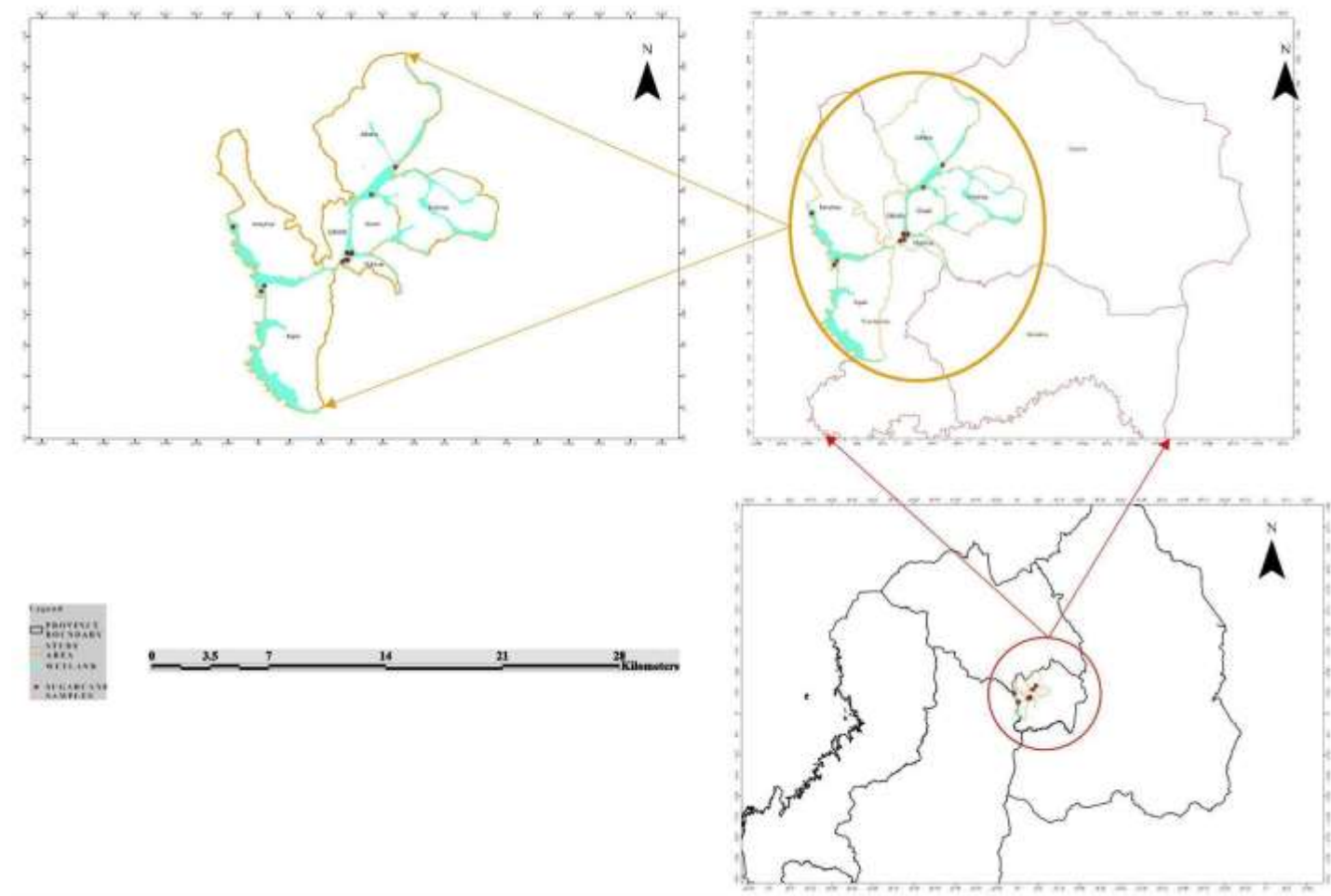


Figure 4. 6. Location of the Nyabugogo wetland and sampled sites in Kigali City, Rwanda. Local administrative boundaries were generated from geodata in Rwanda Spatial Data and Infrastructure under the Rwanda National Land Authority

4.2.2.2. Description of study materials

Within Nyabugogo wetland, crop land occupies more than 54% of the total size of wetland, in contrast to 2,763.4 ha of the natural vegetation predominantly comprising Typhaceae,, Polygonaceae, and Poaceae (EIB and Royal Haskoning DHV, 2016), and 308.1 ha of water bodies, while 1,958.4 ha are designated for river banks and planned eco-tourism activities (ARCOS, 2021). The primary crops cultivated in the wetland include sugarcane which covers more than 3,000 hectares, rice, irrigated tubers (ARCOS, 2021). A significant shift in the use and management of Nyabarongo river and Nyabugogo wetlands begun in 1997 when the Government of Rwanda leased the big part of wetland to Madhivani group for sugarcane plantations (Ansoms 2008). Madhvani group which is an Indian sugar producing company in Rwanda oversees the production of sugar from commercial sugarcane varieties that occupy a substantial area of the wetland. This study focuses on “CO 421 cultivar”, the most common commercial cultivar in the Nyabugogo wetland. The cultivar was released in India in 1950 and was introduced in East Africa via Kenya in 1990 as an exemplary sugarcane variety resistant to biotic and abiotic stresses (Sewaya, 1994). The second involved variety was Pindari, characterized by its natural phenotypical green and red or purple sugarcane cultivars (Shukla et al., 2018). Despite limited information regarding the origins of sugarcane in Rwanda, these Pindari’s cultivars were earlier domesticated by farmers for local consumption and small market supply for direct chewing. However, poor data availability regarding sugarcane varieties and their origins in East Africa is challenging.

4.2.2.3. Sampling method and data collection

Sampling of sugarcane stemborers was conducted in May 2023 during the transition from the rainy to dry season, extended to June, July, and August (dry season) as well as September, October, and November (rainy season). The period from December to April was not surveyed as it coincides with the end of sugarcane harvesting period and heavy rainfall marked by flooding in wetland from March to the early May. Fields of sugarcane were initially visited to identify plots with green, red, and white-powdery (CO 421) sugarcane varieties and without chemical pesticides spray.

For each variety, two plots were selected from individual farmers' fields and these owned by the Madhvani group. The main criteria of plot selection include: having at least 0.5 ha plantation of target sugarcane varieties, being treated with leaves removal (where old leaves are gradually removed by farmers) and non-treated (where old leaves are not removed), accessibility, and obtaining permission from the farm owner or farm manager. For data collection, each selected plot was subdivided into five sampling quadrats of 3m x 3m (Fig. 4. 7). This resulted in 10 sampled quadrats for each sugarcane variety, totaling 30 sampled quadrats. Every quadrat contained an average of 90 sugarcane stems, leading to a total of 900 stems (450 stems / replicate) per plot for each sugarcane variety and an overall total of 2,700 stems across all sanitation treatments, culminating in an average of 5,400 investigated stems. In each sugarcane plot, infested stems were harvested. All data such as number of stemborers, stage, plots, and related information were entered in Kobotoolkit.

Female moths lay eggs in hidden places, after hatching, they fast move to young shoots and feed on soft leaves and tunnel the stems as they grow. During sample collection, we checked frass, holes, wounds, and hidden places for eggs colonies. This approach, informed by earlier studies (Assefa et al., 2010; Otieno et al., 2008; Gofitshu et al., 2018), aimed to detect potential stemborer infestations in surrounding habitats that may serve as alternative host reservoirs. All suspected stems from both sugarcane and wild vegetation were deeply checked and infested stems were harvested, longitudinally dissected and examined for the presence of stemborers at the field. Only 5% of infested stems were carried to laboratory and placed in insectary boxes for further observations (Assefa, 2006). Live stemborers were placed into 30 ml vials containing the artificial diet (Devi and Kaur, 2015), and taken to the laboratory of the Biology Department, at the College of Science and Technology, University of Rwanda, and reared to the adult stage for identification. Deceased stemborers were conserved in 70% ethanol for later identification. In addition, rainfall and temperature data were obtained from the Rwanda Meteorological Agency.

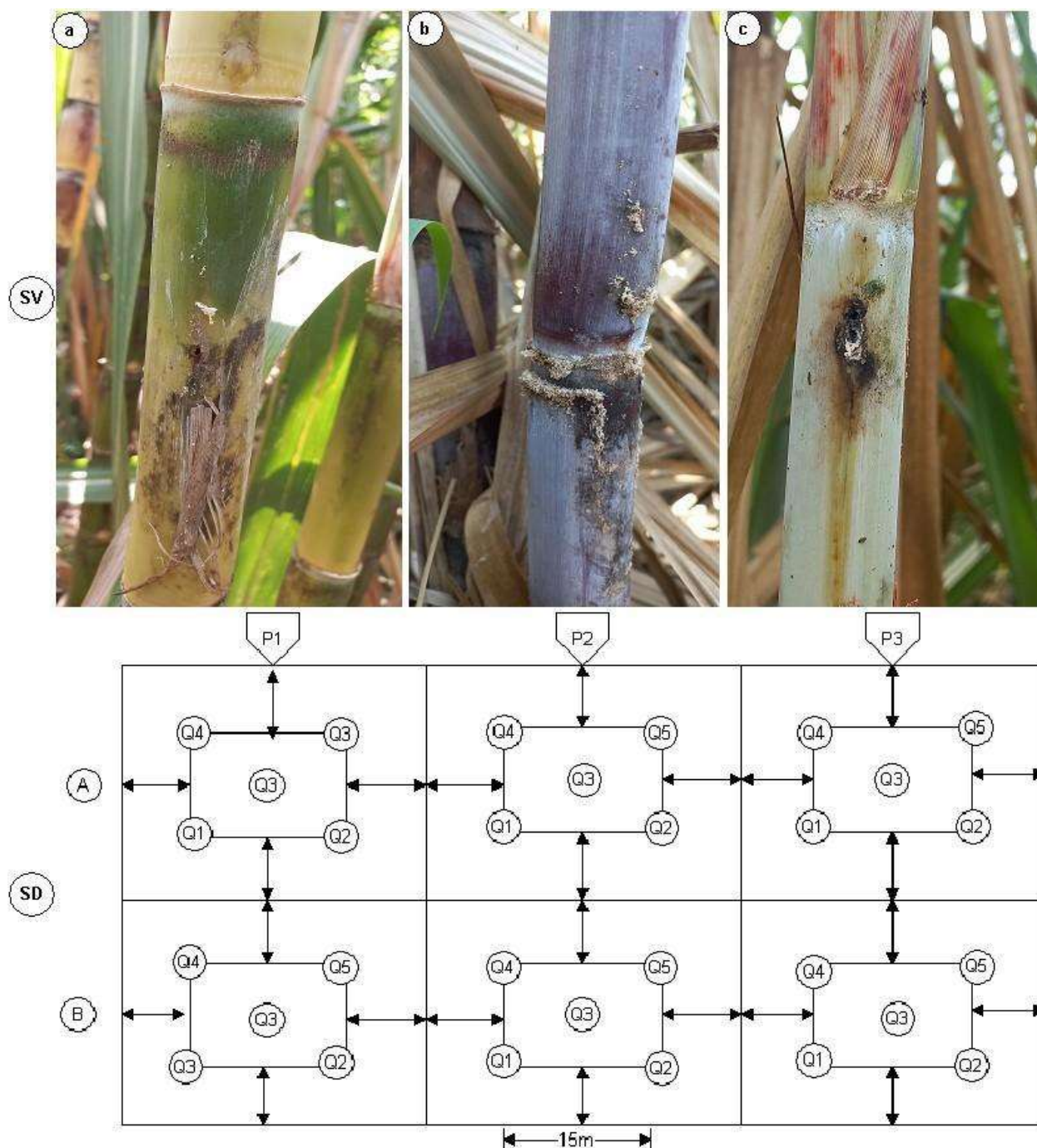


Figure 4. 7. Sampling design for sugarcane varieties. (SV) Sugarcane Varieties, (a) green sugarcane, (b) red sugarcane, (c) CO 421 sugarcane (CO 421); (SD), sampling design, (A) plots of sugarcane with leaves, (B) plots of sugarcane gradually removed leaves; (P1) green sugarcane plot, (P2) red sugarcane plot, (P3) CO 421 sugarcane plot, (Q) quadrat, (Q1-Q5) quadrats sampled in each plot

4.2.2.4. Stemborers identification

Identification process utilized a combination of dissection and microscopic observation of the adult moth's morphological features, in conjunction with a stemborer identification guide (Overholt et al. 2001), and the Lucid Central stemborer identification key (<https://www.lucidcentral.org>). Further, a lepidopteran stemborer expert from the International Centre of Insect Physiology and Ecology (ICIPE) was consulted to verify and confirm the species. Voucher specimens of the identified larvae and adults are preserved at the zoology laboratory, Biology Department, College of Science and Technology, University of Rwanda, under the 2023 sugarcane stemborers collection (Access Code: KNS2023).

4.2.2.5. Data analysis

The abundance of identified species was extracted from Kobo toolkit used during data collection and subsequently utilized for further analysis. The population dynamics of stemborers in sugarcane plantations were calculated using the distribution model that took seasonal variation and considering sugarcane varieties. Relative infestation was determined by dividing the total number infested stems per treatment by the total sampled stems and multiplying by 100. The stemborers infestation ratio per stem was calculated by dividing the total number of stemborers recovered from stems by the total number of infested stems for each treatment. A chi-square test was performed to analyze the association between sanitation treatment and stemborers infestation with a significant level at $p\text{-value} = 0.05$. Additionally, interactions between stemborer population, sugarcane varieties, and weather parameters (temperature and rainfall) were interpreted from the correlation analysis. Pearson's correlation coefficients ($p\text{-value} = 0.05$) calculated to explore these relationships. However, since the data did not meet the assumptions required for Pearson's correlation (parametric), the Spearman's rank correlation was used to assess interaction between temperature, rainfall, and stemborers population dynamics with $r\text{-values}$ ranging from -1 to +1. A negative Binomial Generalized Linear Model (GLM) was used to analyse the effect of individual parameters on the dynamics of sugarcane stemborer population. all analysis was performed using R studio, version 4.30.

4.2.3. Results

In this study, a total of 899 stemborer individuals were recovered from 442 infested stems in 5,400 examined sugarcane stems. Laboratory observations and characterization using the identification keys confirm that the species is characterized by a light brown to dark-gray color with a sclerotized body covered by dark spots which is *Eldana saccharina* (Lepidoptera: Pyralidae) (Fig. 4. 8). Compared to closely related stemborers species, adults *E. saccharina* are small-sized and morphologically similar to *Chilo partellus* Swinhoe 1885 (Lepidoptera: Crambidae) (<https://keys.lucidcentral.org/>). However, they can be distinguished by their pale brown forewings which feature a few central spots and extended markings along the edges.



Figure 4. 8. *Eldana saccharina*, a single species recorded during the study period; (a) larvae; (b) pupae; (c) adult photo taken after raising larvae to adult stage

The results revealed that stemborer infestation was unevenly distributed across different sugarcane varieties and sanitation treatments, indicating potential varietal and management-related influences on pest dynamics.

The green sugarcane variety was the most infested, accounting for 55.5% (N = 499) of the total collection. The red sugarcane variety had relative infestation of 37.6% (N = 338), while the CO 421 sugarcane was the least infested at 6.9% (N = 62) (Fig. 4. 9).

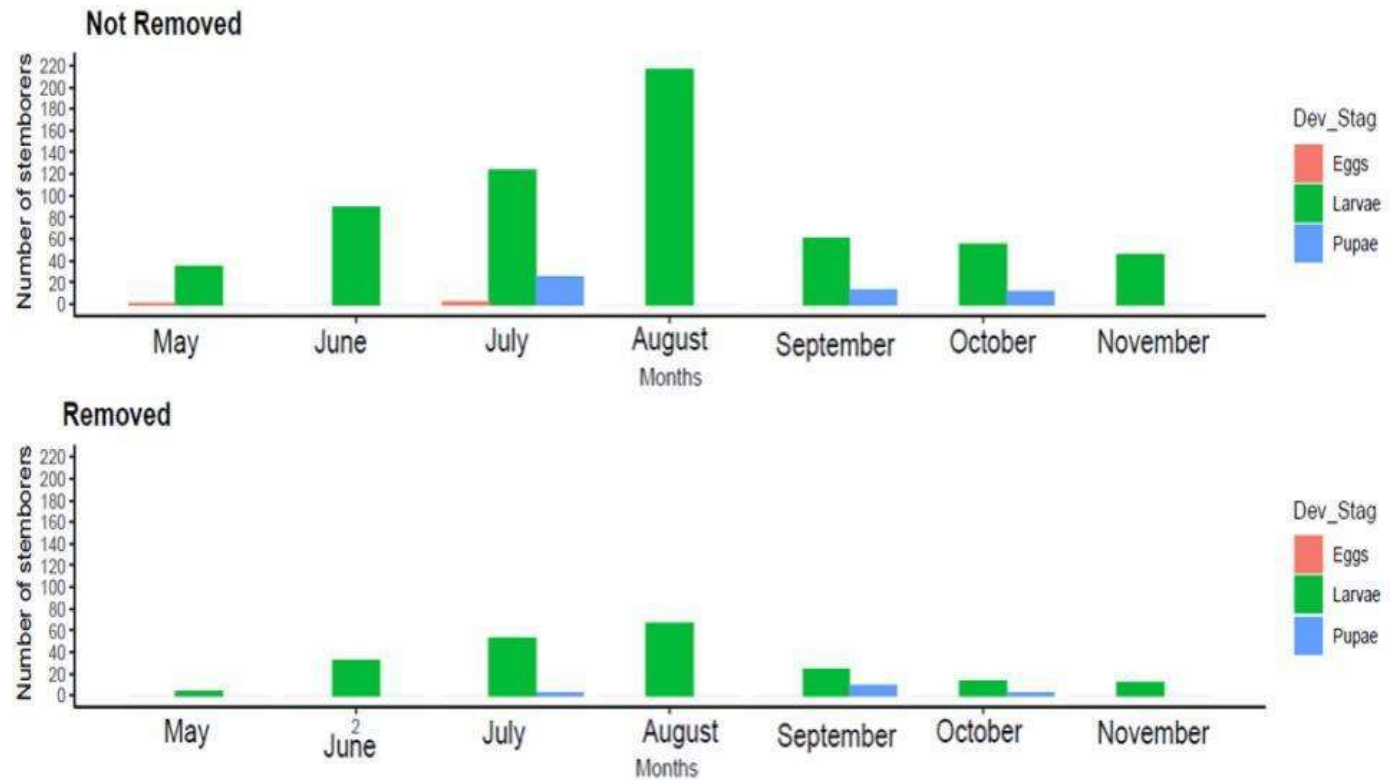


Figure 4. 9. Stemborers in treated (Removed: sugarcane without leaves or the old leaves gradually removed) and non-treated (Not Removed: sugarcane with leaves or the old leaves are not removed) sugarcanes. Dev_Stag: developmental stages

Notably, non-treatment stems were heavily infested with 64.8% (N=583) of the infested stems found in green sugarcane and 35.2% (N=316) (Tab. 4. 4). Of all collected individuals, 92.6% (N = 833) were larvae, 7.1% (N = 64) were pupae, and the remaining 0.3% (N = 2) were egg batches. The eggs batches were collected from sugarcane with leaves in May (N = 1) and July (N = 1).

Table 4. 4. Proportion of sugarcane stems infestation in different varieties with different treatments.

