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Establishment for Laboratory Rearing of *Salpingogaster nigra* (Diptera: Syrphidae) and the disclosure of a new species

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Tese apresentada ao Programa de Pós-Graduação em Biodiversidade e Conservação da Natureza da Universidade Federal de Juiz de Fora como requisito parcial à obtenção do título de Doutor em Ciências Biológicas. Área de concentração: Comportamento ecologia e sistemática.

Orientador: Dr. Alexander Machado Auad

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Porque é do chão que se vê o céu.
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Resumo Geral

Os sirfídeos constituem um grupo de elevada diversidade e importância ecológica, atuando tanto como polinizadores quanto como inimigos naturais de insetos-praga. No gênero *Salpingogaster*, destaca-se *Salpingogaster nigra*, reconhecida como predadora eficiente de cigarrinhas-das-pastagens, uma das principais pragas da pecuária no Brasil. Dessa forma, o uso dessa espécie no controle biológico apresenta grande potencial dentro do manejo integrado de pragas, especialmente por sua capacidade de localizar e consumir ninfas no interior da espuma produzida por esses insetos. No entanto, a aplicação prática desse predador depende do desenvolvimento de protocolos eficientes de criação em laboratório. Neste estudo, verificou-se que o desenvolvimento embrionário de *S. nigra* é fortemente influenciado por fatores abióticos, sendo recomendada a manutenção dos ovos entre 20 e 30 °C com hidração diária para garantir alta viabilidade. Para os adultos, a dieta artificial à base de levedura e mel mostrou-se adequada, permitindo boa sobrevivência, fecundidade e produção de ovos, o que representa um avanço importante para a criação massal dessa fase do ciclo de vida. Por outro lado, o desenvolvimento larval ainda representa o principal desafio para a produção em larga escala da espécie. As dietas alternativas testadas não foram capazes de sustentar o desenvolvimento completo das larvas, e mesmo com o uso de presas naturais, foram observadas altas taxas de mortalidade em condições controladas. Esses resultados indicam que as larvas dependem de condições ecológicas específicas, possivelmente relacionadas à umidade, à espuma produzida pelas ninfas das cigarrinhas e a interações ambientais ainda não reproduzidas em laboratório, o que limita, até o momento, a viabilidade da criação massal. Além dos aspectos biológicos e aplicados, foi descrita uma nova espécie, *Salpingogaster distincta* sp. nov., morfologicamente próxima a *S. nigra*, cujas larvas também foram encontradas predando cigarrinhas-das-pastagens. A caracterização morfológica e molecular dessa nova espécie amplia o conhecimento taxonômico do grupo e evidencia o potencial ainda pouco explorado do gênero como fonte de agentes de controle biológico. De forma geral, os resultados desta pesquisa demonstram avanços importantes na criação de adultos e na compreensão dos fatores que afetam o desenvolvimento inicial de *S. nigra*, ao mesmo tempo em que evidenciam limitações críticas no desenvolvimento larval. A descoberta de uma nova espécie predadora reforça o potencial do gênero *Salpingogaster*, indicando que, apesar dos desafios, esses sirfídeos representam uma alternativa promissora e sustentável para o manejo de cigarrinhas-das-pastagens.

Palavras-chave: Analise-molecular, Controle- biológico, Syrphidae, predador

Abstract

Syrphid flies constitute a group of high diversity and ecological importance, acting both as pollinators and as natural enemies of insect pests. Within the genus *Salpingogaster*, *Salpingogaster nigra* stands out, recognized as an efficient predator of spittlebugs, one of the main livestock pests in Brazil. Therefore, the use of this species in biological control presents great potential within integrated pest management, especially due to its ability to locate and consume nymphs inside the foam produced by these insects. However, the practical application of this predator depends on the development of efficient laboratory rearing protocols. In this study, it was found that the embryonic development of *S. nigra* is strongly influenced by abiotic factors, and maintaining the eggs between 20 and 30 °C with daily hydration is recommended to ensure high viability. For adults, an artificial diet based on yeast and honey proved adequate, allowing good survival, fecundity, and egg production, which represents an important advance for the mass rearing of this stage of the life cycle. On the other hand, larval development still represents the main challenge for large-scale production of the species. The alternative diets tested were not able to sustain the complete development of the larvae, and even with the use of natural prey, high mortality rates were observed under controlled conditions. These results indicate that the larvae depend on specific ecological conditions, possibly related to humidity, the foam produced by the spittlebug nymphs, and environmental interactions not yet reproduced in the laboratory, which limits, to date, the viability of mass rearing. In addition to the biological and applied aspects, a new species, *Salpingogaster distincta* sp. nov., morphologically close to *S. nigra*, was described, whose larvae were also found preying on spittlebugs. The morphological and molecular characterization of this new species expands the taxonomic knowledge of the group and highlights the still largely unexplored potential of the genus as a source of biological control agents. Overall, the results of this research demonstrate important advances in the rearing of adults and in understanding the factors that affect the early development of *S. nigra*, while also highlighting critical limitations in larval development. The discovery of a new predator species reinforces the potential of the genus *Salpingogaster*, indicating that, despite the challenges, these hoverflies represent a promising and sustainable alternative for the management of spittlebugs.

Keywords: Molecular analysis, Biological control, Syrphidae, predator

Summary

GENERAL INTRODUCTION

Traditionally, research has focused on species that stand out in providing a single ecosystem service (e.g., pollinating insects or natural enemies acting as predators or parasitoids for pest control). Although this approach is useful, it may be more efficient to investigate species capable of simultaneously providing both services, such as flies of the family Syrphidae.

Syrphids are flies commonly known as “flower flies” and, within the order Diptera, constitute a family with high diversity and abundance of species. They occur in different habitats, exhibiting a wide range of behaviors or life-history strategies and adaptations (Gilbert & Owen, 1990; Gilbert et al., 1994; Thompson, 1999). They display a great variety of shapes and sizes, often mimicking other insects, especially wasps and bees (Penney et al., 2012). The family comprises 6,674 species in 284 genera distributed among four subfamilies Microdontinae, Eristalinae, Syrphinae, and Pipizinae and is found in nearly all biogeographic regions (Thompson, 1987; Arcaya, 2012). In the neotropical region, 60 genera and approximately 1,600 species are known, and it is likely that many additional species remain undescribed (Thompson, 1999; Arcaya, 2012).

Adults of most species are flower visitors, where they feed on pollen, nectar, or both (Larson et al., 2001). Many of these flower-visiting dipterans are recognized as essential pollinators in natural and agricultural systems (Klecka et al., 2018; Rader et al., 2016). Larvae, however, exhibit an exceptionally broad diversity of feeding regimes, including zoophagy, saprophagy, phytophagy, mycophagy, and coprophagy (Rotheray & Gilbert, 2011). Zoophagy is the ancestral condition of the subfamilies Syrphinae and Pipizinae, which together represent nearly one-third of all Syrphidae species (Mengual et al., 2023).

Some dipterans belonging to this family are important agents in reducing the population density of phytophagous insects considered pests. Aphids are the most commonly consumed prey, including most economically important species. Several syrphids are generalists, such as *Episyrphus balteatus* (De Geer), which has been recorded as a predator of 234 species, whereas other syrphids have been reported as predators of more than 50 species (Rojo et al., 2003; Gilbert, 2005). In contrast, *Fagisyrphus cinctus* (Fallén) has been recorded as a predator primarily of a single aphid species (Rotheray & Gilbert, 2011). Auad (2003) used the aphid *Schizaphis graminum* as prey for *Pseudodorus clavatus* (Fabricius).