Sugarcane variety	Old leaf removal	Total stems	Infested stems	Total stemborers	Relative infestation (%)	Proportion of infested stem
GSC	Not removed	900	148	364	33.7	2.45
	Removed	900	94	135	21.3	1.43
RSC	Not removed	900	112	266	25.3	2.37
	Removed	900	51	72	11.5	1.41
CO 421	Not removed	900	26	50	5.8	1.92
	Removed	900	11	12	2.4	1.09

The Chi-square test analysis of the association between sugarcane sanitation treatments (leaf removal or not) and stemborers infestation yielded the following results: $X^2 = 51.072$, $df = 25$, $p\text{-value} = 0.001568$. The results indicated a statistical significant association between sanitation treatments and the degree of stemborers infestation. In both sugarcanes with leaves and where leaves were gradually removed, the number of larvae showed a gradual increase from May ($N = 40$) to August ($N = 284$), with more pronounced rise in sugarcane with leaves ($N = 680$) compared to sugarcane without leaves ($N = 219$). A subsequent decline was observed during the rainy season from September ($N = 107$) to November ($N = 58$). Besides, pupae were mainly found in July ($N = 28$), September ($N = 22$), and October ($N = 14$). The population dynamics of eggs, larvae, and pupae are detailed in Figure 4. 10.

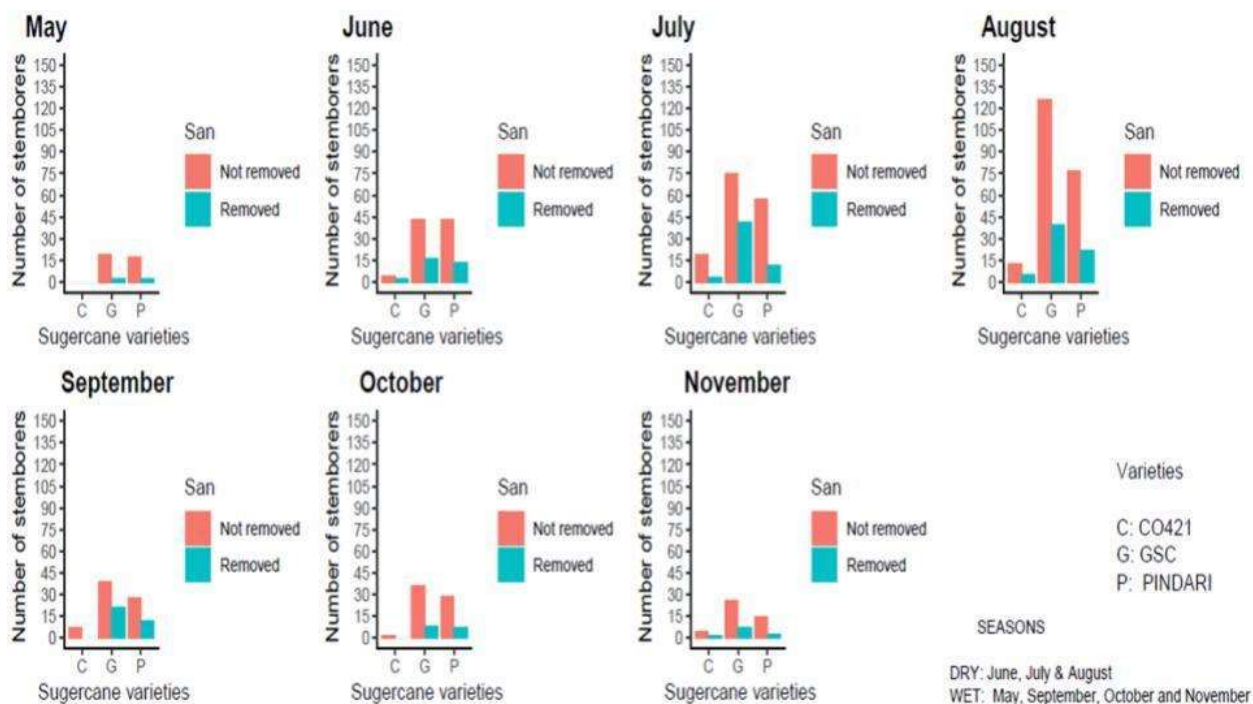


Figure 4. 10. Monthly infestation of stemborer in relation to sugarcane varieties. CO 421 sugarcane (CO 421), green sugarcane (GSC), and red sugarcane (RSC) varieties

The occurrence of *E. saccharina* correlated with both sugarcane treatments and sugarcane varieties as demonstrated in figure 4. 11.

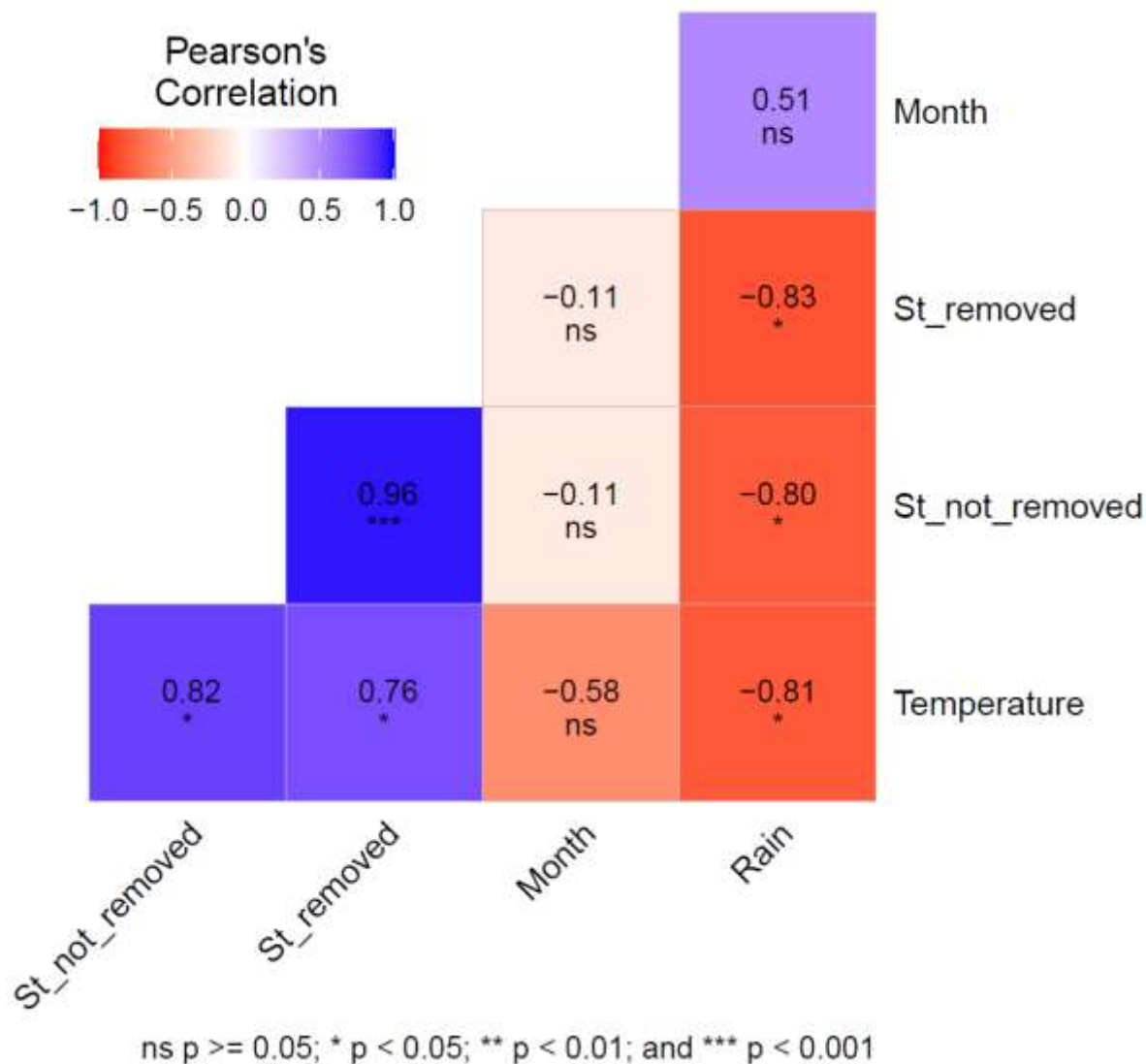


Figure 4. 11. Correlation between sanitation practices, rainfall, temperature and stemborers in sugarcane plantations (St= Sanitation treatment: not removed = old leaves are not removed; removed = old leaves are gradually removed; ns = non-significance; * =Significance range

A post-hoc Dunn's test (p -value = 0.0005486) confirmed that sanitation treatment significantly impacted stem infestation (Fig. 4. 12b). However, the Kruskal-Wallis test analysis ($X^2 = 1.6422$, $df = 2$, p -value = 0.44) indicated no statistically significant difference in stemborer abundance per stem across sugarcane varieties (Fig. 4. 12a).

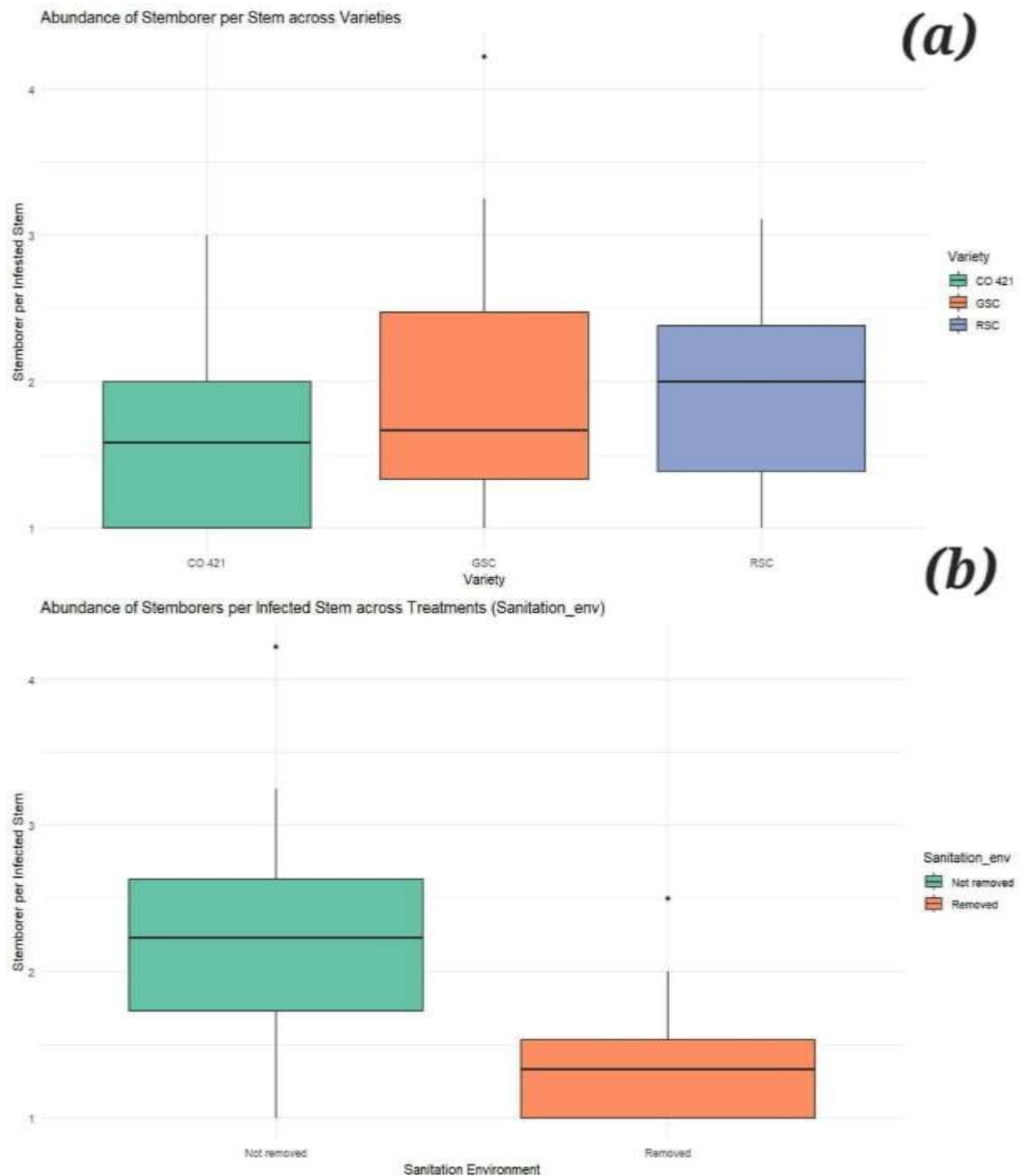


Figure 4. 12. Degree of infestation in a total infestation and per stem. (12a). Abundance of stem borers per infested stems across treatment of leave-removal or not; (12b) abundance of stem borers per stem across varieties

Regarding the preliminary findings on temperature and rainfall, the lowest mean temperatures were recorded in November (20.0°C) and October (20.5°C). The highest mean temperatures were observed in August (23.95°C), July (22.32°C), and June (22.3°C). In terms of rainfall, data collection began in May, marking the transition between the short, heavy rain, and dry seasons. The average rainfall in May was 111.7 mm, decreasing to 1.6 mm in June and reaching 0 mm in both July and August during the dry season. Rainfall gradually increased from 109.5 mm in September to 137 mm in November, coinciding with the short rainy season (Tab. 4. 5).

Table 4. 5. Abundance of stemborers in relation with moth and weather parameters in Rwanda

Month	Temperature variation		Rainfall variation		Stemborers abundance per month
	Mean	Standard deviation	Mean	Standard deviation	
May	22.00	0.94	111.50	8.77	40
June	22.30	0.76	1.60	0.47	122
July	22.32	0.92	0.00	0.00	206
August	23.95	1.02	0.00	0.00	284
September	21.40	0.85	109.50	5.84	107
October	20.50	0.59	124.00	5.47	82
November	20.00	1.16	137.00	6.45	58

The Pearson correlation test showed a significant negative correlation between rainfall and stemborer abundance ($r = -0.80, -0.83, P < 0.05$) for rainfall and positive correlation with ($r = 0.76, 0.82, P < 0.05$) for temperature under different treatment (Fig. 4. 11). Similarly, a Spearman's rank confirmation test indicated a negative correlation ($r\text{-value} = -0.306$) with rainfall and a positive correlation ($r\text{-value} = 0.277$) with temperature. A negative Binomial Generalized Linear Model (GLM) test revealed a statistically significant a negative relationship between rainfall and stemborers abundance ($p\text{-value} = -0.005578$), suggesting that increased rainfall leads to decrease in stemborer abundance. However, no clear linear relationship was found between temperature and stemborer abundance ($p\text{-value} = 0.9571$).

4.2.4. Discussion

The study findings clearly confirm the presence of stemborers in the sugarcane plantations within the Nyabugogo wetland. Notably, a single species of *E. saccharina* found to be widely distributed across the surveyed area. These results are consistent with previous studies, which reported *E. saccharina* as the most prevalent and destructive pest of sugarcane throughout Sub-Saharan Africa (Lucid Keys, 2007; Assefa et al., 2010). This species persists throughout the year, producing multiple generations, a phenomenon facilitated by the continuous availability of its primary host plants (Moolman et al. 2013; Goftishu et al., 2017; Canning et al., 2023). Moreover, alternative host plants are thought to play a crucial role in sustaining stemborer populations during periods of environmental stress or scarcity of preferred host plants (Overholt et al., 2001; Le Rü et al., 2006; Otieno et al., 2008; Calatayud et al., 2008; Moolman et al., 2014; Goftishu et al., 2018). During the survey, four dead stemborers were recovered from symptomatic *Typha*. located in sugarcane plantation farms. although limited, this observation suggests a potential association between stemborers and wild vegetation. These findings underscore the need for more comprehensive surveys to assess the presence and ecological role of stemborers in non-cultivated plant species, as recommended by previous studies (Otieno et al., 2008; Moolman et al., 2014; Goftishu et al., 2018). The absence of live stemborers and the recovery of only dead larvae from non-cultivated plants remain unexplained. One plausible factor could be associated with the low diversity of stemborers in wild vegetation and restricted number of stemborers' wild host plants along the boundaries of the farms (Le Rü et al., 2006; Otieno et al., 2008; Ong'amo, 2009; Moolman et al., 2014; Goftishu et al., 2018).

The mortality of the collected larvae may be linked to pesticide use in these adjacent rice fields as reported that pesticide contamination in irrigation systems can lead to reductions in insect richness ranging from 15% to 77% (Renault et al., 2018; Ito et al., 2020; Raut et al., 2023). Another potential factor is the proximity of sugarcane car washes and garages, which may generate toxic minerals such as copper and iron to the water supply (Monney et al., 2020; Talebzadeh et al., 2021). These heavy metals are known threats to insects species and other arthropods (Ben-Shahar, 2018; Sánchez-Bayo, 2021; Tabassum et al., 2024). To validate these hypotheses, further investigations are recommended. These should include chemical analyses of stemborers and water sources near sugarcane plantations to detect potential contaminants, as well as ecological

assessments of stemborers populations in both cultivated and wild vegetation. Such studies would provide valuable insights into the environmental stressors affecting stemborer survival and distribution, and inform more sustainable pest and land management practices.

In terms of sugarcane preferences, *E. saccharina* exhibited preferences among different sugarcane varieties. This preference is probably influenced by phenology of sugarcane stems and key qualitative factors such as Brix, Pol, juice purity, and sugar content (Abedi et al., 2019). Recent studies on *Sesamia nonagrioides* Lefèbvre, 1827 (Lepidoptera: Noctuidae) revealed that female stemborer performance and oviposition are strongly correlated with secondary metabolite concentration and degree of softness of sugarcane stem (Abedi et al. 2019; Mortazavi Malekshah et al. 2022). This theory explains why females stemborers have the potential to lay hundreds of eggs in colonies on selected hosts (Overholt et al., 2001; Lohman et al., 2024).

Our study revealed an uneven distribution of *Eldana saccharina* populations among different sugarcane varieties, with significantly higher infestation levels observed in the Green and Red varieties compared to the CO 421 variety. This pattern aligns with findings from previous studies, which have shown that susceptibility to stemborers can vary considerably among sugarcane cultivars. For example, a study conducted in Iran reported that the degree of *Sesamia* infestation differed across sugarcane varieties, notably, the study documented substantial yield losses of 98.08, 96.61, and 47.73 kg/ton in the CP69-1062, CP48-103, and IRC99-02 sugarcane varieties, and added that losses attributed to differences in the physico-chemical composition of the cultivars (Ebrahimifar et al., 2023).

Similarly, a study conducted on 16 sugarcane varieties in Louisiana (Southern United States) examined the impact of *Diatraea saccharalis* Fabricius, 1794 (Lepidoptera: Crambidae), and reported comparable trends in infestation rates. The research revealed a strong correlation between the nutritional composition of sugarcane varieties and their attractiveness to female ovipositional moths. These findings reinforce the idea that varietal traits particularly physico-chemical properties play a critical role in pest preference and infestation severity (Penn and Read, 2024). A recent report produced by Indian Institute of Sugarcane Research has provided further insights into physio-chemical composition of sugarcane varieties and their responses to specify the pests and diseases.

The report indicates that the CO 421 sugarcane variety, along with similar cultivars, exhibits higher fiber content (approximately 15%) and moderate sucrose levels in juice polarization (Pol ~13%), traits that are believed to enhance resistance to pests such as stemborers. In contrast, indigenous red and green sugarcane varieties are characterized by softer stems, lower fiber content (ranging from 0% to 14%), and higher sucrose concentrations (Pol ~15%). These physico-chemical differences likely contribute to their increased susceptibility to stemborer infestation (Shukla et al., 2018). This interpretation goes with the model of phytophagous insect host preference where female insects select potential host plants that optimize the chances of their offspring's survival (Jaenike, 1978; West and Paul Cunningham, 2002; Zhou et al., 2020, 2025). These findings underscore the importance of varietal selection in integrated pest management strategies. Further research is recommended to investigate the specific traits that confer resistance or susceptibility to *E. saccharina*, which could guide the development and promotion of more resilient sugarcane varieties.

Our study investigated the relationship between sugarcane sanitation practices, specifically old leaf removal and the degree of stemborer infestation in sugarcane fields. Our findings demonstrate that fields left untreated were significantly more susceptible to stemborer attacks compared to those subjected to regular leaf-removal. Previous studies demonstrated that female stemborers lay eggs in hidden parts of their host plants (Glas et al., 2007; Khan et al., 2007; Calatayud et al., 2008). By eliminating preferred egg-laying sites, such as senescent or damaged leaves, sanitation measures disrupt the pest's life cycle and contribute to population suppression.