Larvae that prey on phytophagous insects are considered highly voracious, although their biological control potential varies among species. The number of aphids consumed during larval development has been estimated for species such as *E. balteatus*, *Allograpta*

obliqua, *Toxomerus marginatus*, and *Ischiodon scutellaris*, with laboratory tests showing variation (from 130 to 1,300 third-instar aphids during larval development) depending on temperature, predator species, and prey species (Tenhumberg, 1995; Hopper et al., 2011).

Some syrphid species are considered natural enemies of scale insects, such as larvae of *Eosalpingogaster* sp., which are dipterans found exclusively in the New World (Mengual & Thompson, 2011). Adults visit flowers to obtain pollen and nectar and are common pollinators, as are all adults of the subfamily Syrphinae.

The larval biology of some species of this genus is known: all are predators of Sternorrhyncha, including several pest species (Rojo et al., 2003). The genus *Eosalpingogaster* was proposed by Hull (1949) as a new subgenus of *Salpingogaster* Schiner. Hull based this decision on the absence of a sharp hook at the anteriores corners of the first abdominal segment and on the slight curvature of vein R4+5.

In contrast, species of the genus *Salpingogaster* have the first abdominal tergum laterally produced into a strong spur and a strongly sinuous R4+5 vein. Species of both groups, *Salpingogaster* and *Eosalpingogaster*, share four characters: (i) vein M1 strongly sinuous; (ii) abdomen strongly petiolate; (iii) complete post-metacoxal bridge; and (iv) femora with ventral spinose setae.

Despite these shared traits, these taxa appear to have distinct natural histories, as they prey on different types of larvae. Species of the genus *Eosalpingogaster* feed primarily on scale insects (Coccoidea), whereas species of the genus *Salpingogaster* exhibit more restricted feeding habits, being consistently observed in predatory association with nymphs of spittlebugs (Cercopidae), as first recorded by Gough in 1910 and later reported in several studies (Paez & Torres, 1985; Guppy, 1913; Rojo et al., 2003; Granobles, 2012; Veríssimo et al., 2018; Veríssimo et al., 2024). This specialization may reflect specific ecological adaptations, functionally differentiating them from other genera within the subfamily Syrphinae.

In pasture areas, where spittlebugs are recurrent pests of economic importance, Brazil has a large proportion of degraded pastures (Cavalcanti et al., 2021), caused especially by complexes of spittlebugs belonging to the family Cercopidae, which are the main pasture insect pests in Latin America (Schöbel & Carvalho, 2019). Kershaw (1913) considered *Salpingogaster nigra* Schiner, 1868, a natural enemy with potential for controlling pasture spittlebugs. Representatives of *Salpingogaster* have demonstrated high potential as biological control agents due to their ability to locate spittlebug nymphs and oviposit within the foam produced by them (Granobles, 2012; Veríssimo et al., 2018), which favors the successful

completion of the predator's life cycle. Upon hatching, larvae exhibit high voracity (Paez & Torres, 1985). Predatory capacity was estimated using the average number of nymphs consumed per larva per day; a total of 24 larvae were used to obtain the observed results, thereby enhancing the potential impact on pest populations.

In Brazil, the occurrence of *Salpingogaster nigra* has been recorded in the states of Bahia, Pernambuco, Rio de Janeiro, São Paulo, Mato Grosso do Sul (Ramos, 1976; Ramos, 1978; Botelho & De Sá, 1980; Ramos Marques, 1987), and Minas Gerais (Veríssimo et al., 2021), based on field observations conducted over several decades.

Laboratory experiments suggest that its predatory efficiency may contribute to the natural regulation of spittlebug populations, reducing the need for chemical interventions and favoring sustainable integrated pest management strategies. Results such as those of Paez & Torres (1985), who evaluated the predatory capacity of *S. nigra* larvae on spittlebug nymphs, support this potential. In their experiment, 24 larvae were individually maintained in plastic cups and fed daily with nymphs of different sizes. Dead nymphs were removed and examined under a stereomicroscope to verify signs of predation. Predatory capacity was estimated based on the average number of nymphs consumed per larva per day. The authors observed that a single larva was capable of consuming up to 17 nymphs during its development, demonstrating the species' potential as a biological control agent.

In this context, the development of protocols for the mass rearing of *Salpingogaster* sp. under laboratory conditions emerges as an essential step for its use in applied biological control programs. Recent studies (Veríssimo et al., 2018; Veríssimo et al., 2024) have sought to optimize parameters such as temperature, humidity, photoperiod, and suitable food substrates for both adults and larvae, with the aim of ensuring high survival, development, and reproduction rates. Large-scale production of this syrphid could enable its strategic release in infested areas, contributing significantly to the population control of spittlebugs in pastures.

The first effort to rear *Salpingogaster* under laboratory conditions until the adult stage was reported by Urich (1912). The most complete account of its life history was provided by Guppy (1913) and Paez & Torres (1985). According to their reports, the egg is approximately 0.75 mm long, elongated, and bright white in color, and is deposited in the foam. The egg stage lasts about two to two and a half days. The apodous larva that hatches destroys the spittlebug nymph by piercing its integument and sucking out its contents.

More than a century after these first reports, Veríssimo et al. (2018) conducted laboratory experiments with *S. nigra* that corroborated the observations made by Guppy (1913) and Paez & Torres (1985).

The resumption of these studies represents a significant advance in knowledge of the species and reinforces its potential as a biological control agent in pastures. However, mass rearing of *S. nigra* still faces important obstacles. During laboratory work conducted by Paez & Torres (1985), problems arose due to contamination of live material. The most common issue was the presence of a nematode that attacked eggs and larvae of *S. nigra*. This nematode was possibly transported to the laboratory in the foam collected in the field and used to maintain larvae in captivity. The same authors also reported a phorid fly (Diptera: Phoridae) attacking pupae in the same experiment. According to Paez & Torres (1985), the lack of knowledge regarding ideal abiotic factors for maintaining live material in captivity led to the loss of a significant portion of it. Larvae rapidly dehydrate under low humidity and high temperatures, whereas excessive humidity causes unpleasant odors that attract contaminants.

Another complicating factor is that spittlebug population density varies seasonally, with well-defined peaks during the rainy season (spring and summer in southeastern Brazil) and a marked decline during the dry season (Auad et al., 2009), making the continuous maintenance of the natural prey difficult. These factors highlight the challenges of maintaining a stable *S. nigra* colony based exclusively on its naturais prey and reinforce the importance of developing or implementing alternative or artificiais diets, especially during periods of limited spittlebug availability.

Guppy (1914) reported attempts to rear the predator during the dry season in Trinidad but was unsuccessful, possibly due to the scarcity of natural hosts needed to maintain prey in the laboratory. In addition, his observations indicate that the experiments were conducted with few replicates due to the low occurrence of the predator in the field, demonstrating that challenges related to rearing this species date back more than a century. Despite the recognized potential of *S. nigra* as a biological control agent of pasture spittlebugs, its laboratory rearing still presents technical and biological constraints that limit its practical application.

Veríssimo et al. (2018) achieved success in studying the biology of the predator during the egg, pupal, and adult stages. However, when monitoring the development of 210 larvae under laboratory conditions, they found that mass rearing was not feasible due to low larval viability, with an average of only 10.04%. This may have resulted from contamination of the nymphs and foam collected in the field by microorganisms or nematodes.

Given this context, this thesis aimed to expand knowledge about the potential of the genus *Salpingogaster* as a biological control agent for spittlebugs, with an emphasis on applied and taxonomic aspects. To this end, abiotic factors influencing embryonic development and egg prediction in *Salpingogaster nigra* were investigated, as well as the efficiency of alternative diets for maintaining adults in the laboratory. Additionally, different larval rearing methodologies were evaluated, seeking to overcome the challenges associated with the mass production of the species under controlled conditions. Finally, a new species of the genus, *Salpingogaster distincta* sp. nov., was described based on morphological and molecular characters, highlighting its potential as a new biological control agent. Thus, this thesis integrates biological, and taxonomic approaches, contributing to the advancement of the sustainable use of hoverflies in integrated pest management.

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Artificial diet for adults and adequate egg maintenance for laboratory rearing of the predator *Salpingogaster nigra* Schiner, 1868 (Diptera: Syrphidae)

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ABSTRACT
This research evaluated the effect of six temperatures and the duration of exposure to hydration on the embryonic development and egg viability of *Salpingogaster nigra* Schiner, 1868 and analyzed an artificial diet suitable for adults of this predator. The viability was significantly lower at 10 °C and stabilised significantly from 20 °C upward, fitting to a quadratic equation. The mean embryonic development was 10.31, 8.44, 3.86, 2.18, and 2.04 days and was significantly less at 25 and 30 °C. The lower threshold temperature and the thermal constant were 7.4 °C and 44.2 d degree, respectively. Eggs were unable to complete development without a daily hydration regime. Adults fed with only honey yielded a lower total number of eggs compared to other diets (yeast + honey or inflorescences + honey). The offered diets did not alter the viability of the eggs or the longevity of adults of *S. nigra*. The results of this study indicated *S. nigra* egg development and viability was adequately maintained between 20-30 °C and with a daily hydration regime, and an artificial diet of yeast + honey was suitable for adults. This information will improve the efficacy of mass rearing techniques for the different stages of *S. nigra*.

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Biological control; predator; pest insect; abiotic factors; biotic factors

Introduction

Several species of hoverfly (Diptera: Syrphidae) stand out for their bioindicator character as pollinators in the conservation of habitats and for their function as pest controllers. According to Veríssimo et al. (2018), the most promising biological control agent for pasture spittlebugs is the predator *Salpingogaster nigra* Schiner, 1868 (Diptera: Syrphidae). On average, each *S. nigra* larva consumes between 12 and 17 spittlebug nymphs in pastures (Guppy, 1913; Páez et al., 1985), and their life cycle is of short duration, with two to three generations during their prey's life cycle (Guagliumi, 1970). Spittlebugs mainly affect forage and are considered a limiting insect pest in the tropical Americas

(Auad et al., 2010; Fonseca et al., 2016; Holmann & Peck, 2002), causing estimated losses of up to US$ 2.1 billion annually worldwide (Thompson, 2004). Among the methods of spittlebug biological control, scientific research has sought the use of entomophagous agents, such as hoverflies are promising.

In the field, abiotic factors such as temperature and relative humidity (RH) limit an insect's permanence in an environment (Chakraborty, 2005). Knowledge of these factors can be exploited in an agricultural context by determining the thermal limits, including the lower threshold temperature (LTT), at which insect species can develop; with knowledge of these limits, one can predict the occurrence and population peaks of an insect in a given area (Lloyd & Barnett, 1983).

Because temperature and humidity are the main abiotic factors influencing insect biology, studying *S. nigra* egg development at different temperatures and exposures to hydration can contribute to the development of more effective rearing techniques to allow this biological control agent to be used efficiently on forage. In addition, the diet offered to the adults is important. According to King et al. (1975), rearing insects on artificial diets has led to advances in integrated pest management practices. The use of artificial diet has the advantage of continuously producing individuals with nutritional and biological uniformity and brings the possibility of automation for eventual mass rearing (Parra, 2002).

Information for the development of mass rearing techniques for the different stages of the predator *S. nigra* are rare. In this context, the present study evaluated the effects of different temperatures and periods of exposure to hydration on the embryonic development and viability of *S. nigra* eggs and evaluated an artificial diet for adults of this predator.

Methods

Bioassay 1: adequate maintenance of S. nigra eggs

Adults of *S. nigra* were collected at Campo Experimental José Henrique Bruschi from the municipality of Coronel Pacheco, Minas Gerais (MG), Brazil, and taken to the entomology laboratory of Embrapa Gado de Leite in the municipality of Juiz de Fora, MG. Adults of the predator and nymphs of the prey, *Mahanarva spectabilis* Distant 1909 (Hemiptera: Cercopidae), were placed in acrylic cages measuring 80 × 55 × 55 cm. Predator eggs were placed in the foam produced by the prey nymphs. Next, 96 eggs per treatment were individualised in microtiter plates lined with filter paper moistened with distilled water. The plates were placed in BOD-type climatized chambers at constant temperatures of 10, 15, 20, 25, 30, and 35 ± 1 °C at 70 ± 10% RH and a 12 h photophase.

The lower threshold temperature (LTT) and thermal constant (K) were estimated by fitting a hyperbolic equation to the results (Bean, 1961) using regression analysis. The linear model was used to estimate the LTT and thermal constant expressed by the equation in day degree (DD).

The parameters were subjected to analysis of variance (ANOVA) and regression analysis, with the nonlinear model selected according to the significance of the regression coefficients ($p < 0.05$) and the determination coefficients (R^2). Statistical analyses were performed using Sisvar 5.0 software (Ferreira, 2014).

In the second test, the effect of hydration exposure period was assessed by placing 50 eggs per treatment in microtiter plates lined with filter paper and transferring them to

climate-controlled chambers. Eggs were then subjected to the following treatments: (1) no water; (2) application of 0.5 mL of distilled water, daily, from fifth day onwards; (3) application of 0.5 mL of distilled water, daily, from tenth dayonward; (4) application of 0.5 mL of distilled water, daily, from the start of the experiment. Egg viability was evaluated daily under a stereoscopic microscope. The data were subjected to ANOVA and the means were compared using the Tukey test ($p < 0.05$).

Bioassay 2: artificial diet for adult S. nigra

To analyze alternative diets for adult *S. nigra*, 30 couple were collected in the José Henrique Bruschi experimental field from the municipality of Coronel Pacheco, MG. They were taken to the entomology laboratory of Embrapa Gado de Leite in the municipality of Juiz de Fora (MG) and kept in 30 × 30 × 30 cm cages at a temperature of 25 ± 1°C and an RH of 70 ± 10% with 12 h photophase. Three treatments with different diets were established: (1) honey + yeast + water (2:2:1); (2) honey + water (2:1), and (3) flowers of *Parthenium hysterophorus* and *Emilia fosbergii* + honey + water (2:1).

The longevity of males and females, the total number of eggs per female, and egg viability were evaluated. A completely randomised design with 10 replicates was used. The data were subjected to analysis using ANOVA, and the means were compared using the Scott-Knott test at 5% probability.