This matches with the study conducted in Uganda demonstrated that the leaf-removal on sugarcane stems and effective field sanitation could successfully lower the pest population and reduce the damage to sugarcane plantations (Masanza et al., 2005), and supports the model that leaf-removal enhances the strength of sugarcane stem and basal internode strength (Xue et al., 2017), thereby increasing the crop resistance to stemborer attacks. also, leaves possess functional physio-chemical properties that herbivores detect, guiding their movement (Fernández et al., 2019). alterations in leaf composition and number of leaves in sugarcane fields could significantly impact sugarcane stemborers population. Our findings did not ignore the previous findings from a study conducted in Argentina which indicated that maintaining the sugarcane residues resulted in lower infestation of *Elasmopalpus lignosellus* (Zeller) (Lepidoptera: Pyralidae) and *Pseudaletia unipuncta* Haworth

(Lepidoptera: Noctuidae). Members from these genera primarily attack sugarcane from the roots (Isas et al., 2016). Further research is warranted to explore sugarcane stemborers by considering their entry points. Besides, findings underscore the importance of integrating cultural practices like leaf-removal sanitation practice into sugarcane pest management strategies and further research to quantify the long-term impact of sanitation treatments on pest dynamics and crop yield, and to optimize protocols for different sugarcane varieties and agro-ecological zones is recommended.

This study examined the influence of rainfall and temperature on the seasonal dynamics of *Eldana saccharina*. Preliminary data showed a gradual population increase from June to August, peaking in the dry season, followed by a decline from September to November during the short rains. These findings align with previous research from Iran, which identified temperature as a key factor regulating the developmental rate of *Sesamia* species in sugarcane fields (Soltani Orang et al., 2014). Supporting evidence from Colombia shows that *Diatraea* species achieved full development and successful reintroduction during the dry season, particularly when temperatures ranged between 16°C and 26°C. This further emphasizes the role of temperature as a critical factor influencing stemborer life cycles and seasonal population dynamics (Valencia Arbeláez et al., 2021). Our results showed a weak correlation between temperature and *Eldana saccharina* populations, likely due to the relatively stable year-round temperatures in the surveyed area. However, precipitation had a noticeable negative impact.

Heavy rainfall may disrupt the pest's life cycle by washing away vulnerable developmental stages such as eggs, larvae, and small adults, a phenomenon supported by existing literature (Shrestha, 2019; Karthik et al., 2022). Studies have shown that rainfall, especially when combined with high relative humidity, enhances the activity of entomopathogenic organisms such as fungi and bacteria. This increased biological pressure can significantly reduce insect populations, including *Eldana saccharina*, by disrupting their development and survival rate (Li et al., 2014; Mishra et al., 2015; McGuire and Northfield, 2020; Majchrowska-Safaryan and Tkaczuk, 2021; Kamga et al., 2022). In Rwanda, month of May marks the transition from heavy rains to the onset of the dry season. Early May often brings flooding in wetlands, while late May is characterized by warmer, sunnier conditions. This transitional period is suspected to trigger the first-generation cycle of *Eldana saccharina* in sugarcane plantations. Our findings suggest that sustainable management strategies

should target early infestations in June to prevent severe outbreaks later in the season. However, given the limited timeframe of this study, these results should be considered preliminary and we recommend multi-years' data collection to better understand the relationship between weather patterns and *E. saccharina* population dynamics, enabling more informed and sustainable pest management decisions.

4.2.5. Conclusion

This study identified *Eldana saccharina* as the sole stemborer species present in sugarcane plantations within the Nyabugogo tropical wetlands. The pest showed a marked preference for indigenous green and red sugarcane varieties over recent introduced industrial variety of CO 421. Sanitation practices, of old leaf removal, were found to reduce *E. saccharina* populations. The results confirm that population of this pest decrease with rain and increase with temperature, and confirm that, the first population start with May and reach the peak in August in Rwandan sugarcane system. While weather conditions play a significant role in stemborers diversity and richness, other ecological factors may also regulate *E. saccharina* populations, including cannibalistic behavior observed in related lepidopteran species like *Spodoptera frugiperda*, natural enemies, and entomopathogenic fungi. Given the short duration of this study, these findings should be considered preliminary. Future research should consider multi-year monitoring to better understand the complex interactions influencing stemborer dynamics and to support the development of sustainable pest management.

5. GENETIC DIVERSITY OF STEM BORER SPECIES FROM DIFFERENT AGRO-ECOLOGIES OF RWANDA: USING ETHIOPIAN POPULATION AS MODEL

5.1. Genetic variation and evolutionary relationships among *Sesamia nonagrioides* lineages in Ethiopian and Rwandan agro-ecologies

Abstract

Understanding the genetic structure and host-use dynamics of *Sesamia nonagrioides* is increasingly important as the species expands across East African agro-ecosystems. This study provides the first targeted assessment of mitochondrial COI diversity and phylogeographic structure of *S. nonagrioides* in Rwanda (N=16) and Ethiopia (N=14), integrating newly generated sequences with earlier continental datasets. Phylogenetic reconstruction and haplotype analyses revealed two geographically cohesive mitochondrial lineages corresponding to country of origin, with no haplotype sharing between regions. Rwanda harbors two ecologically distinct sublineages: one associated with sedge- and grass-dominated wild habitats particularly wetlands and another linked primarily to maize in cultivated landscapes. Ethiopia exhibits deeper structuring, forming multiple sublineages that include two sugarcane-dominated clusters and a wild-host lineage associated with *Echinochloa* and *Pennisetum* species. The close genetic affinity between sugarcane-feeding and wild-host Ethiopian sublineages indicates that sugarcane infestations represent recent host-range expansions rather than a divergent cane-specialist biotype. Genetic diversity was consistently higher in Ethiopia than in Rwanda, and mismatch distributions supported contrasting demographic signatures, from narrow, low-diversity patterns in Rwanda to broader, multimodal distributions in Ethiopia. AMOVA results showed complete mitochondrial differentiation between countries ($\Phi_{ST} \approx 1$), confirming long-term geographic separation of maternal lineages.

Collectively, these findings demonstrate that local habitat configuration, wild habitat connectivity, and plantation management strongly shape population structure at national scales, nested within broader continental patterns shaped by historical climatic processes. The presence of ecologically differentiated lineages implies that populations may respond unevenly to control tactics. Effective management should therefore integrate landscape-level surveillance across wild habitats, farm margins, maize zones, and sugarcane estates to detect spillover, anticipate host-range expansion, and mitigate emerging pest risks. This work advances evolutionary and applied understanding of *S. nonagrioides* in East Africa and highlights the need for continued genomic and landscape-based monitoring with ephasize on their behavioral changes and response to plant phenologies and chemistry.

Key words: Evolutionary relationship, cultivated crop, mitochondrial DNA, stemborer, wild habitat

5.1.1. Introduction

The *Sesamia nonagrioides* Lefèbvre, 1827 (Lepidoptera: Noctuidae), known as Mediterranean corn borer, pink stalk borer or West African pink borer is a moth belonging to the family Noctuidae (Lefèbvre, 1827). The occurrence of *S. nonagrioides* has been recorded across Europe, the Mediterranean, the Middle East, and Africa. Specifically in Portugal, Spain, the Canary Islands, France, Italy, Greece, Cyprus, Turkey, Morocco, Israel, Iran, Syria, Ethiopia, Ghana, Kenya, and Botswana (Esfandiari et al., 2015; Goftishu et al., 2018; Ho and Seshu Reddy, 1983; Moyal et al., 2011). The larvae of this species feeds on a wide range of host plants specifically Poaceae (graminaceous), Thyphaceae, and Cyperaceae (Goftishu et al., 2018; Khan et al., 1997; Le Rü et al., 2006; Moyal, 2014). It is considered as major pest of maize in Mediterranean countries (Goftishu et al., 2019; Margaritopoulos et al., 2007; Naino Jika et al., 2020) and sugarcane in Iran and Ethiopia (Cherghali et al., 2015; Goftishu et al., 2018). Similarly, Goftishu et al. (2018) clearly reported that *S. nonagrioides* utilizes multiple wild and cultivated host plants in Ethiopia and confirmed that the species is associated with grasses, sedges, maize, and sugarcane.

Large geographic distances and substantial physical or ecological barriers between regions can restrict dispersal and promote genetic differentiation, potentially leading to allopatric speciation (Anderson and Weir, 2022; Joshi et al., 2024; Wiens, 2024). In many insect species, including stemborers, even moderate barriers such as shifts in elevation, abrupt changes in vegetation structure, or transitions between agro-ecological zones can significantly reduce movement among populations (Dool et al., 2022). This limited connectivity increases the likelihood of forming distinct genetic lineages, especially when populations remain isolated over long time periods under different environmental regimes (Dool et al., 2022; Suárez et al., 2022). Moreover, populations occupying extensive, continuous continental landscapes often encounter broad gradients in climate, host-plant distribution, and habitat complexity, which may drive localized adaptation and further reinforce genetic differentiation (Suárez et al., 2022). These patterns frequently contrast with those observed in smaller, fragmented, or isolated habitats such as islands, where reduced area and restricted dispersal can intensify genetic structure and accelerate divergence (Broyles et al., 2023; Riesch et al., 2018). Together, these factors highlight the central role of geography, landscape configuration, and ecological heterogeneity in shaping the evolutionary trajectories of widely distributed Lepidopteran pests.

Taxonomically, *S. nonagrioides* was misidentified as *Cossus nonagrioides* Lefèbvre, 1827, *Nonagria hesperica* Rambur, 1837 (De Prins and De Prins, 2024), *Nonagria sacchari* (Wollaston 1858) (Chinese Academy of Sciences, 2024), *S. gracilis* (Butler, 1881) or *Tapinostola gracilis* (Rebel 1899) synonyms (Chinese Academy of Sciences, 2024). Current molecular data supported the species as *S. nonagrioides* and its isolated geographic population clusters in West Africa, East Africa, and the Palearctic regions (Goftishu et al., 2019; Moyal et al., 2011). From these perceptive, the Iranian phylogeographic study confirmed that *S. nonagrioides* species exhibit genetic connection with the East African populations which are mostly found in aquatic and humid land vegetation. Following long term studies (~70 years), the species is adapted to sugarcane plantations (Assefa, 2006; Esfandiari et al., 2015; Goftishu et al., 2019).

Early phylogeographic investigations of *S. nonagrioides* suggested the existence of three genetically distinct, allopatric lineages: The Palearctic lineage (*S. nonagrioides*), the West African lineage (historically *S. botanephaga*, later *S. n. botanephaga*), and an East African lineage recorded in Kenya and Uganda (Moyal et al., 2011). However, this initial study was constrained by limited sampling, covering only four African countries and heavily biased toward Kenyan specimens. Subsequent research expanded the geographic scope and refined analytical approaches. Later, Kergoat et al. (2015) and Goftishu et al. (2019) conducted a comprehensive phylogeographic study incorporating populations from sub-Saharan Africa and the Mediterranean, revealing strong genetic structure and historical isolation likely driven by ecological barriers and Pleistocene climatic oscillations.

Building on these foundations, recent species specific molecular studies employed advanced population genomics and landscape-level sampling to explore evolutionary dynamics within cultivated and wild habitats (Hévin et al., 2024; Goftishu et al., 2019; Margaritopoulos et al., 2007). These analyses confirmed that Ethiopian and Botswana populations of *S. nonagrioides* represent long-established lineages shaped by historical range expansions and secondary contact events (Goftishu et al., 2019). Genetic evidence suggests that host-plant shifts, particularly the adaptation of Ethiopian populations to sugarcane were likely driven by habitat fragmentation and ecological pressures rather than genetic divergence alone (Assefa, 2006; Goftishu et al., 2019).

Collectively, these findings underscore the complex interplay of climatic oscillations, ecological adaptation, and anthropogenic landscape changes in shaping the phylogeographic patterns of *S. nonagrioides* (Goftishu et al., 2019), with significant implications for pest management strategies across its expanding range (Moyal, 2014).

Built on previous phylogeographic insights, the present study reassessed and expanded the datasets reported by Goftishu et al. (2019); Kergoat et al. (2015); Moyal et al. (2011) by incorporating *S. nonagrioides* species previously sampled from Ethiopia and newly collected in Rwanda. These additions are critical since the inclusion of new populations can substantially alter inferred genetic relationships and evolutionary interpretations (Rota et al., 2022). Our objectives were (1) to analyze the origin and genetic identity of *S. nonagrioides* populations from both wild habitats and cultivated crops in Rwanda; (2) to determine the genetic relationships between its populations in Ethiopia, Rwandan and previously characterized lineages from sub-Saharan Africa, and the Palearctic; (3) to characterize the genetic structure of *S. nonagrioides* across its current range, reconstruct its evolutionary history; and (4) to assess the influence of host plants on genetic diversity within Ethiopian and Rwandan populations. This integrative approach would provide a more comprehensive understanding of lineage diversification, host-use dynamics, and historical biogeography in pest species of growing economic and ecological importance.

5.1.2. Materials and Methods

5.1.2.5. Insect Specimens

Larvae of *Sesamia nonagrioides* were collected between 2023 and 2024 in four agroecological zones of the Northern, Eastern, Southern Rwanda, and the capital city of Kigali. Sampling targeted both green sugarcane, red sugarcane, and maize from the cultivated habitats, Napier grass (*Pennisetum purpureum*), *Echinochloa crus-galli*, *Rottboellia cochinchinensis*, *Cyperus strigosus*, *Cyperus fastigiatus*, and *Typha* species in wild habitats. Collections took place in wetlands, marshlands, riverbanks, farms, farm margins, and roadside vegetation to capture the full range of host plants associated with stemborer populations. Population of *Sesamia nonagrioides* in Rwanda was incorporated into previously constructed phylogenetic trees that were originally based on samples from Ethiopia (extracted from NCBI database) (Tab. 5.1), along with newly collected samples from Rwanda to analyse understand the Rwandan population of stemborers' divergence. Ethiopian samples were selected because Ethiopia has experienced a long history of geological changes, leading to significant differentiation from other parts of East Africa (Yemane K. et al., 1985). Additionally, Ethiopia has the highest number of genetic studies conducted on stem borers worldwide, making it a valuable reference for comparison (Moyal et al., 2011; Kergoat et al., 2015; Goftishu et al., 2019).

Table 5. 1. Locations where specimens of *S. nonagrioides* were collected in Rwanda and accession numbers for published sequences of Ethiopia

Acc.no/labels	Country	Locality	Coordinates	Host plant
SEETG3673	Ethiopia	Bahar Dar	08°06'00"N 38°47'00"E	<i>Echinochloa pyramidalis</i>
SEETG4009	Ethiopia	Bahar Dar	08°06'00"N 38°47'00"E	<i>Echinochloa pyramidalis</i>
SEETG5421	Ethiopia	Andasa	11°30'00"N 37°28'00"E	<i>Pennisetum thonbergii</i>
SEETG5422	Ethiopia	Chamoleto	05°55'00"N 37°32'00"E	<i>Typha domingensis</i>
SEETG5449	Ethiopia	Bahar Dar	08°06'00"N 38°47'00"E	<i>Echinochloa colonum</i>

SEETG5450	Ethiopia	Black Water	07°05'00"N 38°28'00"E	<i>Saccharum officinarum</i>
SEETG5451	Ethiopia	Hesbay Nuro	07°57'00"N 38°43'00"E	<i>Pennisetum purpureum</i>
SEETG8506	Ethiopia	Metehara 1	08°54'00"N 39°54'00"E	<i>Saccharum officinarum</i>
SEETG8551	Ethiopia	Metehara 2 (64)	08°50'00"N 39°05'00"E	<i>Saccharum officinarum</i>
SEETG8553	Ethiopia	Kesem (68)	09°08'00"N 39°52'00"E	<i>Saccharum officinarum</i>
SEETG8554	Ethiopia	Walenchiti	08°48'00"N 39°52'00"E	<i>Saccharum officinarum</i>
SEETG8555	Ethiopia	Walenchiti	08°48'00"N 39°52'00"E	<i>Saccharum officinarum</i>
SEETG8556	Ethiopia	Dera	08°21'00"N 39°19'00"E	<i>Saccharum officinarum</i>
SEETG8552	Ethiopia	Melkassa	08°24'00"N 39°22'00"E	<i>Saccharum officinarum</i>
3 Sn	Rwanda	Kigali_Nyabugogo	01°56'16"S 30°02'39"E	<i>Zea mays</i>
4 Sn	Rwanda	Kigali_Nyabugogo	01°56'17"S 30°02'41"E	<i>Saccharum officinarum</i>
10 Sn	Rwanda	Kigali_Nyabugogo	01°56'17"S 30°02'47"E	<i>Saccharum officinarum</i>
23 Sn	Rwanda	Kayonza_Gahini- Muhazi lake	01°50'54"S 30°28'43"E	<i>Zea mays</i>
24 Sn	Rwanda	Kayonza_Gahini	01°45'35"S 30°37'08"E	<i>Zea mays</i>
36 Sn	Rwanda	Kamonyi_Rugalika	01°56'48"S 29°57'31"E	<i>Zea mays</i>
40 Sn	Rwanda	Kamonyi_Rugalika	01°56'17"S 30°30'30"E	<i>Zea mays</i>
46 Sn	Rwanda	Kigali_Gitikinyoni	01°57'04"S 30°00'08"E	<i>Pennisetum purpureum</i>
47 Sn	Rwanda	Kigali_Nyabugogo	01°56'19"S 30°02'42"E	<i>Echinochloa crus-galli</i>
54 Sn	Rwanda	Kigali_Masaka	01°55'60"S 30°02'56"E	<i>Rottboellia cochinchinensis</i>

60 Sn	Rwanda	Kigali_Nyabugogo	01°55'10"S 29°59'11"E	<i>Cyperus strigosus</i>
61 Sn	Rwanda	Kayonza_Murundi	01°40'34"S 30°28'42"E	<i>Cyperus fastigiatus</i>
66 Sn	Rwanda	Kayonza_Gahini	01°45'35"S 30°37'08"E	<i>Typha</i>
75 Sn	Rwanda	Kigali_Nyarugenge	01°57'04"S 30°00'08"E	<i>Pennisetum purpureum</i>
82 Sn	Rwanda	Kigali_Nyarugenge	01°56'17"S 30°02'41"E	<i>Cyperus</i>
93 Sn	Rwanda	Kigali_Kicukiro	01°56'42"S 30°03'43"E	<i>Cyperus</i>

5.1.2.2. DNA Extraction, Polymerase Chain Reaction (PCR), and Sequencing

Samples of insects were sent delivered to The e-DNA consultancy Ltd, an environmental DNA (e-DNA) consultant company registered (registration number of 15508607), Gloucestershire, England) for DNA extraction and PCR. Genomic DNA was extracted from insect specimens targeting the mitochondrial cytochrome c oxidase subunit I (CO1) gene for amplification, as it is the universal marker for DNA barcoding. The PCR amplification carried out under the following conditions: initial denaturation at 95 °C for 10 min, followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min, and extension at 72 °C for 1 min, with a final extension at 72 °C for 5 min (Hebert et al., 2003). The primers used were LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-AACTTCAGGGTGACCAAAAAATCA-3') as described in Folmer et al. 1994), ensuring coverage of the barcode region and comparability with previous studies. The PCR products were subsequently purified and sequenced by Macrogen's Genomics branch in England.

Amplification reactions were optimized for annealing temperature and cycle number to minimize nonspecific products. Successful amplicons were subjected to Sanger sequencing in both directions, generating paired chromatogram files (.ab1) for each specimen. Sequencing quality was assessed using Phred scores and only reads meeting a minimum threshold of Q20 (corresponding to 99% base-calling accuracy) were retained for downstream analysis. Sequences were received as raw sequences, not cleaned, and reverse and forwards segments were separated.

5.1.2.3. Sequences Management Prior to MEGA Analysis

All COI sequences were first processed to generate high-quality consensus reads before downstream analyses in Molecular Evolutionary Genetics Analysis (MEGA 12). Raw Sanger chromatogram files (forward and reverse. ab1) for each specimen were imported into Geneious Prime, where low-quality ends were trimmed based on error-probability thresholds to remove unreliable base calls. Forward and reverse reads were assembled de novo into contigs, and chromatograms were manually inspected to verify base-calling accuracy, resolve mismatches, and correct ambiguous positions where necessary. Final consensus sequences were extracted in FASTA format, checked for expected COI length, translated to confirm the absence of premature stop codons, and curated for naming consistency.

These curated consensus sequences constituted the final dataset imported into MEGA for alignment, model selection, and phylogenetic reconstruction.

5.1.2.4. DNA Sequence Alignment and Data Preparation

Raw DNA sequences obtained from Rwandan and Ethiopian samples were aligned using MEGA 12 (Molecular Evolutionary Genetics Analysis software). Sequences were first imported in FASTA format and examined for quality, including checking for ambiguous bases, gaps, and alignment consistency. Multiple sequence alignment was performed using the ClustalW algorithm implemented in MEGA 12 with default parameters (gap opening penalty = 15, gap extension = 6.66). The alignment was manually inspected and trimmed to remove regions of poor alignment prior to phylogenetic and population-genetic analysis.

5.1.2.5. Phylogenetic Tree Reconstruction

The resulting Neighbor-Joining (NJ) consensus tree was visualized in MEGA 12's Tree Explorer, and *S. nonagrioides* was used to root the tree and marked as SEETG. Branch lengths were calculated in number of substitutions per site, and bootstrap support values $\geq 70\%$ were considered moderate to strong support. Clades were interpreted in relation to geographic origin (Rwanda vs Ethiopia) and host plants (maize vs wild vegetation). Host plants relationship analysis was limited to Rwanda since the Ethiopian specimens were only 4 from sugarcane plantation.

To determine the most appropriate nucleotide substitution model, the dataset was subjected to model selection in MEGA 12 (Analysis → Models), and the optimal model was chosen based on the lowest Bayesian Information Criterion (BIC) and the highest maximum likelihood score. This best-fit model was then applied during phylogenetic reconstruction. Phylogenetic relationships among samples were inferred using the neighbor-joining (NJ) tree method implemented in MEGA 12 under the selected substitution model, with rates among sites modeled as gamma-distributed or uniform depending on the recommended configuration. The analysis used 1,000 bootstrap replicates to assess node support, and gaps were treated using pairwise deletion unless indicated otherwise. The resulting NJ tree was visualized in MEGA's Tree Explorer, where *SEETG5451 Sn* served as the outgroup for rooting, and branch lengths were expressed in substitutions per site.

Bootstrap values of 70% or higher were interpreted as moderate to strong support, and the final tree topology was examined to evaluate clade structure in relation to both geographic origin (Rwanda vs. Ethiopia) and host-plant association (maize vs. wild vegetation).

5.1.2.6. Genetic Diversity and Population Structure Analysis

Genetic diversity analyses were conducted in MEGA 12 using the Diversity module to assess variation both within and between defined populations. Two hierarchical levels of population structure were evaluated: (i) geographic groups consisting of samples from Rwanda and Ethiopia, and (ii) host-plant-associated groups within Rwanda (Tab. 5. 1). Population assignments were created in MEGA through the “Data → Select Taxa/Groups” function, ensuring that each sequence was placed into its appropriate biological category before downstream analysis.

Within-population genetic diversity was estimated using the “Compute Mean Diversity Within Subpopulations” procedure in the Diversity module. The statistics calculated included nucleotide diversity (π), haplotype diversity (H_d), the number of segregating sites (S), and the average number of nucleotide differences (k). These analyses were performed under the p-distance substitution model, which allows direct comparison between the estimated diversity value (d) and nucleotide diversity. Gaps and missing data were treated with pairwise deletion, and variance around each estimate was assessed using 1,000 bootstrap replicates. This approach enabled comparisons of diversity between the two geographic populations as well as between the maize-feeding and wild-vegetation-feeding groups within Rwanda.

To quantify genetic divergence between populations, mean interpopulation diversity was estimated using the “Compute Mean Interpopulation Diversity” option. This analysis provided two key metrics: D_{xy} , the average number of nucleotide substitutions per site between two populations, and D_a , the net nucleotide divergence after accounting for within-population variation. Net divergence was calculated using the expression $D_a = D_{xy} - \frac{\pi_1 + \pi_2}{2}$. These measures were used to evaluate the extent of genetic separation between Rwanda and Ethiopia, as well as between the different host-plant-associated groups.

Population differentiation was further examined using AMOVA (Analysis of Molecular Variance) was performed in `pegas::amova` using a DNA distance matrix (model “N”), with 9,999 permutations:-based Φ -statistics generated through the “Compute Φ -statistics of Differentiation” function. Analyses were conducted under the p-distance model using 1,000–10,000 permutations to assess statistical significance. Φ_{ST} values, together with their corresponding P-values, provided a measure of the proportion of total genetic variation attributable to differences between populations, allowing robust assessment of differentiation across both geographic and ecological groupings.

Finally, overall genetic diversity across the entire dataset was estimated using the “Compute Mean Diversity in Entire Population” option, which provided broader context for interpreting within- and between-population patterns. This comprehensive framework allowed a detailed evaluation of genetic structure across geographic regions and host-plant associations.

5.1.3. Results

5.1.3.1. Phylogenetic Structure of *Sesamia nonagrioides* Populations

The phylogenetic analysis demonstrates that Rwandan and Ethiopian populations of *S nonagrioides* form two geographically distinct evolutionary lineages. In Rwanda, the lineage further separates into two well-supported ecological subclades. The first subclade comprises individuals associated with wetland grasses and sedges, including *Panicum repens*, *Echinochloa pyramidalis*, *Typha* spp., *Pennisetum purpureum*, and *Cyperus* species represented by 93 Sn, 75 Sn, 66 Sn, 47 Sn, 61 Sn, 60 Sn, 54 Sn, and 46 Sn. The second subclade is composed exclusively of maize-feeding individuals from cultivated fields, represented by 23 Sn, 24 Sn, 36 Sn, and 3 Sn, after removing sugarcane- and sedge-associated samples that belong to the wild-vegetation clade (Fig. 5.1).

Ethiopian populations form three distinct but related subclades rather than a single host-associated grouping. The first subclade contains individuals feeding on *Echinochloa* and related grasses (e.g., IDs 3673, 5449, 4009). The second subclade clusters elephant-grass-associated and mixed-host individuals (e.g., SEETG8552–8556 and 5451).

The third subclade consists of sugarcane-feeding populations (e.g., STG 8551, 8506, 8553), which cluster together but do not exhibit deep differentiation from wild-host lineages. Collectively, these patterns show that while Rwanda exhibits clearer ecological partitioning between wetland and maize groups, Ethiopia exhibits a three-subclade structure reflecting host-use overlap and strong connectivity between sugarcane plantations and adjacent wild habitats (Fig. 5. 1).

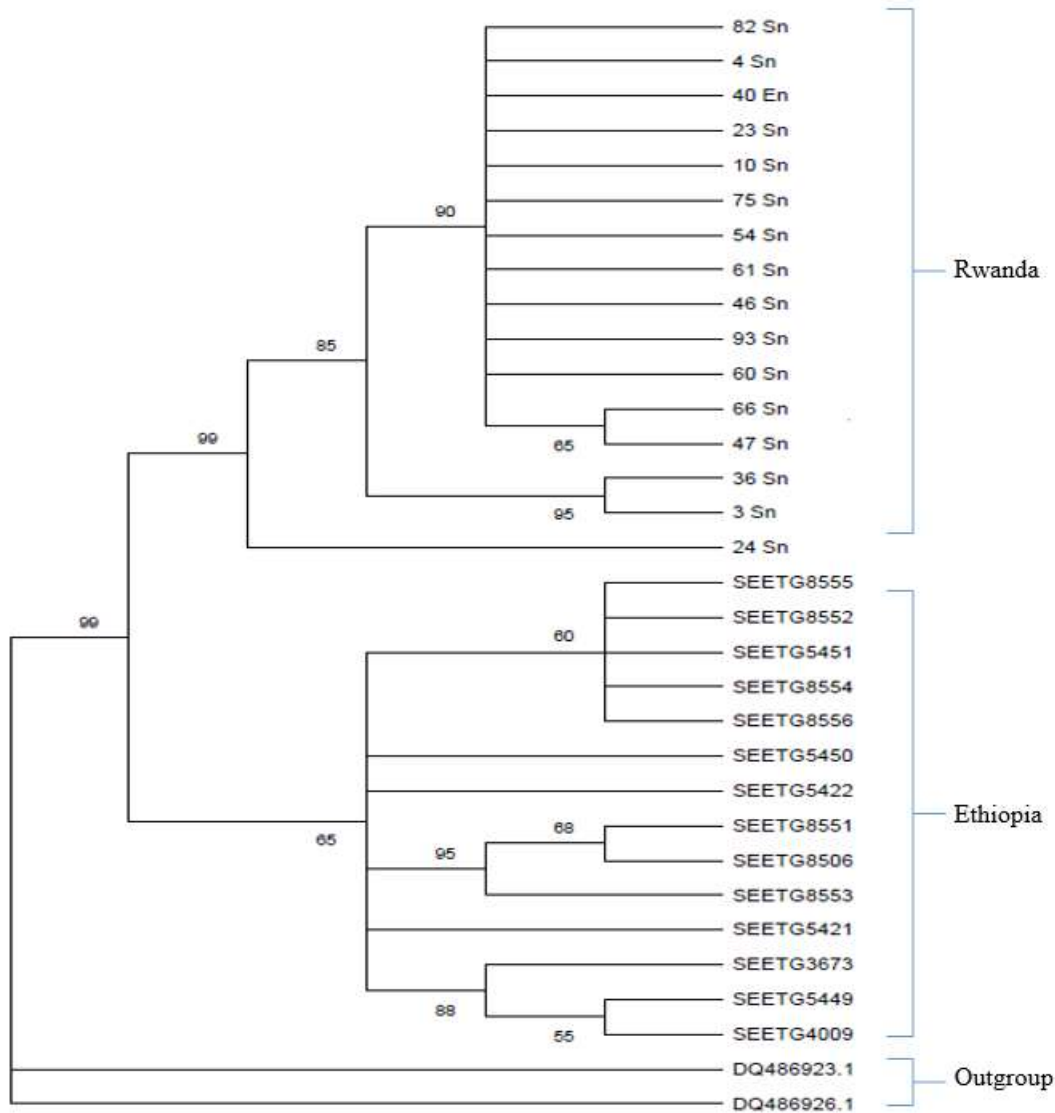


Figure 5. 1. The evolutionary history's Neighbor-Joining consensus tree generated from MEGA12 utilizing up to 4 parallel computing threads. Branches corresponding to partitions reproduced in less than 50% trees are collapsed.

The evolutionary distances were computed using the p-distance method with 10000 repetitions and are in the units of the number of base differences per site

5.1.3.2. Haplotype Composition and Country-Level Partitioning (CORE Dataset)

Haplotype reconstruction from the CORE alignment revealed eight distinct haplotypes across the 30 individuals, supported by 160 segregating sites. These haplotypes exhibited a completely country-exclusive distribution: Rwanda possessed three haplotypes, whereas Ethiopia possessed

five haplotypes. Importantly, there was no haplotypes were shared between the Rwandan and Ethiopian *S. nonagrioides* population (Fig. 5. 2).

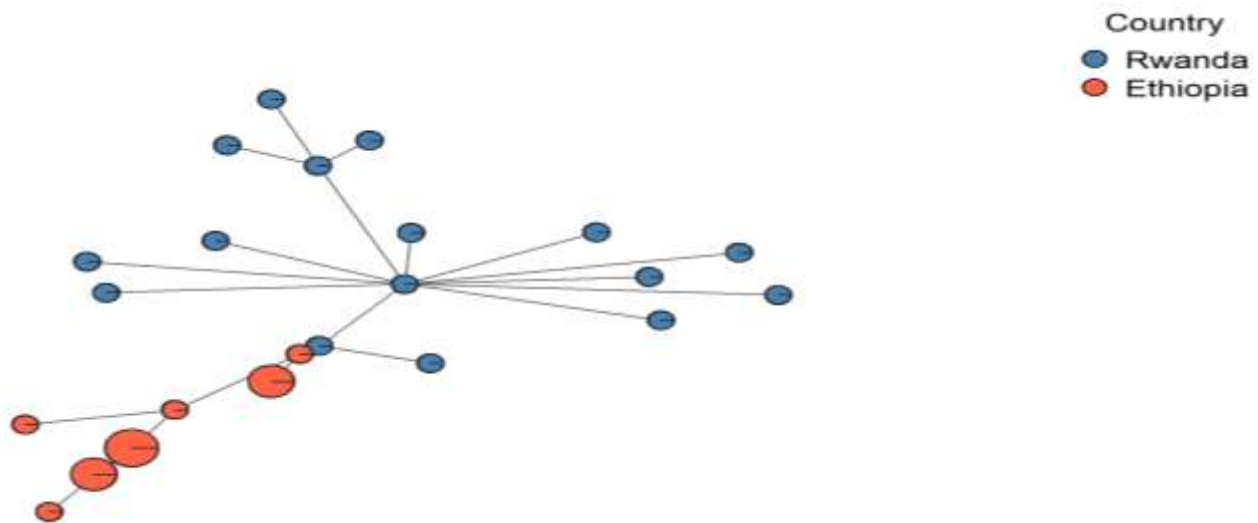


Figure 5. 2. TCS haplotype network based on the 744 bp mitochondrial COI sequences of lepidopteran stem borers sampled in Rwanda and Ethiopia. Each circle represents a unique haplotype, with the size proportional to the number of individuals sharing that haplotype. Colors indicate the country of origin (blue = Rwanda, red = Ethiopia). Lines between haplotypes represent single mutational steps

This stark partitioning indicates that the mitochondrial lineages of Ethiopian and Rwandan *Sesamia* moths are completely distinct within the examined COI region. The absence of shared haplotypes strongly supports pronounced geographic structuring and historical isolation of maternal lineages between the two regions.

The haplotype network analysis further corroborated this pattern: in the CORE network, all nodes were purely country-specific, with no mixed pie charts, demonstrating total mitochondrial separation.

5.1.3.3. Patterns of Genetic Diversity (FULL vs. CORE Datasets)

Genetic diversity statistics were computed separately for each country under both the FULL and CORE datasets. In the FULL alignment, Rwanda exhibited high haplotype diversity ($H_d = 0.8917$) but low nucleotide diversity ($\pi = 0.00382$), accompanied by a strongly negative and statistically

significant Tajima's D ($D = -2.433$; $P < 0.05$). This pattern implies an excess of low-frequency variants, often associated with recent demographic expansion or purifying selection. Ethiopia showed moderately high haplotype diversity ($H_d = 0.8681$) and much higher nucleotide diversity ($\pi = 0.01243$) than Rwanda but with a non-significant positive Tajima's D ($+1.232$), suggesting a more stable or heterogeneous lineage structure.

After trimming to the CORE region, Rwanda's diversity dropped sharply, with fewer haplotypes ($n_{hap} = 3$), substantially lower H_d (0.3417), and very low π (0.00264). Tajima's D became non-significant (-1.002), indicating that once uneven alignment ends were removed, support for an excess of rare alleles diminished. Ethiopia, in contrast, retained moderately high diversity in the CORE dataset ($H_d = 0.7692$; $\pi = 0.00829$) with D values near zero, indicating a more stable pattern of mitochondrial variation. Overall, Ethiopia consistently exhibited greater nucleotide diversity than Rwanda across both datasets, confirming deeper or more varied mitochondrial lineages within the Ethiopian population.

5.1.3.4. Mismatch Distribution Patterns

Mismatch distributions, computed from pairwise DNA distances, displayed contrasting demographic signatures between countries. In the CORE dataset, the Rwandan mismatch distribution was relatively narrow, consistent with its low nucleotide diversity and fewer haplotypes (Fig. 5. 3). This pattern aligns with the reduced variation observed once end-gaps were removed.

In contrast, Ethiopian mismatch distributions were broader, reflecting the population's higher H_d and π values and supporting a scenario of deeper lineage divergence or larger long-term effective population size. These patterns were consistent whether examined in bar-plot or line-plot form.

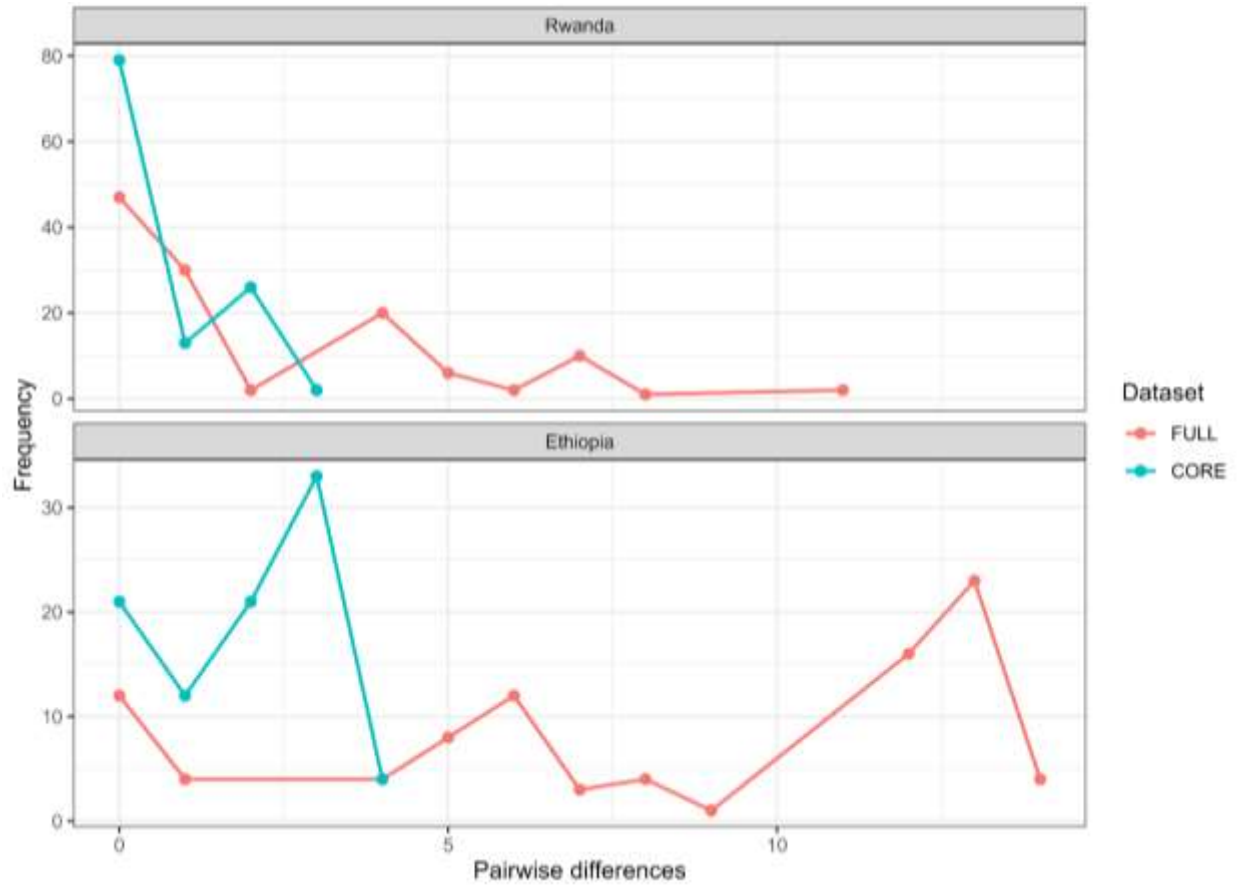


Figure 5. 3. Pairwise-difference mismatch distributions for *S. nonagrioides* in Rwanda and Ethiopia using FULL and CORE datasets. Rwanda displays narrow, steep distributions indicative of low haplotype and nucleotide diversity, while Ethiopia exhibits broader, multimodal patterns consistent with greater genetic diversity and deeper demographic structure

5.1.3.5. Population Differentiation (AMOVA)

Analysis of Molecular Variance (AMOVA) confirmed extremely strong genetic differentiation between Ethiopia and Rwanda. In the FULL dataset, the among-population component accounted for almost all molecular variance ($\sigma^2 \approx 53,411$), yielding $\Phi_{ST} \approx 1$ ($P < 0.0001$).