Results

Adequate maintenance of S. nigra eggs

The best-fit curves for the regression between temperature and egg viability of *S. nigra* were obtained using a second-degree polynomial equation. The average percentage of

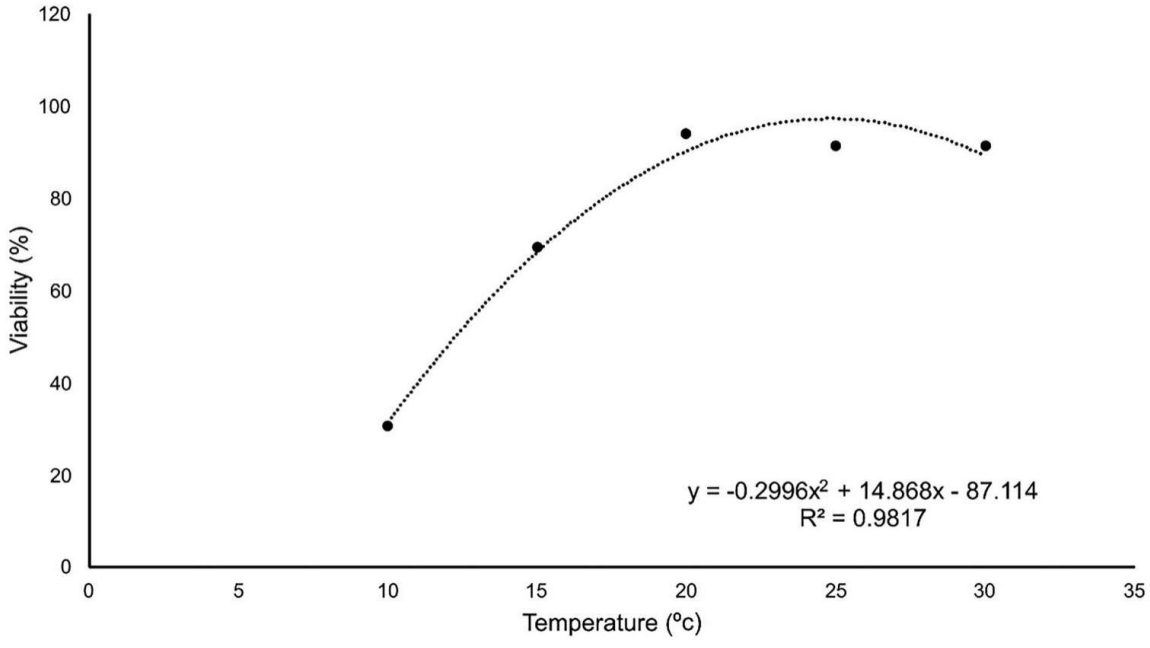

Figure 1. Regression curves adjusted for the viability of *S. nigra* eggs maintained at constant temperatures of 10, 15, 20, 25, and 30 ± 1 °C, RH 70 ± 10%, and 12 h photophase.

Table 1. Duration (days) and viability (%) of S. nigra eggs subjected to different temperatures and hydration periods.

	Temperature (°C)						ANOVA	
	10	15	20	25	30	35	(*p*)	(f)
Duration	10.31 ± 0.37 D n = 29	8.44 ± 0.25 C n = 67	3.86 ± 0.08 B n = 90	2.18 ± 0.08 A n = 88	2.04 ± 0.02 A n = 88	_	0.000	359.912
Viability	30.5 ± 4.71 C n = 96	69.5 ± 4.75 B n = 96	94 ± 2.48 A n = 96	91.6 ± 2.83 A n = 96	91.6 ± 2.83 A n = 96	0	0.000	42.341

	Humidity (mL/day)				ANOVA	
	0 mL/day	0.5 mL after 5 days	0.5 mL after 10 days	0.5 mL/day	(*p*)	(f)
Viability	0 A n = 50	0 A n = 50	0 A n = 50	100 B n = 50	0.000	120.000

Means with distinct letters in the rows indicate significant differences by Tukey's test ($p < .05$); (-) indicates the absence of data due mortality. (n) = number of individuals; *P* and F are values of variance analysis followed by a Tukey's test.

egg viability obtained was 30.5, 69.5, 94.0, 91.6, 91.6, and 0% when they were kept at the respective temperatures of 10, 15, 20, 25, 30, and 35 °C (Figure 1). No significant difference in egg viability was observed at temperatures of 20, 25, and 30°C (Table 1).

The average duration of embryonic development of *S. nigra* was observed to be shorter at temperatures in the range of 25 to 30 °C (Table 1), which followed a regression curve adjusted to a second-degree polynomial equation (Figure 2), with a variation of 10.31 to 2.04 days depending on the increase in temperature. Notably, it was not possible to determine the embryonic development of *S. nigra* kept at 35 °C due to 100% mortality at that temperature.

Based on the linear regression equation (Figure 3), the LTT was estimated at 7.4°C, with a thermal constant of 44.2 DD. The treatments with hydration restriction promoted mortality of 100% (Table 1); however, those with daily hydration were observed to be 100% viable.

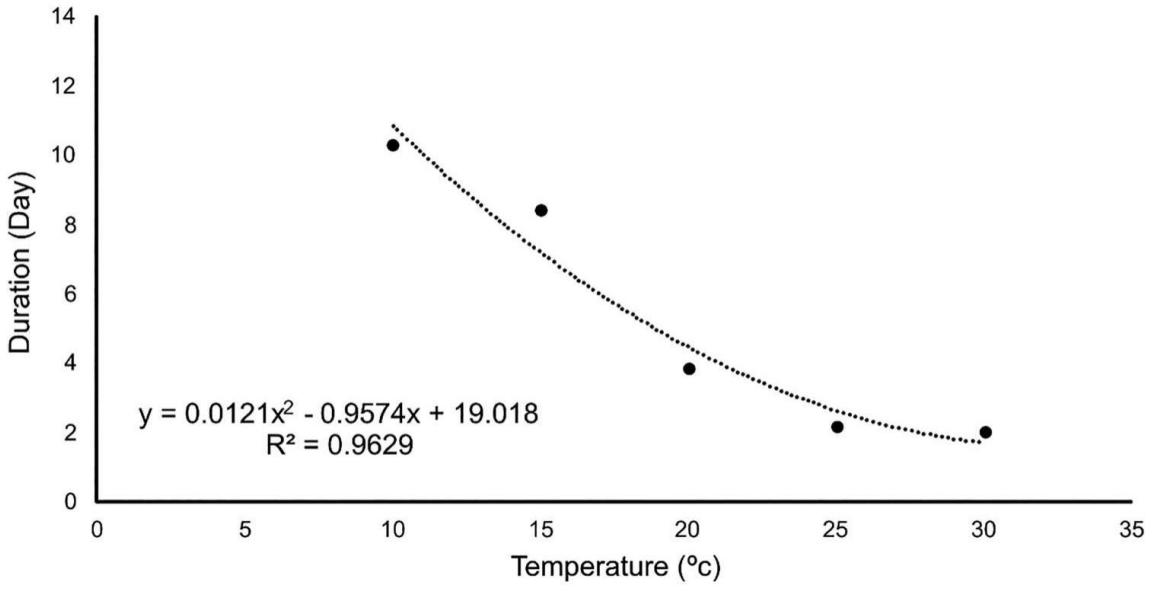

Figure 2. Regression curves adjusted to the mean duration of development of *S. nigra* eggs kept at constant temperatures of 10, 15, 20, 25, and 30 ± 1 °C, RH 70 ± 10%, and photophase of 12 h.