Importantly, the CORE dataset free of alignment produced an equally pronounced result, with among-population $\sigma^2 \approx 12,539$ and $\Phi_{ST} \approx 1$ ($P < 0.0001$) (Tab. 5. 2).

Table 5. 2. Genetic diversity statistics for Rwandan and Ethiopian *Sesamia* populations based on FULL (744 bp) and CORE (224 bp) COI datasets

Dataset	Country	n	L (bp)	nhap	Hd	π	Tajima's D	P (normal)	P (beta)
FULL	Rwanda	16	744	9	0.8917	0.00382	-2.433	0.0150*	0.00023*
FULL	Ethiopia	14	744	7	0.8681	0.01243	+1.232	0.2179	0.2474
CORE	Rwanda	16	224	3	0.3417	0.00264	-1.002	0.3164	0.3468
CORE	Ethiopia	14	224	5	0.7692	0.00829	-0.056	0.9551	0.9857

Note: In a table, n is the number of sequences per population; L is alignment length (bp); nhap is the number of distinct haplotypes; Hd is haplotype diversity; and π is nucleotide diversity (average number of nucleotide differences per site). Tajima's D tests departures from mutation–drift equilibrium, with negative values indicating an excess of rare variants and positive values suggesting an excess of intermediate-frequency variants. P (normal) and P (beta) are the two significance tests provided by pegas, evaluating whether Tajima's D differs significantly from neutral expectations. Significant values indicate demographic changes or selection acting on the mitochondrial lineage. * shows the significant values.

The congruence between FULL and CORE analyses demonstrates that the observed population differentiation is biologically robust rather than an artifact of missing data. The CORE dataset strengthens this conclusion by ensuring that the comparison is based exclusively on shared, high-quality sequence positions. Together with the complete segregation of haplotypes by country, these results show that Rwandan and Ethiopian *Sesamia* populations form two fully distinct mitochondrial lineages with no detectable mixing or shared maternal ancestry (Tab. 5. 3).

Table 5. 3. Analysis of Molecular Variance (AMOVA) comparing Ethiopian and Rwandan *Sesamia* populations based on the FULL (744 bp) and CORE (224 bp) COI datasets

Dataset	Among-pop variance (σ^2)	Within-pop variance (σ^2)	Φ_{ST}	P (among)
FULL (744 bp, with gaps)	53,411.47	6.694	~1.000	0
CORE (224 bp, no gaps)	12,538.53	1.467	~1.000	0

Note: Among-population variance (σ^2) represents the amount of genetic variation attributed to differences between the two countries, while within-population variance (σ^2) reflects variation among individuals within each country

5.1.4. Discussion

5.1.4.1. Overview and placement of our findings in the continental context

Results of the phylogenetic analysis revealed that the Rwandan specimens from different vegetation mosaics and agro ecologies are almost identical to each other and different from Ethiopian model samples. However, the number of specimens used were small to conclude without wide samples from neighboring countries Uganda, DRC, Burundi, Tanzania, and other countries we share similar ecosystems. This makes it difficult to generalize about relationship between Rwandan population of *S. nonagrioides* and populations from other parts of Africa.

Our phylogeny of *S. nonagrioides* from Rwanda and Ethiopia resolves two geographically cohesive lineages that correspond to country of origin. Within Rwanda, we observe two well-supported subclades: (i) a lineage tied to wetland grasses and sedges (*Cyperus*, *Typha*, *Pennisetum*, *Echinochloa*, *Rottboellia*) (with a few sugarcane individuals clustering within it), and (ii) a lineage dominated by maize Ethiopian population presented three sublineages, one comprising *Echinochloa/Pennisetum* feeders, a second mixed group dominated by sugarcane with *Typha* present, and a third pure sugarcane lineage. These patterns indicate phylogeographic separation between countries, complemented by ecological partitioning within countries.

At the continental scale, Gofitishu et al. (2019) reported that *S. nonagrioides* diversity is structured into two major clades with extensive geographic overlap and that broad patterns most likely reflect Pleistocene climatic oscillations and expansion from refugia rather than contemporary isolation by distance or crop history. Our country-focused results fit within this broader narrative by revealing local structure nested inside the larger, historically shaped lineages. However, Rwanda and Ethiopia data may not reflect the total structure of regional structure of *S. nonagrioides* where restricted representation can exaggerate or obscure genetic structure. Therefore, these findings should be regarded as preliminary until supported by broader, landscape-level sampling.

5.1.4.2. Host-plant use: local ecological partitioning versus continental-scale HAD

Across Africa, Gofitishu et al. (2019) reported that there is no clear host-associated differentiation (HAD) when pooling many countries and hosts; shared haplotypes occurred on multiple host families (Poaceae/Cyperaceae/Typhaceae), arguing against stable host races. In contrast, our

Rwanda–Ethiopia focus reveals host-linked clusters at a local scale: in Rwanda, a wetland-grass/sedge lineage sits apart from a maize-dominated lineage; in Ethiopia, sugarcane forms one entire sublineage and dominates another.

We interpret this as ecological partitioning without deep lineage splits as recent study has reported that is about host sorting inside a single species rather than continent-wide HAD (Sezonlin et al., 2006). This reconciles our findings with Gofitshu et al. (2019) at large scales, gene flow and historical mixing obscure host signals, but at local scales, habitat mosaics and resource continuity in plantations can structure populations enough to appear as host-based clades in a country-restricted tree (Assefa, 2006; Calatayud et al., 2016; Elameen et al., 2024).

5.1.4.3. Ethiopia: sugarcane colonization and its genetic meaning

Previous studies showed that the emergence of *S. nonagrioides* as a sugarcane pest in Ethiopia is best explained by host-range expansion from wild hosts rather than a distinct “sugarcane biotype,” noting minimal COI differentiation between sugarcane and wild-host samples and shared haplotypes across hosts (Assefa, 2006; Calatayud et al., 2016; Gofitshu et al., 2019). Our tree supports and extends that view. Ethiopia shows (i) a grass/sedge lineage (e.g., *Echinochloa*, *Pennisetum thonbergii*), (ii) a mixed sugarcane + *Typha* lineage, and (iii) a pure sugarcane lineage. The sister relationships among these Ethiopian sublineages, and the proximity of sugarcane taxa to wild-host taxa, are consistent with recent host colonization events from wild habitat-associated pools into sugarcane, rather than long-standing isolation of a cane-specialist taxon.

Functionally, continuous cane availability, reduced natural-enemy pressure, and habitat simplification in plantations likely maintain these sugarcane-dominated clusters (Esfandiari et al., 2015; Gofitshu et al., 2019). However, due to the limited sample-size of this study, our data should serve as preliminary and recommend expanded researches.

5.1.4.4. Rwanda: wetland–cropland contrast and landscape filters

In Rwanda, *S. nonagrioides* occurs in two sympatric but ecologically distinct lineages: a wild habitat grass/sedge group and a maize-dominated group. Given that Gofitshu et al (2019) did not

detect continental HAD, the Rwandan split is best viewed as landscape-mediated sorting within one evolutionary lineage. Wetlands (papyrus, sedges, riparian grasses) offer perennial refugia and metapopulation continuity, whereas maize fields are seasonal, spatially patchy, and human-managed. This environmental contrast can generate recurrent clade-level structure in country-level trees without implying reproductive isolation, precisely the nuance emphasized by host use plasticity and weak host-linked genetic barriers at broad scales (Assefa, 2006; Esfandiari et al., 2015; Goftishu et al., 2019; Sezonlin et al., 2006).

5.1.4.5. The Rift system and historical climate

Goftishu et al. (2019) argued that Pleistocene climate cycles better explain the major clades of *S. nonagrioides* than simple isolation-by-distance or modern agriculture; they also observed that the East African Rift is not a strict barrier, with closely spaced localities sometimes belonging to different clades. Our dataset was limited to Ethiopia and Rwanda with findings that is congruent with this view. The country-level structure we detect most plausibly reflects post-refugial population sorting layered onto contemporary habitat mosaics rather than strong, ancient vicariance between Rwanda and Ethiopia. Where wetlands and wild habitats are extensive and connected (e.g., lake shores, riverine marshes), they likely act as source habitats feeding both wild-host and adjacent crop populations, a dynamic consistent with the secondary contact/admixture scenarios invoked continent-wide (Cherghali et al., 2015; Le Rü et al., 2006; Moyal, 2014).

5.1.4.6. Demographic signals and diversity

The recent continental study on *S. nonagrioides* genetic diversity reported inferred demographic expansion (unimodal mismatch, negative Tajima's *D* and Fu's *F_s*) in both major clades and higher diversity in wild-host populations than in cultivated-host populations (Goftishu et al., 2019). In our material, the Ethiopian wild-host sublineage (*Echinochloa/Pennisetum*) and the Rwandan wild habitat particularly wetlands's sublineage are more internally diverse than the maize/sugarcane sublineages, qualitatively echoing the pattern that wild-host pools retain more genetic variation while crop-associated clusters appear younger/less diverse, consistent with recent host shifts and bottlenecks (Forbes et al., 2017; Mutamiswa et al., 2023; Toussaint et al., 2012).

5.1.4.7. Implications for pest management

Building on Gofishu et al. (2019), who warned that genetically structured stemborer populations can respond unevenly to control tactics and highlighted the emerging threat of *S. nonagrioides* to East African sugarcane following host-range expansion, our findings provide more geographically nuanced implications for Rwanda and Ethiopia. In Ethiopia, the detection of two sugarcane-dominated sublineages points to multiple recruitment pathways from adjacent wetland habitats, indicating that cane estates function as open ecological systems rather than isolated production units. Effective management should therefore prioritize strengthening vegetation buffers and host-plant monitoring along wetland margins, restoring or reinforcing natural enemy complexes weakened within plantation environments, and implementing landscape-level surveillance that jointly tracks population dynamics across cereal farmlands and nearby wild vegetation as previously recommended (Assefa et al., 2010; Ntiringanya et al., 2026; Otieno et al., 2008; Sokame et al., 2019).

In Rwanda, the coexistence of a wild habitat particularly wetland-derived lineage and a maize-associated lineage underscores the potential for seasonal spillover between habitats; thus, surveillance and interventions should be synchronized across wild habitat and maize fields. By monitoring sedge and grass hosts near maize zones and aligning crop-residue management practices may reduce stemborers inter-season carry-over (Ntiringanya et al., 2026, 2025).

Besides, the weak evidence for broad-scale host-associated differentiation suggests that management strategies should rely more on behavioral and ecological leverage points such as crop phenology, developmental stages, habitat-edge configuration, and the continuous availability of cereal crops rather than expecting strong genetic barriers to limit host switching (Ntiringanya et al., 2026, 2025).

5.1.4.8. Limitations and future work

Our reflections are COI-based trees and country-focused stemborers population from limited number of host plants. Gofishu et al. (2019) demonstrated that patterns can change as sampling broadens and that inference of genetic structure (e.g., F_{ST}) is sensitive to sample size. Future work

that analysis phylogeography and evolutionary relationship of Ethiopian-Rwandan stemborers population should therefore include: (1) Regional trail that express similar or connected historical mechanism of evolution; (2) Multilocus or genomic markers to test for cryptic structure beyond COI; (3) periodical sampling across seasons to quantify wild vegetation to crop stemborers population structure; (4) Landscape genetics to test how wild habitat extent, urban and infrastructure expansion, and agriculture intensification and cropping calendars shape movement hypotheses explicitly raised for stemborers population in not only Rwanda-Ethiopia, but in East Africa.

5.1.5. Conclusion

This study provides the first focused evaluation of the genetic diversity and phylogeographic structure of *S. nonagrioides* in Rwanda and Ethiopia, revealing clear regional differentiation that refines the broader continental picture of the species. While earlier work attributes major lineage splits to deep Pleistocene divergences, our results point to more recent, landscape-driven structure operating at micro and medium-scale habitats. In Rwanda, populations separate into two distinct ecological lineages, one tied to sedge- and grass-dominated by wetlands population and the other associated with maize in cultivated fields. Ethiopian populations exhibit even greater structuring, with multiple sublineages including two linked to sugarcane and another connected to *Echinochloa* and *Pennisetum* hosts. The close genetic affinity between sugarcane-feeding and wild-host Ethiopian lineages indicates that sugarcane infestations likely reflect a recent host-range expansion rather than a divergent biotype, underscoring the species' marked host-use plasticity.

Collectively, these patterns highlight the strong influence of wild habitat connectivity, habitat configuration, and plantation management on population dynamics. They also emphasize that ecologically and genetically differentiated lineages may respond unevenly to control tactics. Effective pest management should therefore integrate surveillance across both wild and cultivated habitats, with particular attention to wetlands, farm edges, cereals systems, and sugarcane estates where *S. nonagrioides* is emerging as a significant threat. Overall, this work advances understanding of the species' evolutionary ecology in East Africa and underscores the need for continued landscape-level monitoring to anticipate further host-range expansion and mitigate future pest risks.

5.2. Genetic diversity and phylogenetic relationships of *Eldana saccharina* Walker (Lepidoptera: Pyralidae) from different agroecological zones of Ethiopia and Rwanda

Abstract

Understanding the genetic and ecological differentiation of *Eldana saccharina* populations is critical for predicting host-use dynamics and improving pest management strategies in East Africa. Using COI sequences and Maximum Likelihood phylogenetic reconstruction, we examined evolutionary relationships between Ethiopian and Rwandan populations of *E. saccharina*. The phylogeny revealed a clear geographic split, with all Ethiopian individuals forming a distinct and well-supported monophyletic clade, markedly separated from the Rwandan lineage. Internal substructure was detected in both countries, although samples from Ethiopia exhibited tight genetic similarity, while individuals from Rwanda were grouped into ecologically meaningful subclades associated with maize and wild vegetation. The pairwise distance estimates further supported this divergence, showing high distance between-population differentiation and low distance within-population variation, confirming long-term geographic isolation between the two countries. These findings demonstrate that *E. saccharina* populations from Ethiopia and Rwanda represent genetically distinct evolutionary units shaped by geographic barriers, ecological heterogeneity, and anthropogenic activities. The strong genetic structure and host-associated patterns underscore the need for region-specific monitoring and management strategies that account for both evolutionary divergence and dynamic host-use behavior.

Keywords: *Eldana saccharina*, phylogeography, haplotypes, host plants, mitochondrial DNA

5.2.1. Introduction

The African sugarcane stemborer, *Eldana saccharina* Walker (Lepidoptera: Pyralidae), is an indigenous African moth widely distributed across sub-Saharan Africa, associated with a broad range of wild sedges, grasses, and cultivated cereals (Assefa, 2006; Conlong, 2001; Girling, 1978). Historically, the species has garnered significant attention due to its role as a major pest of sugarcane in eastern, western, and southern Africa, where it causes extensive damage and yield loss (Assefa, 2006; Ntirenganya et al., 2025; Zhou, 2016). Although *E. saccharina* has been documented feeding on maize, sorghum, and other few wild vegetation in several parts of Africa (Assefa, 2006; Sampson and Kumar, 1985), the regional difference in host distribution, reflects a much broader pattern of ecological and geographic differentiation within the species.

Understanding the evolutionary relationships among *E. saccharina* populations is a critical prerequisite for predicting pest expansion, designing effective biological control programs, and assessing the risk of host shifts into major food crops like maize, and related cereals (Assefa, 2006). In this regard, prior phylogenetic studies have consistently demonstrated genetic differences among populations of *E. saccharina* from West, East, and southern Africa, with mitochondrial COI sequence variation separating the species into three well-defined continental clades (Assefa, 2006; Girling, 1978). These genetic differences have been linked to variations in behavior, host plant preference, larval feeding selection, and natural-enemy guild composition across the continent (Assefa et al., 2015, 2010; Mazodze and Conlong, 2003). These biological and ecological variations highlight the complex evolutionary history of *E. saccharina* and its capacity for adaptation under different environmental and agro-ecological conditions.

Importantly, the phylogeographic reconstruction by Assefa et al. (2006) revealed that Ethiopian populations represent a distinct clade that is genetically distant from southern and West African groups. Ethiopian specimens collected from west of the Rift Valley (Lake Tana region) formed two closely related haplotypes that differed substantially from all other African populations, suggesting a long-term evolutionary separation likely driven by major geological and climatic events associated with the formation and expansion of the East African Rift system (Assefa et al., 2006).

Other studies have indicated that geological uplift, rift formation, and the fragmentation of wetland systems have previously been shown to shape the population connectivity in several African lepidopteran species, including *B. fusca* (Sezonlin et al., 2005; Assefa et al., 2015). These processes act as natural barriers to dispersal, creating opportunities for genetic divergence and regional lineage formation.

In contrast to the Ethiopian lineage, *E. saccharina* populations from southern Africa exhibit minimal genetic diversity across broad geographic ranges, reflecting more continuous wetland–grassland habitats and fewer historical landscape barriers (Assefa, 2006; Assefa et al., 2009). However, ecological shifts driven by climate change, anthropogenic wetland modification from natural to agricultural intensification have increasingly facilitated host range expansion within species. Examples include recent colonization of sugarcane by previously maize-associated *B. fusca* in Ethiopia and southern Africa, illustrating how ecological differences can accelerate host use transitions and promote localized adaptations (Assefa et al., 2015). Similar processes are likely to influence *E. saccharina* across ecozones where land-use pressures and climatic variability alter the distribution of natural host plants (Assefa et al., 2015).

Rwanda represents a particularly dynamic ecological and agricultural setting which call for the understanding of such population processes. Historically dominated by wild wetland vegetation and sedges, many of its valley wetlands have undergone rapid agricultural transformation, with sugarcane gradually removed or reduced in the profit of maize which is becoming the dominant crop in reclaimed wetland systems. Given that *E. saccharina* is ancestrally associated with sedges and wetland grasses (Conlong, 1994; Lucid Keys, 2007), this ecological shift creates conditions favorable for the emergence of new host-associated lineages adapted to maize, a scenario consistent with the observed clade subdivisions in other East African stemborers (Assefa, 2006; Gofishu et al., 2019; Ong’amo, 2009). The proximity of wetland refugia, combined with year-round plant availability and diverse cropping systems, further increases the likelihood of ecological diversification within Rwandan populations (Assefa, 2006).

Despite the morphological knowledge of *E. saccharina* in different parts of Africa, the evolutionary relationships between regional populations remains unexplored.

No study to date has conducted a comparative genetic analysis of *E. saccharina* in East Africa, particularly for ecologically distant countries like Ethiopia and Rwanda, specifically linking Ethiopian and Rwandan regions, despite their contrasting geological histories, agro-ecological contexts, and host-plant transitions. Given Rwanda's recent shift from wild wetland vegetation and sugarcane toward intensified maize cultivation and ecoparks in urban wetlands, and Ethiopia's distinct highland and wetland ecologies west of the Rift Valley, a comparative assessment is crucial for understanding whether the two countries harbor independently evolving lineages or share deeper ancestral affinities. These countries were selected because their sharply contrasting ecological and climatic regimes offer an ideal natural gradient for assessing how ecological and environmental divergence shapes population differentiation.

This study therefore investigates the genetic diversity and evolutionary relationships of *E. saccharina* populations from Ethiopia and Rwanda using phylogenetic tools. By integrating field-collected COI sequence data, phylogeographic evidence, and ecological context, this work aims to (1) determine the extent of genetic divergence between the two regional populations, (2) assess how ecological and geological factors may have shaped this divergence, and (3) evaluate whether ecological shifts may be driving emerging host-associated substructure.

Clarifying these genetic relationships will enhance our understanding of *E. saccharina* population divergence in Rwanda and Ethiopia and provide insights necessary for the eastern African region and global-specific pest management, ecological monitoring, and predictive modeling under ongoing land transformation to agricultural intensification and climate change.

5.2.2. Materials and Methods

5.2.2.1. Insect Specimens

Larvae of *E. saccharina* were collected between 2023 and 2024 from four agroecological zones of the Northern, Eastern, Southern, and the Kigali capital city of Rwanda. Sampling targeted green and red sugarcane and maize from the cultivated habitats. It was also extended to Napier grass (*Pennisetum purpureum*), *Echinochloa crus-galli*, *Rottboellia cochinchinensis*, *Cyperus strigosus*, *Cyperus fastigiatus*, and *Typha* species sampled from wild habitats.

Collections took place in wetlands, marshlands, riverbanks, farms, farm margins, and roadside vegetation to capture the full range of host plants associated with stemborer populations. These locations were selected due to the accessibilities and presence of host plants. To analyse the species divergence and (Tab. 5. 4).

Table 5. 4. Geographic locations where specimens of *E. saccharina* were sampled in Rwanda and Ethiopia. Labels are the names given to sequences to easier the characterization and differentiation of specimens

Label	Country	Location	Coordinate	Host plant
411 Es	Ethiopia	Lake Awasa	07°03'00"N 38°28'00"E	<i>Cyperus dives</i>
412 Es	Ethiopia	Lake Tana	11°22'00"N 31°39'00"E	<i>C.papyrus</i>
413 Es	Ethiopia	Lake Tana	11°22'00"N 31°39'00"E	<i>C.papyrus</i>
414 Es	Ethiopia	Metehara	08°49'00"N 39°58'00"E	<i>C. dives</i>
27 Es	Rwanda	Kayonza_Lake Muhazi	01°51'17"S 30°29'14"E	<i>Zea mays</i>
34 Es	Rwanda	Kamonyi_Rugalika	02°01'03"S 29°51'59"E	<i>Zea mays</i>
41 Es	Rwanda	Kigali_Gasabo_Nyacyonga	01°53'06"S 30°04'23"E	<i>Panicum repens</i>

55	Es	Rwanda	Kamonyi_Bishenyi	01°59'01"S 29°58'51"E	<i>Echinochloa pyramidalis</i>
87	Es	Rwanda	Kigali_Kicukiro_Masaka	02°01'18"S 30°11'19"E	<i>C.papyrus</i>
91	Es	Rwanda	Kigali_Kicukiro_Rwandex	01°56'14"S 30°02'53"E	<i>Typha sp</i>
97	Es	Rwanda	Kigali_Nyarugenge_Gitikinyoni	01°57'04"S 30°00'11"E	<i>Nappier grass</i>

5.2.2.2. DNA Extraction, Polymerase Chain Reaction (PCR), and Sequencing

Genomic DNA was extracted from insect specimens targeting the mitochondrial cytochrome c oxidase subunit I (CO1) gene for amplification, as it is the universal marker for DNA barcoding. The PCR amplification was carried out under the following conditions: initial denaturation at 95 °C for 10 min, followed by 40 cycles of denaturation at 94 °C for 1 min, annealing at 60 °C for one minute, and extension at 72 °C for one minute, with a final extension at 72 °C for five minutes (Hebert et al., 2003). The primers used were LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-AACTTCAGGGTGACCAAAAATCA-3') (Folmer et al., 1994) as described in Goftishu et al. (2016), ensuring coverage of the barcode region and comparability with previous studies. The PCR products were subsequently purified and sequenced by MacroGen's Genomics branch in England.

Amplification reactions were optimized for annealing temperature and cycle number to minimize nonspecific products. Successful amplicons were subjected to Sanger sequencing in both directions, generating paired chromatogram files (.ab1) for each specimen. Sequencing quality was assessed using Phred scores and only reads meeting a minimum threshold of Q20 (corresponding to 99% base-calling accuracy) were retained for downstream analysis (Hebert et al., 2003). Sequences were received as raw sequences, not cleaned, and reverse and forwards segments were separated.

5.2.2.3. Sequences Management Prior to MEGA Analysis

All COI sequences were first processed to generate high-quality consensus reads before downstream analyses in MEGA. Raw Sanger chromatogram files (forward and reverse. ab1) for each specimen were imported into Geneious Prime v2023, where low-quality ends were trimmed based on error-probability thresholds to remove unreliable base calls (Kearse et al., 2012). Forward and reverse reads were assembled de novo into contigs, and chromatograms were manually inspected to verify base-calling accuracy, resolve mismatches, and correct ambiguous positions where necessary. For samples with poor-quality reverse reads that failed to assemble reliably (e.g., samples 39, 87, and 97), only high-quality forward sequences were retained to avoid introducing erroneous consensus calls. Final consensus sequences were extracted in FASTA format, checked for expected COI length, translated to confirm the absence of premature stop codons, and curated for naming consistency. These curated consensus sequences constituted the final dataset imported into MEGA for alignment, model selection, and phylogenetic reconstruction.

5.2.2.4. DNA Sequence Alignment and Data Preparation

Raw DNA sequences obtained from the samples collected from Rwanda and Ethiopia were aligned using MEGA 12 (Kumar, et al., 2024). Sequences were first imported in FASTA format and examined for quality, including checking for ambiguous bases, gaps, and alignment consistency. Multiple sequence alignment was performed using the ClustalW algorithm implemented in MEGA 12 with default parameters (gap opening penalty = 15, gap extension = 6.66). The alignment was manually inspected and trimmed to remove regions of poor alignment prior to phylogenetic and population-genetic analysis.

5.2.2.5. Phylogenetic Tree Reconstruction

To determine the most appropriate nucleotide substitution model, the dataset was subjected to model selection in MEGA 12 (Analysis → Models) (Kumar et al., 2024), and the optimal model was chosen based on the lowest Bayesian Information Criterion (BIC) and the highest maximum likelihood score. This best-fit model was then applied during phylogenetic reconstruction. Phylogenetic relationships among samples were inferred using the Neighbor-Joining (NJ) method in MEGA 12 under the selected substitution model, with rates among sites modeled as

gamma-distributed or uniform depending on the recommended configuration. The analysis used 10,000 bootstrap replicates to assess node support, and gaps were treated using pairwise deletion unless indicated otherwise. The resulting Neighbor-Joining Tree (NJT) was visualized in MEGA 12's Tree Explorer (Kumar et al., 2024), where *S. nonagrioides* (Lepidoptera: Noctuidae) served as the outgroup for rooting due to its closer and indirect relationship with *E. saccharina*, and branch lengths were expressed in substitutions per site. Bootstrap values of 70% or higher were interpreted as moderate to strong support, and the final tree topology was examined to evaluate clade structure in relation to both geographic origin (Rwanda vs. Ethiopia) and host-plant association (maize vs. wild vegetation).

5.2.2.6. Genetic Diversity and Population Structure Analysis

To assess genetic diversity and variation within and between defined populations, stemborer individuals were assigned to groups based on their collection site and host plant, and these classifications were arranged and visualized in MEGA 12 before conducting the analyses.

Two hierarchical levels of population structure were evaluated: (i) geographic groups consisting of samples from Rwanda and Ethiopia, and (ii) host-plant-associated groups within Rwanda, which included specimens recovered from maize (27 Es and 34 Es) and wild-vegetation (41 Es, 55 Es, 87 Es, 91 Es, and 97 Es).

The within-population genetic diversity was estimated using the “Compute Mean Diversity Within Subpopulations” procedure in the diversity module. The calculated statistics included nucleotide diversity (π), haplotype diversity (H_d), the number of segregating sites (S), and the average number of nucleotide differences (k). These analyses were performed under the p-distance substitution model, which allows direct comparison between the estimated diversity value (d) and nucleotide diversity. Gaps and missing data were treated with pairwise deletion, and variance around each estimate was assessed using 1,000 bootstrap replicates. This approach enabled comparisons of diversity between the two geographic populations as well as between the maize-feeding and wild-vegetation-feeding groups in Rwanda.

To quantify genetic divergence between populations, mean interpopulation diversity was estimated using the “Compute Mean Interpopulation Diversity” option. This analysis provided two

key metrics: D_{xy} , the average number of nucleotide substitutions per site between two populations, and D_a , the net nucleotide divergence after accounting for within-population variation. Net divergence was calculated using the expression $D_a = D_{xy} - \frac{\pi_1 + \pi_2}{2}$. These measures were used to evaluate the extent of genetic separation between Rwanda and Ethiopia, as well as between different host-plant-associated groups.

Population differentiation was assessed using AMOVA (Analysis of Molecular Variance), which calculates Φ -statistics, the molecular analogue of F-statistics based on genetic distance, data-based Φ -statistics generated through the “Compute Φ -statistics of Differentiation” function.

The Φ -statistics were chosen due to the few samples that may provide non-significance results if F_{st} is applied. Analyses were conducted under the p-distance model using 1,000–10,000 permutations to assess statistical significance. Φ_{ST} values, together with their corresponding P-values, provided a measure of the proportion of total genetic variation attributable to differences between populations, allowing robust assessment of differentiation across both geographic and ecological groupings.

Finally, the overall genetic diversity across the entire dataset was estimated using the “Compute Mean Diversity in Entire Population” option, which provided broader context for interpreting within- and between-population patterns. This comprehensive framework allowed a detailed evaluation of genetic structure across geographic regions and host-plant associations.

5.2.3. Results

5.2.3.1. Phylogenetic Structure of *E. saccharina* Populations

During the analysis, 11 species where 7 individuals were collected from Rwanda and only four were sampled in Ethiopia. The rooted phylogeny revealed a clear and well-resolved geographic separation between specimens of *Eldana saccharina* from Ethiopia and Rwanda. Using *S. nonagrioides* as an outgroup, all four specimens from Ethiopia isolates (411 Es, 412 Es, 413 Es, 414 Es) and clustered into a single monophyletic clade with moderate bootstrap support (72). Within this group, two well-supported sister pairs were recovered: 411/414 (bootstrap 65) and 412/413 (bootstrap 59), indicating internal substructure and lineage diversification in Ethiopia (Fig. 5.4).

In contrast, all specimens from Rwanda (27 Es, 34 Es, 41 Es, 55 Es, 87 Es, 91 Es, 97 Es) isolates and formed a geographically cohesive clade (bootstrap 100) that was completely distinct from the Ethiopian lineage. Multiple subclades were recovered within Rwanda, including the maize-associated pair 27/34 (bootstrap 73), the wetland-associated pair 55/87 (bootstrap 85), and a distinct triplet composed of 91/97 with 41 *Eldana saccharina* as a sister lineage (supports 92→88→66) (Fig. 5. 4).

These patterns indicate finer-scale ecological or microgeographic structure within the Rwandan population. In nutshell, the topology demonstrated two geographically monophyletic lineages of Ethiopia and Rwanda with no evidence of haplotype mixing, consistent with long-term geographic isolation and limited gene flow.

lineages or as closely allied external branches (Fig. 5. 5).

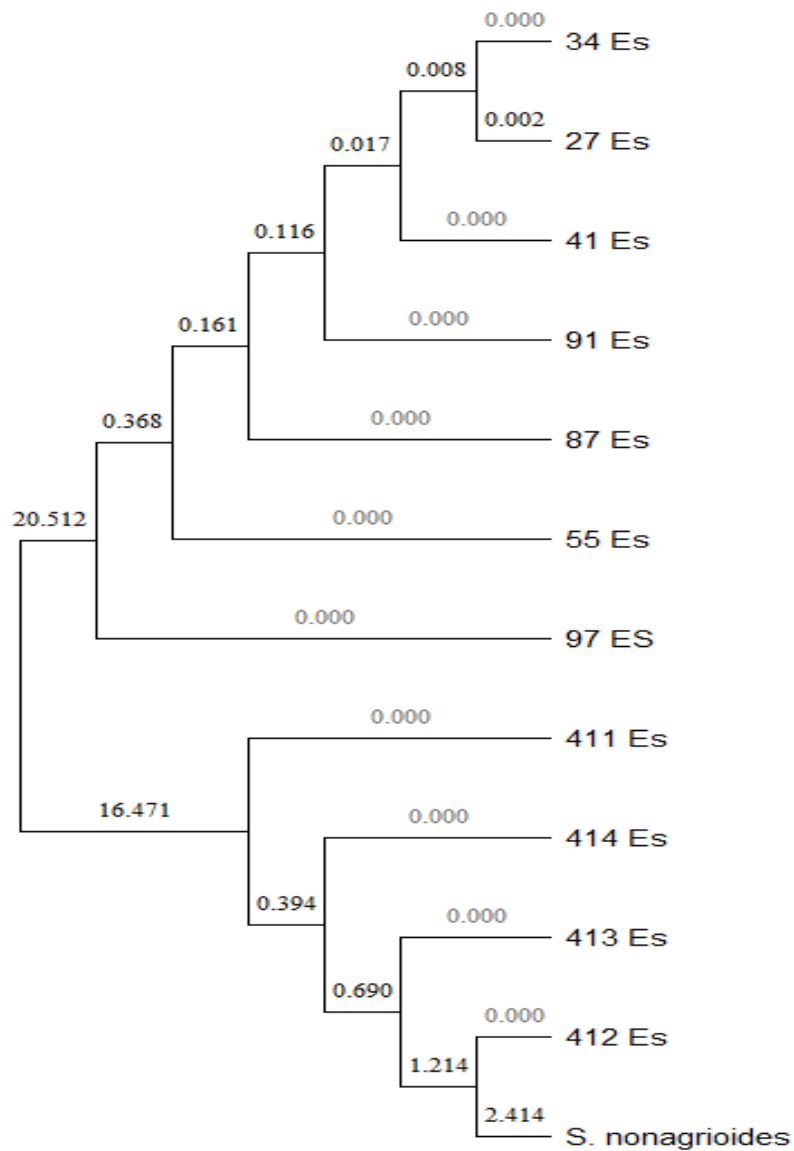


Figure 5. 5. The evolutionary history inferred by using the Neighbor-Joining method

The comparative maximum Parsimony (MP) analysis yielded three equally most-parsimonious trees, all exhibiting highly consistent topologies (tree length = 424; CI = 0.985849; RI = 0.992806). The high consistency and retention indices indicate minimal homoplasy and strong phylogenetic signal within the COI dataset (Fig. 5. 6).

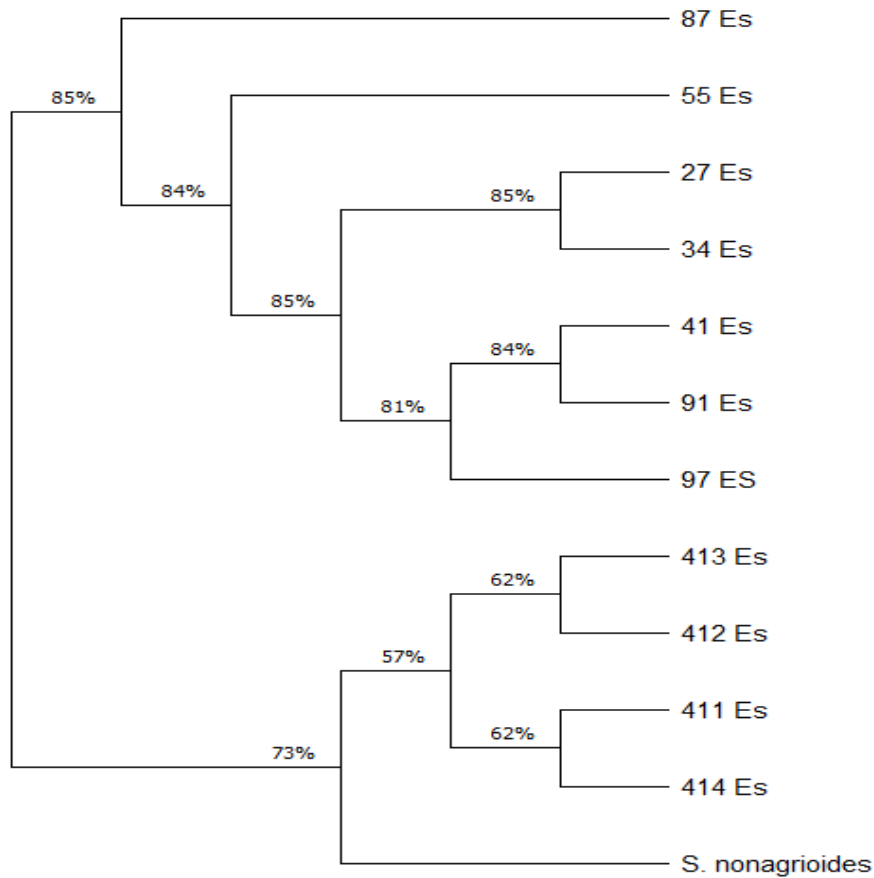


Figure 5. 6. Figure 5. 1. The evolutionary history was inferred using the Maximum Parsimony method. Tree #1 out of 3 most parsimonious trees (length = 424) is shown. The consistency index is 0.985 (0.977), the retention index is 0.992 (0.992), and the composite index is 0.978 (0.970) for all sites and parsimony-informative sites (in parentheses). The MP tree was obtained using the Subtree-Pruning-Regrafting (SPR) algorithm.

5.2.3.2. Sequence Characteristics and Polymorphism

Across the 14 sequences (697 bp region), 626 sites were retained after excluding gaps, and 20 variable sites were detected (sequence conservation = 0.971).

Two fully conserved regions (Sites 60–169 and 243–337) were identified with significant homozygosity ($P = 0.0282$ and $P = 0.0478$), reflecting stretches of strong evolutionary constraint. InDel analyses identified 12 informative InDel sites contributing to six InDel haplotypes, with an InDel haplotype diversity of 0.879 and an average InDel length of 1.359 bp.

Tajima's D was positive but not significant ($D = 0.2155$, $P > 0.10$), indicating no deviation from neutral expectations in the demographic history of the combined populations. The pairwise distance data quantifying the magnitude of separation have indicated that *E. saccharina* populations represent genetically distinct evolutionary units in Ethiopia and Rwanda (Tab. 5. 4).

5.2.3.3. Within-Population Diversity and Between-Population Divergence

The mean pairwise nucleotide diversity (π) was low in both populations, with Rwanda exhibiting slightly higher within-population diversity ($\pi = 0.021 \pm 0.005$) than Ethiopia ($\pi = 0.017 \pm 0.005$). Confidence intervals overlapped extensively (Rwanda: 0.0105–0.0328; Ethiopia: 0.0059–0.0286), indicating that the observed difference was not statistically significant. Interpopulation analyses revealed divergence between Ethiopia and Rwanda. The mean interpopulation diversity (D_{xy}) was 0.200 ± 0.010 , substantially exceeding within-population values and corresponding to ~20% average nucleotide difference between the two regional populations. Net nucleotide divergence was similarly high ($D_a \approx 0.181$), indicating that most of the observed genetic differentiation reflects true historical separation rather than within-population variation.

This scale of divergence ($D_{xy} \approx 10\times$ within-population π) was consistent with extremely high AMOVA-based differentiation, previously estimated as $\Phi_{ST} \approx 0.90$ – 0.97 ($P < 0.001$), indicating near-complete genetic isolation between Ethiopian and Rwandan lineages. This shows that pairwise distances within the putative Rwandan cluster (97_Es, 91_Es, 87_Es, 55_Es, 41_Es, 34_Es, 27_Es) are uniformly low (mean = 0.0186, range = 0.002–0.056, $n = 21$ comparisons), indicating shallow within-population divergence. Distances within the Ethiopian cluster (413_Es, 412_Es, 411_Es, 414_Es) are similarly low (mean = 0.0127, range = 0.003–0.020, $n = 6$), again consistent with limited intra-regional variation. By contrast, between-cluster distances are strikingly larger (mean = 1.099, range = 1.060–1.122, $n = 28$), demonstrating a deep genetic split between Ethiopian and Rwandan lineages that is related to the NJ (Fig. 5.II.2) and MP (Fig. 5. 6) tree topologies.