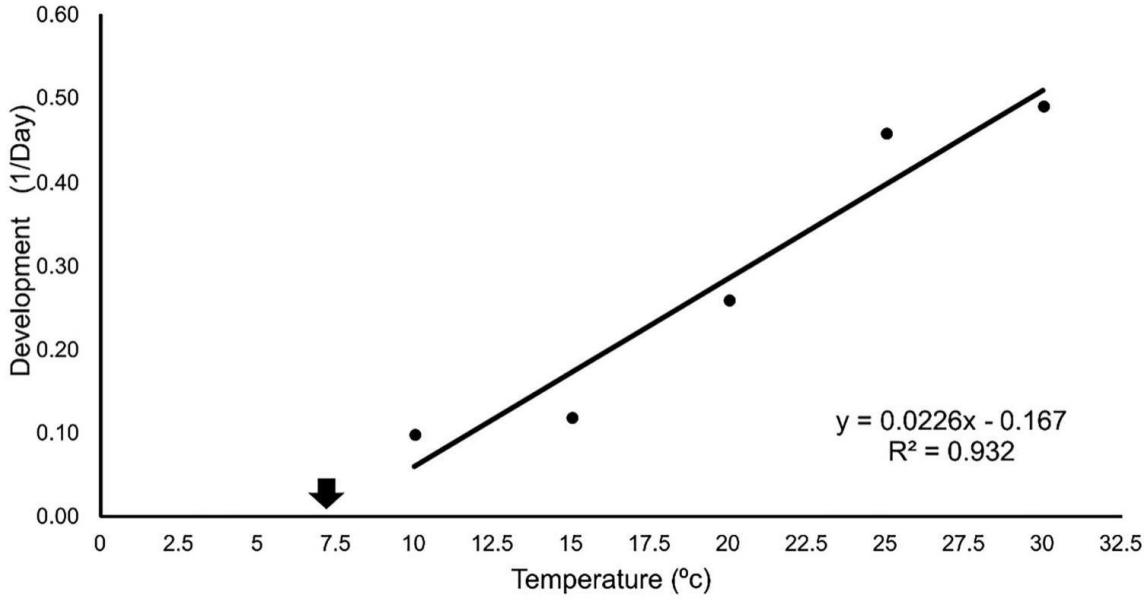

Figure 3. Relationship between development rate and temperature of *S. nigra* eggs kept at constant temperatures of 10, 15, 20, 25, and 30 ± 1 °C, RH 70% ± 10%, and photophase of 12 h.

Table 2. Salpingogaster nigra adult longevity (days), total eggs perfemale, and eggs viability (%) when fed different diets.

	Diets			ANOVA	
Female longevity	Yeast + Honey	Honey	Flowers + Honey	(*p*)	(f)
	14.66 ± 2.86 A	14.42 ± 2.71 A	16.83 ± 3.53 A	0.8636	0.148
	n = 10	n = 10	n = 10		
Male longevity	18.88 ± 3.44 A	14.42 ± 2.41 A	21.66 ± 2.91 A	0.3699	1.049
	n = 10	n = 10	n = 10		
Eggs total/female	89.00 ± 17.17 B	32.14 ± 12.51 A	112.33 ± 35.25 B n = 10	0.0565	3.356
	n = 10	n = 10			
Eggs viability	93.84 ± 4.06 A	98.63 ± 1.20 A	99.28 ± 0.42 A	0.4395	0.859
	n = 10	n = 10	n = 10		

Averages with different letters in the rows indicate significant differences using the Scott-Knott test ($p < .05$); (n) = number of repetitions; *P* and F values from the ANOVA followed by the Scott-Knott test.

Artificial diet for adult S. nigra

Adults fed with only honey yielded a lower total number of eggs than those on the other diets (yeast + honey or inflorescences + honey). The viability of the eggs was more than 93% and not was affected by the diets offered. The diets offered too not affected the longevity of the males and females of *S. nigra* (Table 2).

Discussion

Knowledge of *S. nigra*'s thermal and humidity requirements is important, as it advances our understanding of predator dynamics, helps establish breeding protocols, and improves the efficiency and effectiveness of control programmes.

The results of this study are consistent with those observed on the viability of eggs of the hoverfly *Sphaerophoria rueppellii* Wiedemann, 1830 (Diptera: Syrphidae), which were able to develop properly in a temperature range between 20 and 30°C (Amorós-Jiménez et al.,

2012). According to Tonelli et al. (2018), the foam of the nymphs of the prey (spittlebugs from pastures) has a relatively constant internal temperature of 25.2 ± 0.63 °C, creating a thermal microhabitat suitable for the survival of the pest insect. At that temperature, in the current study, the viability of the predator's eggs was above 90%, showing a close relationship between the predator *S. nigra* and the spittlebugs.

Following the general rules valid for poikilothermic animals, the greater metabolic activity of eggs in the higher temperatures. Beserra et al. (2006) report a reduction in the period of development of *Aedes aegypti* Linnaeus, 1762 (Diptera; Culicidae) with thermal elevation, identifying 34 °C as yielding the shortest period of development. Laws and Belovsky (2010) mention that physiological and developmental processes of insects can be directly affected by temperature, as they have limited capacity for thermoregulation. Frazier et al. (2006) and Savage et al. (2004) reported that species that have adapted to high temperatures have a fast development period and, consequently, a reduced generation time (Roff, 2002). The results of this research corroborate this hypothesis but are limited to 30°C.

In studies on the impact of temperature, knowing the thermal limits of the predator and target prey is important for the development of biological control, as the temperature influences the performance of both organisms. According to Vucic-Pestic et al. (2011) and Dell et al. (2014), temperature affects the physiological and ecological characteristics of insects, influencing prey-predator interaction. According to Honek (1996), the lower thermal limit of development decreases with increasing latitude. Thus, tropical species have a higher LTT, close to 13.7 °C, while temperate species have an LTT of 7.9 °C. However, for the *S. nigra* found in the tropical climate region, the LTT value for the egg stage is close to that reported for temperate climate insects. Milanez et al. (1983) recorded an LTT of 10.2 and 10.4 °C, respectively, for the pasture spittlebugs *Notozulia entreriana* Berg, 1879 and *Deois flavopicta* Stal, 1854. The present result strengthens the hypothesis that the predator *S. nigra* and pasture spittlebugs have similar thermal thresholds.

Considering a region with a monthly average temperature of 25°C, it is possible to estimate, with the current research results, a number of 1.8 days for the embryonic development of *S. nigra*. Veríssimo et al. (2018) consolidate the results obtained for thermal constant, demonstrating in their study that the average period of embryonic development of this predator under study was 2.7 days. This result is important in aiding the planning of mass rearing in the laboratory.

The present research evidenced the requirement of a daily hydration condition for eggs. These results explain why the females lay their eggs in the foam produced by the nymphs of their prey (spittlebugs), in which a microclimate is formed that reduces the risk of the predator's eggs drying out or overheating (Whittaker, 1970; Zedadra, 2019). According to Amorós-Jiménez et al. (2012), the eggs and larvae of the hoverfly *S. rueppellii* are susceptible to low RH. Similar results are observed in the present study, in which the hydration restriction promoted 100% embryo mortality.

Thus, the results of the present research make clear the thermal and hydration limitations for mass rearing of *S. nigra*. However, we must take into account that these insects have strategies to overcome adverse conditions for their development. This is confirmed in the observation that female *S. nigra* lay their eggs in the foam produced by the spittlebug nymphs, their main prey, a strategy to keep their eggs in an environment without risk of overheating. In addition, the foam provides constant moisture for the embryonic

development process, meeting the predator's requirement. In this context, it should be noted that the pest's peak of development coincides with the rainy season, which certainly favours the viability of the predator's eggs, which, according to the results of this research, require daily hydration.

Artificial diets have been evaluated for rearing adult *S. nigra*. An important factor for improving natural enemies is the artificial diet for adults, due to both its cost effectiveness and easy preparation and its positive effects on the biological parameters of this species of hoverfly compared to its natural diet. Since adult common hoverflies (*S. nigra*) in the natural environment require nectar and pollen providing these nutritional requirements in an artificial diet in agricultural ecosystems can help establish and maintain populations of this predator.

According to Pinheiro et al. (2012), glucose enhances the longevity of *Episyrphus balteatus* De Geer, 1776 (Diptera: Syrphidae). A study by Venzon et al. (2006) with *Chrysoperla externa* Hagen, 1861 (Neuroptera: Chrysopidae) also found that diets supplemented with honey provided greater longevity. For *S. nigra*, longevity was not altered by the inclusion of yeast or inflorescences in honey-based diets. Adults of *S. nigra* fed on yeast + honey significantly provided equal production with the components of nature (inflorescences + honey) and produced significantly more eggs (2.8 times) than the diet with only honey. This result corroborates findings that, based on research carried out by Hagen (1950), that diets containing only carbohydrates were not nutritionally suitable for oviposition, making it necessary to use a mixture of hydrolyzed yeast and honey. It is believed that the constituents present in yeast (50% protein, 31.5% carbohydrates, 6% fat, 6%–8% nucleotides, 7%–8% minerals and vitamins) (Reed & Nagodawithana, 1991) contributed to the higher fecundity of *S. nigra* in the current study. Zucoloto (1987) and Chan et al. (1990) report that these constituents contribute to the development of *Ceratitis capitata* Wiedemann, 1824 (Diptera: Tephritidae). The importance of the protein constituent associated with carbohydrate sources in the artificial diet for laboratory rearing of the predator (*S. nigra*) is evident.

Conclusion

For the mass rearing of the predator *S. nigra*, it is recommended to keep the eggs in the range of 20–30 °C with a daily hydration regimen. In addition, the artificial diet of yeast + honey is suitable for mass rearing this predator.

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Challenges in artificial diet formulation and larval rearing of *Salpingogaster nigra* (Diptera: Syrphidae)

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Abstract

This study evaluated larval rearing methodologies for *Salpingogaster nigra* (Diptera: Syrphidae), an important predator of spittlebugs, aiming to support its application in biological control programmes. Artificial and natural diets were assessed using four rearing methods under laboratory and greenhouse conditions. Artificiais diets did not support development to the pupal stage, and high larval mortality occurred even when natural prey was provided. These findings indicate substantial limitations for mass rearing of *S. nigra* under controlled conditions, highlighting the need for further methodological refinement and the importance of reporting negative results to inform future research.

Keywords: biological control; mass rearing; artificial diet; Syrphidae; spittlebugs

The increasing demand for sustainable production systems, combined with progressively stricter restrictions on the use of chemical pest control agents, has intensified the search for alternative pest management strategies (Ragland et al., 2008). In this context, biological control has emerged as a promising option.

In certain situations, natural enemies can establish stable populations capable of tracking fluctuations in target species and adapting to prevailing local environmental conditions; however, the success of biological control programmes increasingly relies on the mass rearing of insects under laboratory conditions, which allows the maintenance of viable populations and the large-scale release of effective and highly specific control agents.

The syrphid fly *Salpingogaster nigra* Schiner, 1868 (Diptera: Syrphidae) has emerged as an important biological control agent of spittlebugs (Veríssimo et al., 2018), pests responsible for significant losses in intensive livestock systems. This predatory species is widely distributed in Brazil (Guagliumi, 1968), exhibits high fecundity and a short life cycle (Veríssimo et al., 2018), allowing two to three generations to occur during the life cycle of its prey, in addition to showing efficient predatory specificity in pasture environments. Paéz et al.

(1986) reported that, after monitoring 24 larvae of *S. nigra*, each larva consumed an average of 18 prey nymphs.

Much of the accumulated knowledge on the biology of this predatory insect has resulted from transferring individuals from the field to laboratory conditions, where they were maintained under controlled environments and fed natural diets during the larval stage and alternatives diets during adulthood (Veríssimo et al., 2024), allowing the establishment of small laboratory colonies. However, the low larval viability observed under laboratory conditions still limits the large-scale reproduction of *S. nigra*. Therefore, the objective of this study was to evaluate alternative methodologies to optimise larval survival of this species under non-natural conditions, aiming at its potential application in integrated spittlebug management programmes.

Adults of *S. nigra* and nymphs of the prey *Mahanarva spectabilis* Distant, 1909 (Hemiptera: Cercopidae), were collected at the José Henrique Bruschi Experimental Field, in Coronel Pacheco, Minas Gerais, Brazil, and transported to the entomology laboratory of Embrapa Dairy Cattle, in Juiz de Fora, Minas Gerais. The insects were placed in acrylic cages measuring 80 × 55 × 55 cm and fed with flowers of Parthenium hysterophorus and Emilia fosbergii and honey + water (2:1). Oviposition occurred within the foam produced by prey nymphs. Eggs were subsequently individualised until close to larval eclosion, after which larvae were fed different diets under distinct rearing methods.

For an initial selection of a promising diet for *S. nigra*, two alternatives diets based on Salles (1992) for *Anastrepha fraterculus* were compared, using either mealworm larvae (Tenebrio sp.; diet 1) or spittlebug nymphs (diet 2) as protein sources (Table 1). Mealworms and spittlebug nymphs were killed by freezing, dehydrated in an oven at 40 °C for 48 h, and subsequently ground using an analytical mill (IKA A11). Diet preparation involved homogenising lyophilised brewer's yeast, fermented wheat germ and distilled water in a blender. Methylparaben (Nipagin™, diluted to 10% in ethanol) and sodium benzoate (Vetec; dissolved in 20 mL of distilled water) were then added. Bacteriological agar (Alphatec) was dissolved in 200 mL of distilled water and heated under constant stirring until boiling. The agar was added to the blender along with the other components and the ground protein sources, and mixed for 2 minutes until homogeneous. Before solidification, the diets were poured into Petri dishes (10 cm × 2 cm), forming a 1 cm thick layer. Then, filter papers containing 15 viable predator eggs were placed on top of the diets. The eggs had been previously incubated in the laboratory at 25 °C, and near hatching, placed in 10 plates containing each diet (Fig. 1A).

As a natural diet, spittlebug nymphs were used. Second to fifth-instar *M. spectabilis* nymphs were collected from the elephant grass cultivar 'Kurumi' at the Embrapa Dairy Cattle Experimental Field and transported to the entomology laboratory. During collection, nymphs were removed from the plant base using a fine brush, placed in beakers containing roots and maintained on plants in the laboratory and greenhouse until the beginning of the experiments. Bioassays were conducted using four experimental methods that differed in container size and environmental conditions (temperature, humidity, and photoperiod), which were either controlled in the laboratory or fluctuating under greenhouse conditions.