Because the values were estimated under the Maximum Composite Likelihood model with pairwise deletion (730 sites), the units are substitutions per site (and may exceed 1 due to correction for multiple hits) (Tab. 5. 5);

nonetheless, the order-of-magnitude contrast between within- and between-region distances robustly support strong geographic structuring and comparatively low within-population diversity in each region.

Table 5. 5. Estimates of Evolutionary Divergence between Sequences (* shows significant values)

	97_Es	91_Es	87_Es	55_Es	41_Es	34_Es	27_Es	413_Es	412_Es	411_Es	414_Es
97_Es											
91_Es	0.004										
87_Es	0.040	0.044									
55_Es	0.014	0.013	0.042								
41_Es	0.002	0.004	0.056	0.011							
34_Es	0.004	0.006	0.054	0.009	0.002						
27_Es	0.007	0.002	0.042	0.011	0.013	0.011					
413_Es	1.122*	1.098*	1.102*	1.082*	1.109*	1.101*	1.117*				
412_Es	1.122*	1.098*	1.102*	1.082*	1.109*	1.101*	1.117*	0.003			
411_Es	1.122*	1.098*	1.118*	1.082*	1.109*	1.101*	1.117*	0.017	0.020		
414_Es	1.098*	1.075*	1.095*	1.060*	1.087*	1.079*	1.094*	0.011	0.014	0.011	

5.2.3.4. Haplotypes

A total of 10 mitochondrial COI haplotypes ($H = 10$) were identified across the shared high-coverage core alignment (375 bp) generated from 11 *E. saccharina* sequences. These haplotypes were defined by 13 segregating sites within the gap-free region. Haplotype diversity was high in both populations, with Rwanda showing $Hd = 0.952 \pm 0.005$ and Ethiopia $Hd = 1.000 \pm 0.005$, indicating a high probability that two randomly selected sequences from each country belong to different haplotypes.

Nucleotide diversity was comparable between populations, with $\pi = 0.00965$ in Rwanda and $\pi = 0.00933$ in Ethiopia, reflecting moderate sequence variation within each population based on pairwise nucleotide differences.

Population-wise haplotype distribution revealed strong geographic structuring. Of 10 haplotypes detected, six were exclusive to Rwanda and four were exclusive to Ethiopia, with no shared haplotypes between the two countries. Rwanda haplotypes were represented by multiple individuals in some cases (e.g., one haplotype with frequency = 2), whereas all Ethiopian haplotypes were singletons. This complete haplotype separation is illustrated in the haplotype network and population frequency table (see Haplotype Distribution Table 5. 6), supporting significant phylogeographic differentiation between the two populations.

Table 5. 6. COI haplotype distribution of stemborer populations from Rwanda and Ethiopia

Haplotype (H)	Sequence IDs (Es codes)	Country	Frequency (n)	Remarks
H1	97_Es	Rwanda	1	Private haplotype
H2	91_Es	Rwanda	1	Private haplotype
H3	87_Es	Rwanda	1	Private haplotype
H4	55_Es	Rwanda	1	Private haplotype

Haplotype (H)	Sequence IDs (Es codes)	Country	Frequency (n)	Remarks
H5	41_Es, 34_Es	Rwanda	2	Most frequent Rwanda haplotype
H6	27_Es	Rwanda	1	Private haplotype
H7	413_Es	Ethiopia	1	Private haplotype
H8	412_Es	Ethiopia	1	Private haplotype
H9	411_Es	Ethiopia	1	Private haplotype
H10	414_Es	Ethiopia	1	Private haplotype

Total haplotypes (H) = 10; Rwanda = 6 haplotypes (n = 7 sequences); Ethiopia = 4 haplotypes (n = 4 sequences); Shared haplotypes = 0

5.2.3.5. Ecological Differentiation Within Rwandan population

The phylogeny showed that ecological associations correspond closely to genetic structure within Rwanda. Maize-feeding isolates (27 Es, 34 Es) formed a distinct subclade, while wetland or wild-vegetation feeders (55 Es, 87 Es) grouped into a separate strongly supported lineage. The mixed cluster (41, 91, 97 Es) likely reflects an additional microhabitat or host-associated lineage (Fig. 5. 4). These patterns suggest that host plant specialization contributes to population subdivision even within a single geographic region.

5.2.4. Discussion

Results of the phylogenetic analysis revealed that the Rwandan specimens from different vegetation mosaics and agro ecologies are almost identical to each other and different from Ethiopian model samples. However, the number of specimens used were small to conclude without wide samples from neighboring countries Uganda, DRC, Burundi, Tanzania, and other countries we share similar ecosystems. This makes it difficult to generalize about relationship between Rwandan population of *E. saccharina* and populations from other parts of Africa.

The Ethiopian population formed a tightly clustered and genetically cohesive group, indicating a high degree of similarity among individuals collected from this region. This observation agrees with earlier findings by Assefa (2006) and Assefa et al. (2009), who reported that Ethiopian populations of *E. saccharina* constitute a distinct genetic lineage. A comparable pattern has been documented in other insect taxa with geographically restricted dispersal, where landscape barriers limit gene flow and shape population genetic structure, resulting in a positive correlation between genetic and geographic distance, a process commonly referred to as isolation-by-distance (IBD) (Vasiliev and Greenwood, 2023; Smith et al., 2023). In contrast, the Rwandan population showed a greater internal structuring, with multiple well-supported subclades reflecting ecological differentiation between maize and wild vegetation populations.

5.2.4.1. Geographic isolation, landscape history, and lineage separation

The marked separation between Ethiopian and Rwandan lineages is consistent with long-term geographic isolation shaped by the East African Rift system and associated tectonic and tensional movements, which have repeatedly reconfigured river basins, wetlands, and dispersal corridors for grass and sedge-associated insects (Goftishu et al., 2019). Comparable, continent-scale phylogeographic breaks and region-specific lineages have been documented in *E. saccharina*, with distinct population units aligned to West Africa, the Rift Valley, and East/Southern Africa (Assefa, 2006; Assefa et al., 2009; Conlong, 1994; Girling, 1978), underscoring how the great rift valley and topographic compartmentalization promote genetic differentiation and limit gene flow across biogeographic blocks. Foundational work on *E. saccharina* likewise shows that geographically separated populations differ in behavior, host use, and natural-enemy guilds differences that are

most parsimoniously explained by historical isolation across major landscape barriers and ecozones formed or intensified by rift tectonics (Assefa et al., 2010; Girling, 1978).

Beyond macro-geology, regionally distinct agricultural histories reinforce this isolation. Surveys in Ethiopia revealed a characteristic assemblage of cereal stemborers and sugarcane borers structured by highland agroecologies (Assefa, 2006; Goftishu et al., 2019), while East African studies emphasize wetland mosaics and wild host complexes contiguous with cropped fields alternative settings that predict divergent selective regimes and, over time, divergent lineages. Early biological and distributional work from Uganda already noted that *E. saccharina* occurs widely in wild hosts and that crop-feeding forms arose in East Africa, anticipating present-day country-level lineage structure and the persistence of wild vegetation as a demographic source for crop infestations (Assefa, 2006; Girling, 1978; Le Rü et al., 2006a).

5.2.4.2. Climate change and natural hazards

Superimposed on the tectonic template is contemporary climate change, which alters rainfall regimes, wetland hydrology, and thermal niches (Lourenço and Rozel, 2023). Where rainfall patterns become more seasonal or erratic, wetlands may contract or change phenology, re-shaping the temporal match between stemborers larval development and host quality (Maicher et al., 2018; Ntiringanya et al., 2025). Across the region, empirical studies repeatedly show that *E. saccharina* dynamics track temperature and moisture, with warmer and drier conditions often coinciding with higher damage pressure in cane systems and with population pulses in maize cycles patterns that can intensify divergent selection among populations differentially exposed to drying lowlands versus cooler highlands (Mutamiswa et al., 2023; Mwalusepo et al., 2015).

In Rwanda's sugarcane system, for instance, recent work associates *E. saccharina* abundance with thermal and seasonal signals, consistent with climate-linked shifts in habitat suitability and host availability in wetland sugarcane systems (Ntiringanya et al., 2025). Such relationships plausibly explain the evolutionary event and shifting of wild stemborers to cultivated crops such as maize (Mulcahy et al., 2023). At this point, the divergence and isolated lineage of Rwandan and Ethiopian populations indicate that historically isolated lineages changed over many years, a pattern that would be exacerbated if climate change differentially filters habitats and phenologies across regions and further constraining gene flow among established lineages and reinforcing the

country and regional-levels splits seen in our dataset. Similar trend was reported in other stemborers like *S. nonagrioides* (Esfandiari et al., 2015; Gofishu et al., 2019).

5.2.4.3. Historical host shifts and Agricultural intensification

In Ethiopia, historical and contemporary surveys document sugarcane estates, smallholder cane fields, and adjacent wetlands or field margins as components of a broader ecological complex that can support *Busseola–Eldana* stemborer assemblages (Asmare et al., 2014; Assefa, 2006; Gofishu et al., 2018). However, the *E. saccharina* populations analysed in the present study were collected exclusively from wild habitats, particularly from natural grasses occurring in wetlands and unmanaged field margins, with no specimens obtained from cultivated sugarcane or maize.

This wild-host association indicates that the genetic cohesion observed within the Ethiopian population likely reflects long-term persistence in semi-natural ecosystems rather than recent expansion within plantation agro-systems. Landscape configuration in such habitats including wetland connectivity, riparian corridors, and natural grass belts may facilitate localized dispersal while still maintaining regional isolation. Region-wide syntheses further indicate that many noctuid and pyralid stemborers remain associated with specific host guilds and vegetation belts; when land-use intensification restructures these belts, host availability and community composition shift accordingly, creating conditions for distinct, locally adapted clades to emerge (Assefa, 2006; Esfandiari et al., 2015; Gofishu et al., 2016).

A consistent theme in African stemborer ecology is the progression from indigenous wild hosts (Cyperaceae, Poaceae, Typhaceae families) to cultivated plants (Le Rü et al., 2006b; Moyal, 2014; Ong’amo, 2009). For *E. saccharina*, early accounts and continent-wide reviews agree that the species is indigenous to wild sedges and grasses (Kfir et al., 2002; Moyal, 2014). Evidence from Eastern Africa (Assefa, 2006) and parts of West Africa (Sampson and Kumar, 1985) points to region-specific host shifts and behavioral differentiation. Field studies in East Africa document extensive use of wild host plants in wetlands and riverine ecosystems, highlighting that many pest species persist in natural habitats and periodically move into cultivated crops. These ecological dynamics promote local adaptation to specific hosts and crop phenologies, creating opportunities for the development of host-associated lineages within countries over relatively short evolutionary timescales (Assefa, 2006; Girling, 1978).

Against this backdrop, Rwanda's agricultural transformation provides a plausible narrative for clade formation observed in our study. *E. saccharina* that were historically native to wild wetland vegetation and, later, associated with sugarcane in marshes, encountered a rapid expansion of maize into former wetland sugarcane zones. This transition, coupled with wetland drainage associated with maize intensification, would be expected to generate strong and consistent selection pressures favoring maize-adapted stem borer assemblages, while remnant wetland patches continue to support lineages associated with wild host plants.

Such dual habitat structuring has been repeatedly documented in East African agro-ecological mosaics, where agricultural expansion reorganizes host availability without fully eliminating semi-natural refugia (Goftishu et al., 2018; Ong'amo et al., 2018).

In these landscapes, wetlands and unmanaged grass habitats that retain abundant native vegetation can function as reservoir environments for *E. saccharina*, shaping its spatial distribution across adjacent cultivated systems (Ntirenganya et al., 2025). This form of habitat partitioning may influence how stemborer populations are distributed within agricultural fields either diffusing or locally concentrating pest pressure depending on landscape connectivity and host plant configuration, although confirming such dynamics would require direct infestation and abundance data across ecological niches.

5.2.4.4. Ethiopia vs. Rwanda: contrasting eco-evolutionary contexts

The phylogeographic reconstruction reveals contrasting lineage configurations between Ethiopian and Rwandan populations of *E. saccharina*, reflecting differences in landscape structure and land-use history. Ethiopian samples cluster into comparatively cohesive and closely related haplogroups, forming geographically structured but shallowly differentiated clades. However, given Ethiopia's vast geographic scale and status as one of Africa's major biodiversity hotspots, the limited number of sampling localities represented in this study constrains broad phylogeographic inference. The Ethiopian dataset should therefore be interpreted as indicative rather than conclusive, providing a baseline framework for future range-wide population genetic assessments.

In contrast, Rwandan populations exhibit finer phylogenetic structuring, with multiple sub-lineages distributed across short geographic distances. This pattern is consistent with Rwanda's highly heterogeneous agro-ecological setting, characterized by intensively cultivated valleys, fragmented marshlands, and rapid wetland conversion for maize production and urban eco-park development. The close spatial juxtaposition of croplands and semi-natural marsh habitats creates sharp ecotonal gradients that can restrict dispersal while simultaneously exposing populations to divergent host regimes.

Empirical studies across eastern and southern Africa demonstrate that *E. saccharina* occupies sedge-dominated wetlands but can also exploit cultivated hosts such as sugarcane and maize where available, reflecting considerable ecological plasticity (Assefa, 2006; Ntirenganya et al., 2025). Within rapidly transforming landscapes, such plasticity can promote localized adaptation and lineage subdivision, a process reflected in the host-associated and geographically partitioned clades observed in the Rwandan phylogeny.

5.2.4.5. Limitations and recommendations

Although the Ethiopian lineage appeared distinct, uniform, and more stable than Rwandan population, interpretation must be approached with caution because the number of Ethiopian and Rwandan specimens included in this analysis were relatively small. Additionally, important neighboring regions must be represented in the sampling to strengthen the geographic resolution of African lineages. To more fully understand the evolutionary relationships, population structure, and potential contact zones between regions populations, future work should incorporate broader sampling across the geologically transformed habitats of similar histories and ecosystems across more countries. Expanding the geographic scope of sampling will help clarify whether the Ethiopian group represents a locally confined lineage or part of a broader African clades with deeper genetic connectivity or subdivision as recommended by Assefa (2009).

5.2.5. Conclusion

The present study demonstrates substantial genetic diversity of *E. saccharina* populations between Ethiopia and Rwanda, reflecting an evolutionary history strongly shaped by both ancient tectonic processes and more recent landscape transformations within the region. The complex uplift and rifting dynamics of the Ethiopian Highlands and the broader East African Rift System appear to have created long-standing geographic and ecological discontinuities that limited gene flow and promoted early lineage separation. These historical barriers are now increasingly reinforced by contemporary drivers such as wetland loss, agricultural intensification, and rapid urban expansion, which further fragment suitable habitats and isolate local populations despite the weak host-associated genetic differentiation.

Understanding whether the observed lineages primarily reflect ancient tectonic-driven isolation or more recent anthropogenic fragmentation will require expanded sampling from Ethiopia, Kenya, Uganda, and Rwanda, combining natural vegetation with special focus on older, ecologically stable wetland systems, sugarcane plantation, and crops particularly maize and related cereals within and beyond the region. Besides, the observed within species variations patterns in Ethiopia and Rwanda highlight the combined influence of deep climate change and accelerated landscape modification are reported to be major factors shaping the diversification and current local population structures of *E. saccharina* in both countries.

6. GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

6.1. General discussion

To investigate Rwandan stemborers population divergence and genetic diversity as well as relationship of species from wild habitats and cultivated crops, Ethiopian samples were used for Phylogeographic analyses of *S. nonagrioides* and *E. saccharina* as selected species. The results revealed strong genetic structuring between Rwandan and Ethiopian populations, providing important insights into the evolutionary history and spatial dynamics of these selected stemborer pests. Using mitochondrial COI markers, no shared haplotypes were detected between the two countries, indicating long-term geographic isolation and restricted gene flow. These findings confirm that phylogeography is a powerful approach for linking genetic variation with historical, ecological, and geographic processes, and it plays a crucial role in understanding pest evolution and designing effective management strategies (Avise, 2000). Previous studies in African stemborers have similarly demonstrated the usefulness of mitochondrial DNA in resolving population structure and evolutionary relationships (Assefa et al., 2006; Sezonlin et al., 2006; Esfandiari et al., 2015).

For *S. nonagrioides*, the results indicate clear differentiation between Rwandan and Ethiopian populations, with additional ecological partitioning within Rwanda. Rwandan populations were structured into distinct lineages associated with wetland habitats and cultivated maize systems, reflecting local adaptation to heterogeneous agro-ecological conditions. In contrast, Ethiopian populations exhibited higher genetic diversity and more complex phylogenetic structuring, consistent with a longer evolutionary history and broader host associations. These patterns support the hypothesis that widespread phytophagous insects may consist of genetically differentiated populations shaped by ecological gradients and host use (Assefa et al., 2006; Martel et al., 2003). The divergence between the two regions is likely linked to historical climatic fluctuations, particularly Pleistocene oscillations, which have been shown to influence the genetic structure of African stemborers and promote isolation in ecological refugia (deMenocal, 2004; Sezonlin et al., 2006; Gofishu et al., 2019).

Similarly, *E. saccharina* populations showed strong phylogeographic separation between Rwanda and Ethiopia population, with evidence of microhabitat-driven differentiation within Rwanda. Distinct genetic clusters were associated with maize-based systems and wetland vegetation, indicating that landscape heterogeneity and agricultural expansion are key drivers of diversification. Ethiopian populations, by contrast, appeared more cohesive and closely linked to wild and semi-natural habitats, suggesting long-term ecological stability. These findings align with previous studies demonstrating that habitat fragmentation, host shifts, and agricultural intensification can drive genetic divergence and population structuring in insect pests (Getu, 2012; Tamiru et al., 2008; Kebe et al., 2017).

Overall, the comparative analysis confirms that Rwandan *S. nonagrioides* and *Eldana saccharina* population represents a valuable evolutionary reference system for interpreting the divergence stemborer populations. While Rwandan population shows the recent evolutionary history, Ethiopian populations used as model reflect deeper historical structuring and long-term persistence in diverse habitats. Rwandan populations exhibit more recent ecological differentiation driven by landscape modification and agro-ecosystem complexity. These results have important implications for pest management, as genetically distinct populations may respond differently to control measures, highlighting the need for region-specific and landscape-based integrated pest management strategies that incorporate both ecological and molecular insights.

6.2. Overall conclusion and conclusion

Stemborer species were more diverse among wild plants than in the cultivated fields. The wild species however occurred in limited number of host plants suggesting that they are monophagous than pest species, contrary to fewer earlier reports in which they were presented as polyphagous. This study further confirmed variation in host use pattern and distribution among different stemborer species. Wild habitats may however accommodate more unknown stemborer species and there is need to extend similar surveys to other areas, particularly in the wetlands as the most affected areas by anthropogenic activities, to generate more information on the general species diversity and host use pattern.

Variation in seasonal distribution stemborers data confirm that wild habitats play a crucial role in keeping the yearly population of stemborers in non-cropping seasons where conditions are not favorable. Non-cropping season showed higher diversity in wild vegetation that host stemborers, explaining that maize is a best host plant since their presence reduce wild vegetation reduction. There is therefore need of managed, since the wild habitats conserve is rich and sustain populations of natural enemies that would eventually regulate stemborers and other pest population, we recommend a revised wild-cultivated habitat management system that combine both wild and cultivated crops environment that support push-pull and intercropping systems.