Elephant grass seedlings were planted in 1 L plastic pots filled with soil collected from the Embrapa Dairy Cattle Experimental Field in Coronel Pacheco, totalling 10 pots. Pots were covered with voile fabric to prevent the escape of predator nymphs and prey larvae. Plants and insects were maintained either in a greenhouse (Method 1) (Fig.1B) or in the laboratory (Method 2) (Fig.1C). The third method consisted of elephant grass seedlings planted in ten 500 mL plastic pots filled with commercial substrate. Pots were covered with lids containing a central opening to allow plant growth, with a layer of gauze fixed around the opening to prevent insect escape (Fig.1D). The fourth method involved elephant grass seedlings planted in ten 500 mL plastic pots filled with soil collected from the same experimental field. After 30 days, lateral cuts of approximately 5 × 5 cm were made in the pots to create a restricted environment that facilitated observation of *S. nigra* larval development (Fig. 1E).

After 30 days of plant growth, all methods were subjected to daily infestation with pasture spittlebug nymphs. Filter papers containing 15 viable predator eggs were then placed beneath the foam produced by the prey. Predator eggs were previously incubated in the laboratory at 25 °C until close to hatching, totalling 600 eggs. Prey nymphs were replenished daily to ensure continuous food availability for larvae.

It was found that the alternative diet was unsuitable for the larval development of *S. nigra*. Although larvae fed on the diet, none reached the pupal stage. Consequently, in an attempt to better replicate field conditions, predator larvae were supplied with their natural prey, spittlebugs. However, regardless of the rearing container or whether environmental conditions were controlled (climate chambers) or fluctuating (greenhouse), only 20%, 10%, and 0% of larvae reached the pupal stage under rearing Methods 1, 2, and 3, respectively. To better understand the causes of low viability, in the method 4 maintained predator larvae and prey in smaller containers (0.5 L) to allow daily observation under a stereomicroscope. This method confirmed extremely high larval mortality, with 100% mortality observed shortly after larval eclosion.

Although rearing methods have been established for aphidophagous syrphid species (Li et al., 2023), no such methodology has yet been defined for *S. nigra*. Previous studies on this species report results based on adults collected in the field (Paez & Torres, 1984), but no larval rearing methodology has been established. The present study represents the first attempt to develop a detailed method for the mass rearing of larvae of this predator.

Preliminary studies by Paez & Torres (1985) also reported high larval mortality when natural prey (pasture spittlebugs) was provided, corroborating the results of the present research. These authors additionally reported the presence of nematodes attacking *S. nigra* larvae.

Low viability under controlled conditions may be related to the inadequacy of the methodologies tested, as field observations during the sampling period revealed population peaks of up to 100 individuals within two hours of active searching in areas heavily infested with spittlebugs, despite climatic variability and prey availability constraints. It is also hypothesised that low viability may be associated with the high fecundity of females (approximately 25 eggs per day; Veríssimo, 2018).

Nevertheless, as this represents the first study in Brazil evaluating artificiais diets and larval rearing methodologies for *S. nigra*, the results remain preliminary, and adjustments to the physical characteristics of the diet may be explored in future studies. For such refinements to be effective, it is essential that studies reporting unexpected or negative results be published, as these provide valuable information to guide subsequent experiments towards improved outcomes. According to Cohen (2018), negative results often do not arise from experimental design failures. Thus, by transparently reporting the limitations and challenges encountered in the larval rearing of *S. nigra*, this study not only reinforces the relevance of this species as a biological control agent of spittlebugs but also contributes to the scientific recognition of underreported data. These findings provide an essential foundation for future research aimed at developing more efficient methodologies for the mass production of this important predator.

It is evident that both alternative and natural diets tested exhibited significant limitations in supporting larval development of *S. nigra*, demonstrating that, to date, laboratory rearing of this predator is not feasible. Its practical use currently relies on the collection of adults from the field and their maintenance in the laboratory on artificiais diets, as described by Veríssimo et al. (2024), as well as on maintaining eggs under optimal conditions defined by the same authors. This approach allows the inoculative release of eggs with the aim of enhancing biological control efficacy under field conditions.

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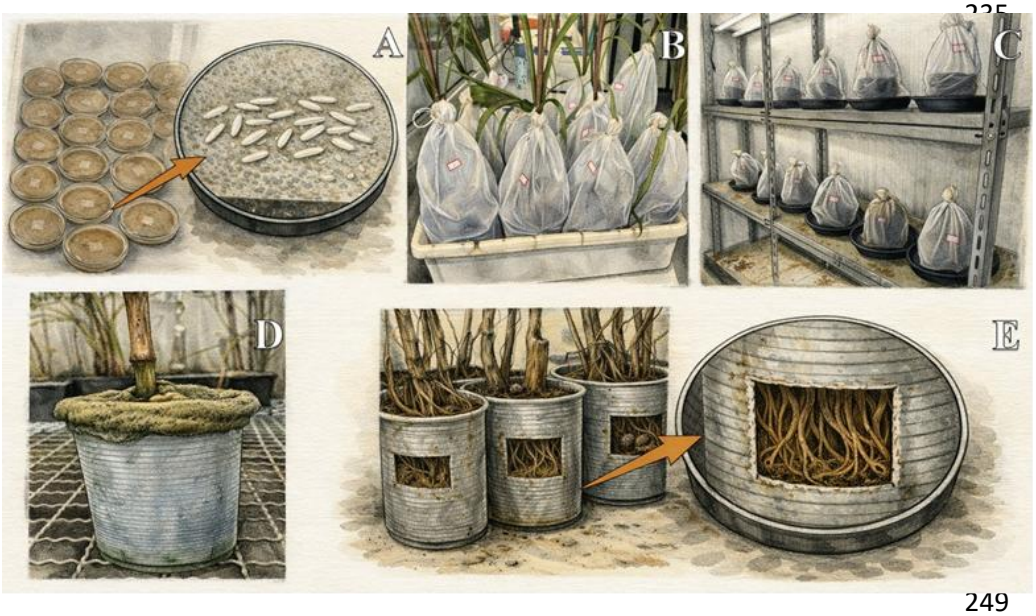
Table 1. Components of the artificiais diets used for larval development of *Salpingogaster nigra* under laboratory conditions, based on Salles (1992) for Anastrepha fraterculus.

Ingredients	**Diet 1**	**Diet 2**
Brewer's yeast	60 g	60 g
Wheat germ	60 g	60 g
Distilled water	800 ml	800 ml
Agar	20 g	20 g
Sodium benzoate	1 g	1 g
Nipagin	8 g	8 g
Mealworm meal	100 g	-
spittlebugs meal	-	100 g

Figure.1: A-Viable eggs on artificial diet; B-Elephant grass pots infested with eggs of the predator *Salpingogaster nigra*, covered with voile fabric, kept in a greenhouse; C-Pots maintained in a fitotron-type climatic chamber at 25 ± 2 °C, 70 ± 10% relative humidity, and a 12 h photophase; D-Plastic pots 500 ml covered with gauze; E-Pots with lateral openings of approximately 5 × 5 cm.