The majority of recorded species are *B. fusca*, *C. partellus*, and *S. clamistis* that are common stemborers pests in East Africa. Other species like these from Tortricidae, Acrapex, calex, and others were more associated with wild vegetation and possibly but rare in crop. Aforementioned pest species have confirmed to shift from their native habitats. In addition, new emerging pests like FAW and Thaumatotibia, commonly known as the false codling moth (FCM) and fruit-feeder were found to expand their ecologies and host plants by feeding on plant stems due to unknown yet reasons, suggesting deep and repeated investigation for early IPM warning.

The majority of the unknown stem borer species were recovered from host plants growing along the streams and other wetlands. Unfortunately, most of wetlands are earmarked for rehabilitation as demand for agricultural activities increases, and these will ultimately result in habitat loss. Conservation of ecologically important habitats is the only sustainable way of maintaining stem borer species and their respective host plants. There is therefore need to educate the local

communities on the ecological benefits of wetlands, other wild vegetation, and involve them in planning and execution of conservation programmes.

Besides, Rwandan *S. nonagrioides* and *E. saccharina* population shown high genetic diversity and strong divergence of species with wild vegetation and cultivated crops lineages that present clearly separated phylogenetic trees where cultivated crops lineage observed as recent diverged group, and confirming the host expansion and shifting behavior.

6.3. Future Research Outlook

Understanding the combined effects of climate change and habitat fragmentation on biodiversity remains a critical frontier in ecological research, particularly for stemborer systems. The IPCC Sixth Assessment Report (AR6) highlights that climate change has already altered terrestrial, freshwater, and marine ecosystems globally, affecting species distributions, phenology, and ecological interactions across multiple scales where stemborers as other insects respond so fast.

In Rwanda and East Africa, habitat fragmentation driven by agricultural expansion, urbanization, and other land-use change practices significantly modifies ecosystem structure and microclimatic conditions, reducing habitat quality and connectivity within agroecosystems. FAO reports emphasize that biodiversity underpins essential ecosystem services such as pest regulation, pollination, and soil fertility (FAO, 2020), yet these services are increasingly threatened by habitat loss and degradation. At the regional scale, climate change is projected to drive range shifts, redistribution of species, and the emergence of new pest complexes, with the IPCC confirming that rising temperatures and changing rainfall regimes are already forcing species to shift their geographic ranges and seasonal cycles, often toward higher elevations or latitudes (IPCC, 2023). These dynamics are expected to exacerbate food insecurity and agricultural instability by introducing pests into new agroecological zones and altering crop–pest interactions.

In these pressing environmental stresses, understanding stemborers species responses to environmental change, which operate across ecological and evolutionary timescales is key. It is predicted that stemborers species may respond to these stresses through:

- 1) **Phenotypic plasticity**, enabling short-term adjustment to changing climatic conditions;
- 2) **Behavioral and ecological shifts**, such as expanding ecological niches, changing host plant use, and phenology changes.
- 3) **Genetic adaptation**, enhancing tolerance to new environmental conditions.

However, in pressing fragmented landscapes, reduced connectivity can limit adaptive capacity while simultaneously promoting population isolation.

Over time, this isolation may lead to genetic divergence, incipient speciation, or extinction particularly in highly specialized insect taxa such as stemborers. The IUCN list of species status reports that climate change and habitat fragmentation has already contributed to local species in danger to extinctions (<https://www.iucnredlist.org/en>). With this Ecosystem restructuring and shifts, stemborers will keep accelerating their adaptation strategies under continued environmental stress (Mutamiswa et al., 2022).

In this regards, future stemborers research priorities should therefore include:

- 1) **Integrating continuous ecological, genetic, and climate datasets** to assess species distribution shifts across scales;
- 2) **Long-term monitoring of population structure and gene flow** to detect adaptive changes and early-stage speciation;
- 3) **Modeling interactions between climate change and habitat fragmentation** to predict stemborers pest emergence and outbreaks;
- 4) **Strengthening ecosystem-based and landscape-level management approaches**, including habitat connectivity restoration and revised nature-based solutions such as push-pull;
- 5) **Aligning research with global frameworks (CBD-KM commitments, IPCC, FAO, UNFCCC)** to ensure policy relevance and contribution to climate adaptation improved IPM strategies.

Ultimately, advancing knowledge on how climate change and habitat fragmentation jointly influence species distribution, ecological interactions, and evolutionary processes will be essential for developing climate-resilient, biodiversity-based pest management systems. Such approaches are critical for sustaining agricultural productivity, conserving biodiversity, and supporting ecosystem services in East Africa and beyond under rapidly changing environmental conditions.

7. REFERENCES

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8. APPENDICES

Appendix 1. Field sampling figures



Figures above show maize (right) and sugarcane (left) sampling

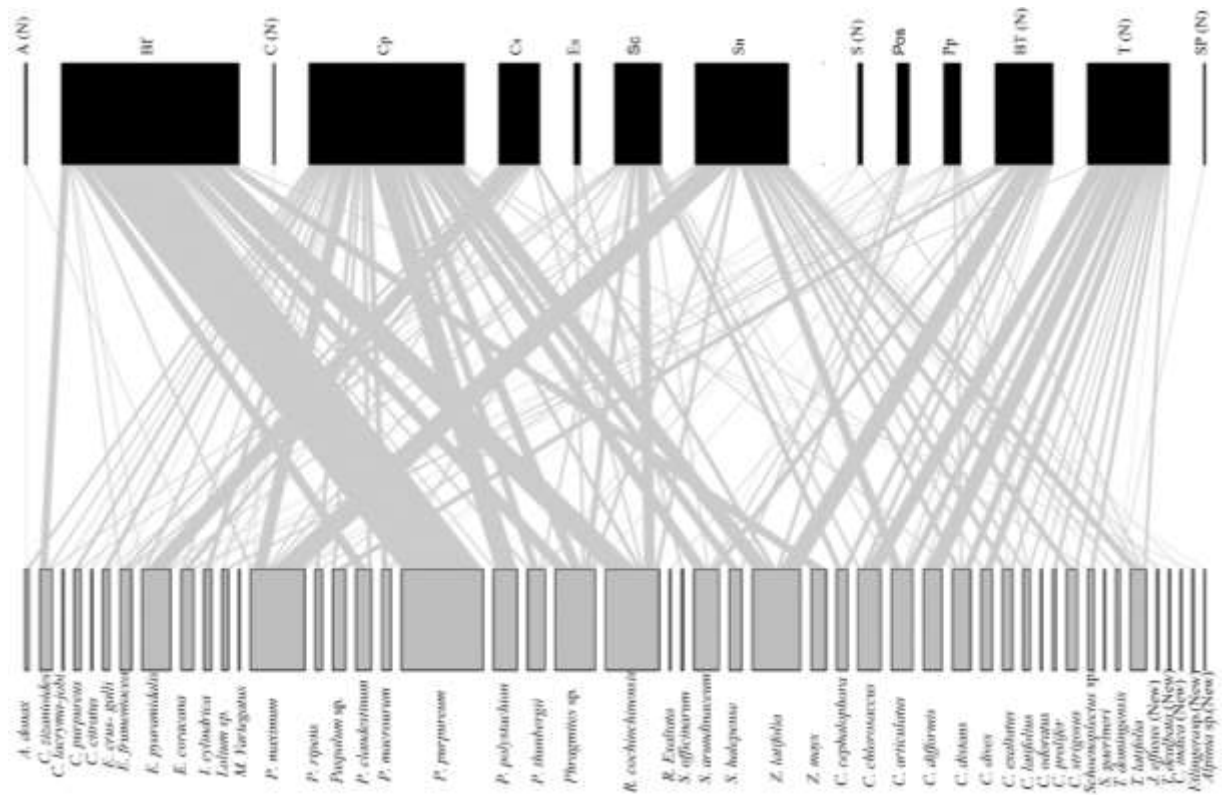


Stages accounted during the field collection. Larvae (right) were recovered in ornamental plants in Kigali, in soil in Kamonyi District (left). Pupae were from Mulindi wetland, near Masaka.

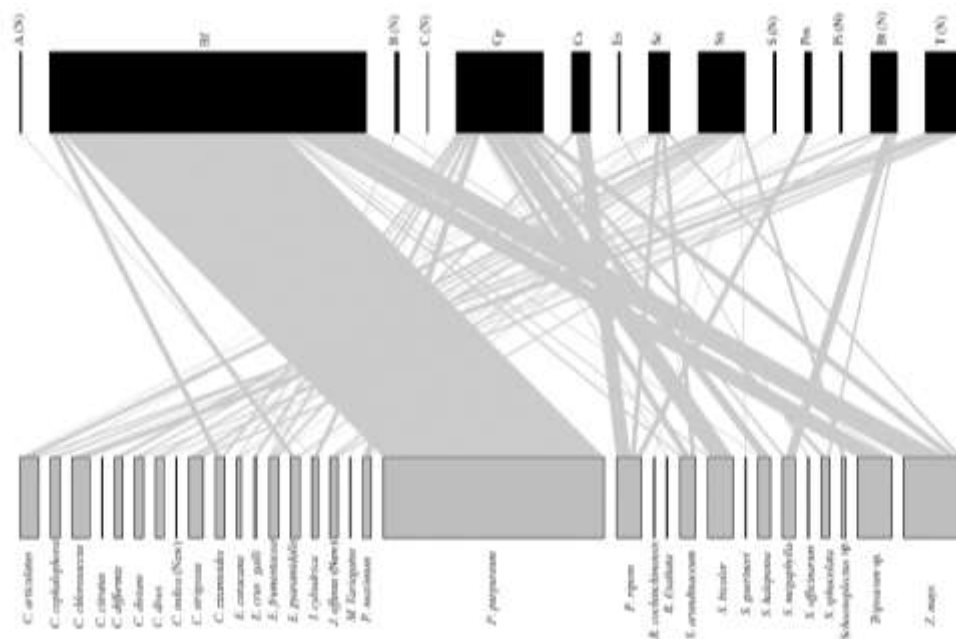


Mass rearing applied artificial feed by original host plant (figure on the top) and artificial feed (figure on the bottom)

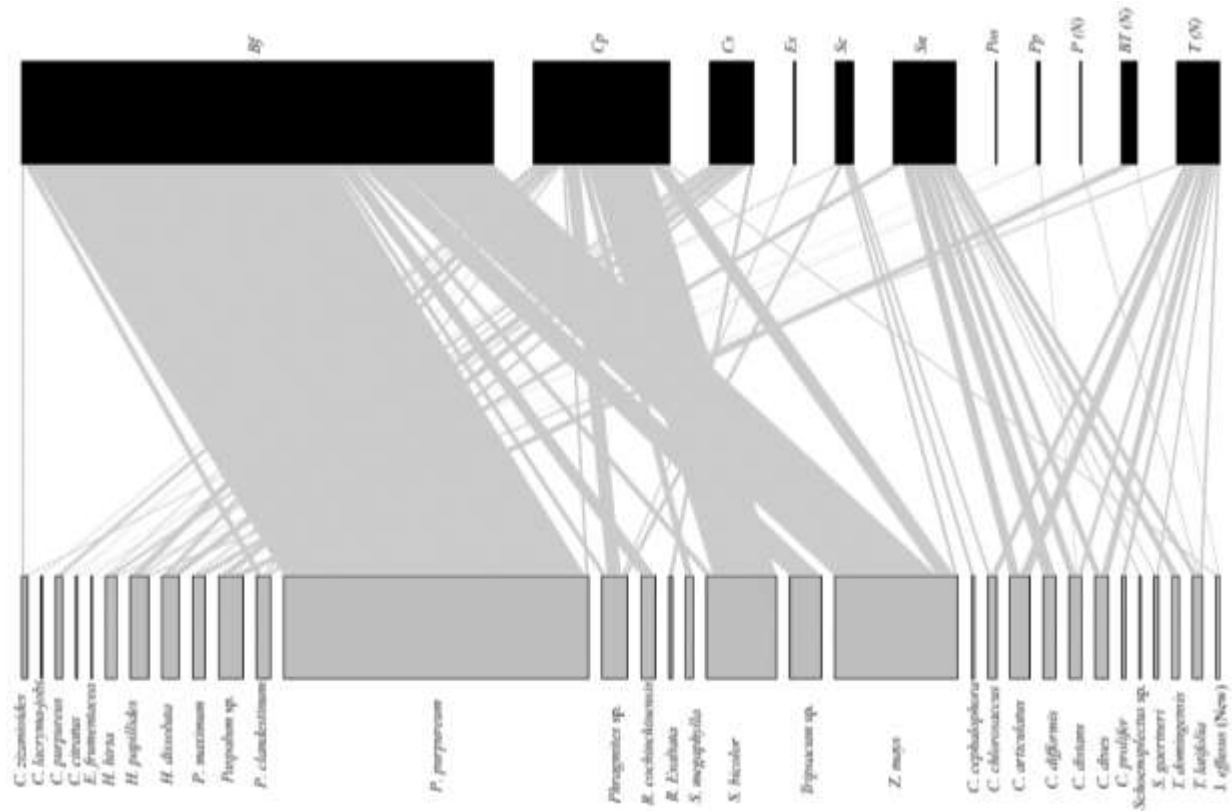
Appendix 2. Figures of Host plant-stemborer interaction network per agroecology (Figure 3. 4)



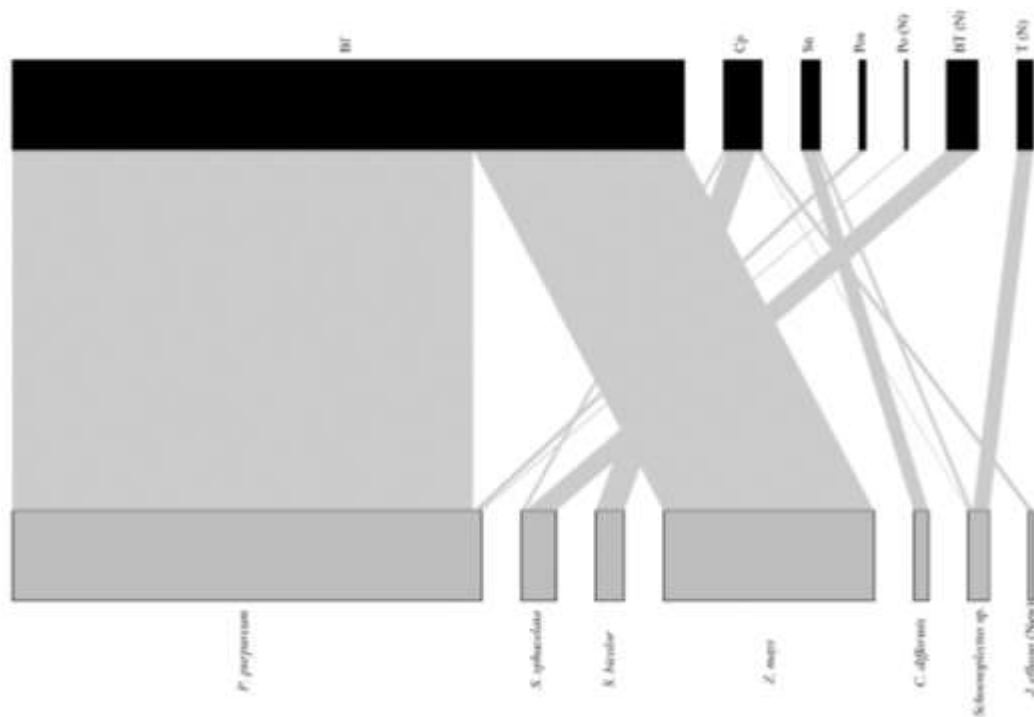
(a) Stemborers-host plants interaction in Kigali City



(b) Stemborers-host plants interaction in Southern Province (Kamonyi midland)

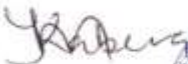


(c) Stemborers-host plants interaction in Eastern Province (Kayonza highland)



(d) Stemborers-host plants interaction in Northern Province (Burera highland)

Appendix 3. Permit and support

 Republic of Rwanda Ministry of Environment	RWANDA ENVIRONMENT MANAGEMENT AUTHORITY (REMA)	 REMA Rwanda Environment Management Authority
Kigali, on <u>29 JUL 2024</u> N° <u>1525</u> /DG/2024		
NTIRENGANYA Elie PhD Student conducting research at College of Science and Technology University of Rwanda, Kigali, Nyarugenge Email: elientirenganya1@gmail.com Tel: +250792404871		
Dear Sir,		
Re: Authorization for exporting biological samples for DNA extraction.		
Reference is made to your application requesting to export dead lepidopteran stemborers for DNA extraction in the Republic of Korea for the purpose of conducting your research project entitled "Diversity and Ecology of Lepidopteran Stem Borers in Rwanda with special focus on species genetic diversity of stem borer from three agroecological zones of Rwanda at three altitudinal gradients", which has the purpose to understand the genetic diversity and elucidating the evolutionary relationships among stemborer populations across regions and host plants in Rwanda;		
Reference is also made to the Law No. 064/2021 of 14/10/2021 governing biological diversity in Rwanda, especially in its article 27 on import and export of genetic resources stating that "<i>Import or export of genetic resources is subject to a permit.</i>"		
Considering the above, we hereby authorize you to export 50 tissue samples of dead Lepidopteran stemborers for DNA extraction and sequencing from Rwanda to the Republic of Korea, solely for research purpose. The samples must be handled in accordance with all relevant regulations and ethical guidelines set forth by Rwanda and the Republic of Korea.		
Sincerely,		
 Juliet KABERA Director General <u>Cc:</u> - Hon. Minister of Environment - Hon. Minister of State for the Environment - CEO and Member of Cabinet/RDB - Vice-Chancellor, UR 		
Inyota House, KG 7 Ave, Kigali PO Box 7436 Kigali - Rwanda Tel: (+250) 788303593, Hotline: 3989 Website: www.rema.gov.rw Email: info@rema.gov.rw X: @REMA_Rwanda		



UNIVERSITY of
RWANDA

CENTER OF EXCELLENCE IN
BIODIVERSITY AND NATURAL
RESOURCE MANAGEMENT



Center of Excellence in Biodiversity &
Natural Resource Management
A UNESCO Category 2 Centre

Huye, 26 June 2024

Ref: CoEB/055/2024

To:

Director General (DG)
Rwanda Environment Management Authority (REMA)
KIGALI - RWANDA

Dear DG,

Re: Support for the request to export DNA Samples of Lepidopteran Stemborers for Advanced Genetic Analysis by Mr NTIRENGANYA Elie

I am writing to express my full support for Mr. NTIRENGANYA Elie, a PhD student enrolled in the Climate Smart Agriculture and Biodiversity Conservation program at Haramaya University, Ethiopia. As his supervisor, I have closely overseen his research from its inception to the present. NTIRENGANYA is working on "Diversity and Ecology of Lepidopteran Stem Borers in Rwanda with special focus on species genetic diversity of stem borer from three agroecological zones of Rwanda at three altitudinal gradients (low altitude in eastern Rwanda, middle altitude in central part of Rwanda and high altitude in northern Rwanda). The main purpose is to explore the origin, genetic diversity, and evolutionary relationships of stemborers of significant impact in maize and wild plants in Rwanda.

Given the complexity and importance of this research, it is essential to conduct advanced genetic analyses that require specialized facilities that are not available here in Rwanda. To this end, I do not hesitate to any support or facilitation that can be given to NTIRENGANYA to export DNA samples of Lepidopteran stemborers to where state-of-the-art genomic sequencing technologies and expertise are advanced.

The outcomes of this research will provide valuable long-term data on lepidopteran stemborer ecology, contributing significantly to our understanding and control of these pests which will be instrumental in informing pest management policies and practices, enhancing food security, and supporting sustainable agriculture in Rwanda.

Yours sincerely,

Venuste Nsengimana (PhD)
Director
Centre of excellence in Biodiversity and Natural Resource Management
College of Science and Technology
University of Rwanda

Digitally signed by
Venuste NSENGIMANA
Date: 2024.07.06
13:05:25 +02'00'

Email: coeb@ur.ac.rw

Website: www.coebiodiversity.ur.ac.rw