A new species of *Salpingogaster* Schiner, 1868 (Diptera: Syrphidae) from Brazil, a potential control agent of spittlebugs (Hemiptera: Cercopidae)

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Abstract

A new species of *Salpingogaster* is described from southern Brazil, *S. distincta* Mengual, Veríssimo & Auad sp. nov. The new species is very similar to the widespread *S. nigra* Schiner. We describe the new species in full and provide images and DNA barcodes of the closest species to differentiate them. Moreover, RAPD analysis was performed to test its value to distinguish *Salpingogaster* taxa. Larvae and adults of the new species were collected in fields of elephant grass, *Cenchrus purpureus* (Schumach.) Morrone.

Keywords: Flower flies, hover flies, forage grass, DNA barcoding, RAPD analysis.

Introduction

Flower flies (Diptera: Syrphidae), also known as hover flies, occur in all biogeographic regions except Antarctica (Thompson et al., 2010). The family includes over 6,500 species distributed across approx. 210 genera (Thompson et al., 2010; Evenhuis and Pape, 2025). The greatest species richness is found in the Neotropical Region, with approximately 1,660 known species and 124 genera. This number may be considerably higher due to the lack of studies in several areas of South America, where many species remain undescribed (Thompson et al., 2010; Carvalho-Filho et al., 2019).

Syrphid adults are important pollinators in agro- and natural ecosystems (Inouye et al., 2015; Rader et al., 2016, 2020; Ssymank et al., 2008; Ssymank and Kearns, 2009) as they feed on pollen and nectar. Further, their larvae present a very diverse array of natural histories: there are saprophagous (consume decaying organic matter), phytophagous (consume geophyte plant tissues), or microphagous (aquatic species that live in ponds and streams and consume debris and bacteria) (Rotheray and Gilbert 2011). In addition, some larvae are predators of phytophagous insects with potential use as biological control agents (Arcaya et al., 2017; Bellefeuille et al., 2019; Moerkens et al., 2021; Nelson et al., 2012; Tenhumberg, 1995), including those of the genus *Salpingogaster* Schiner, 1868 (Rojo et al., 2003; Veríssimo et al., 2018).

Salpingogaster occurs from the southern U.S.A. to southern Brazil and northern Argentina and comprises 35 described species, although there are several undescribed taxa for this genus (Thompson et al., 2010). Members of the genus *Salpingogaster* are easy to identified among other Syrphinae based on the following morphological characters: postpronotum bare, wing vein M1 abruptly and strongly sinuate, wing vein R4+5 strongly sinuate, metafemur with distinct rows of spinose setae on apical half, and abdominal tergite 1 produced laterally into a strong spina (Thompson et al., 2010; Mengual et al., 2018).

All known larvae of *Salpingogaster* feed on spittlebugs (Hemiptera: Cercopidae) (Páez et al., 1985; Rojo et al., 2003; Lastra et al., 2007) and one of the most common species, *Salpingogaster nigra* Schiner, 1868, is a predator of spittlebugs on sugar cane and other grasses of the family Poaceae (Rojo et al., 2003; Thompson et al., 2010; Veríssimo et al., 2018).

Despite their broad distribution and importance as effective natural enemies of spittlebugs pests (Guagliumi, 1971; Koller, 1988; Ramos Marques, 1988; Castro et al., 2005), the genus is in urgent need of a taxonomic revision, as the last identification key was published by Curran (1941). Curran (1941) included the 27 known species at the time,

including those of the genus *Eosalpingogaster* Hull, 1949. At one time considered a subgenus of *Salpingogaster*, *Eosalpingogaster* feeds mainly on scale insects (Hemiptera: Coccoidea) (Rojo et al., 2003; Pérez-Bañón et al., 2013) and it was recently revised (Mengual and Thompson, 2011).

Salpingogaster nigra is likely the species within the genus with the broadest distribution, so far recorded from central Mexico, West Indies, south to Peru, Bolivia, Brazil and northern Argentina (Thompson, 1981; Maza et al., 2023). The morphology of the immature stages (egg, larva and pupa) of *S. nigra* was superficially described by Guppy (1913) and in more detail by Páez et al. (1985), and the puparium was described in detail by Pérez-Bañón et al. (2013). Moreover, the potential of the genus as biological control agent has been mostly studied using this species (see Guppy, 1914, 1915; Pickles, 1932, 1933, 1938; Ramos, 1978, 1984; Sotelo and Cardona, 2001). Unfortunately, its potential as pest biological control agent is reduced by encyrtid parasitoids (Hymenoptera: Encyrtidae) (de Santis and de Sureda, 1988) and by the maintenance of the insect colonies (immatures and adults) in captivity (Lastra et al., 2007; Veríssimo et al., 2018).

The search for *Salpingogaster nigra* specimens to study their potential as control agents of spittlebugs, which are the main pest of forage grasses (Poaceae) throughout Latin America (Alvarenga et al., 2017), led to the discovery of a new *Salpingogaster* species that feeds on spittlebug nymphs. In this study, we aim to describe this new species and characterize it morphologically and molecularly, to facilitate its determination and to promote studies on its biology and potential as biological control agents of pests.

Material and methods

Sampling

During the seasonal occurrence of spittlebugs (September to March), second and third instar larvae were collected manually from the base of elephant grass *Cenchrus purpureus* (Schumach.) Morrone clumps, as well as adults belonging to the genus *Salpingogaster* using an entomological hand-net in an infested area with *Mahanarva spectabilis* (Distant, 1909) (Hemiptera: Cercopidae) in the experimental field of Embrapa Gado de Leite in Coronel Pacheco (21.5495° S 43.2665° W), Minas Gerais, Brazil. The predator and prey were in an area of *C. purpureus* monoculture. The adults and larvae collected in the field were put in cages in the lab for rearing. Additional specimens of *M. spectabilis* were collected from greenhouses to feed the colonies.

Morphology and photography

Morphological terminology follows Van Steenis et al. (2023). Thompson (1999) and Mengual et al. (2018) were used to identify the genus of the adults, and Pérez-Bañón et al. (2013) for the identification of the larvae. The morphological study was undertaken with a Leica® M165C stereomicroscope equipped with a camera lucida. Photographs of pinned specimens were taken with a Canon EOS 7D® camera mounted on a P–51 Cam-Lift (Dun Inc., VA, U.S.A.) and by use of Adobe Lightroom ® ver. 26.2. These images were then focus stacked with the software Helicon Focus ver. 8.3 (HeliconSoft, Kharkiv, Ukraine). Later, the stacked images were edited with Adobe Photoshop ® ver. 26.2.

All body measurements were taken using a reticule in a Leica® M165C microscope. Body length was measured from the anterior oral margin to the posterior end of the abdomen, in lateral view. Wing length was measured from the wing tip to the basicosta.

Specimens were softened in a wet chamber for 24 hours prior male genitalia were dissected. The terminalia were then immersed in a 10% KOH solution for 10 minutes at 56 °C, followed by a 15-minute bath in purified water and another 10 minutes in 96% ethanol before they were stored in a microvial with glycerine.

The material studied is deposited in the following institutions:

CNC: Canadian National Collection of Insects, Arachnids and Nematodes, Ottawa, Canada.
NCB: Naturalis Biodiversity Center, Leiden, the Netherlands.
NMB: Naturhistorisches Museum, Basel, Switzerland.
ZFMK: Museum Koenig Bonn, Leibniz-Institut zur Analyse des Biodiversitätswandels, Bonn, Germany.

For studied primary types, the contents of each label are enclosed in double quotation marks (“ ”), italics denote handwriting, and the individual lines of data are separated by a double forward slash (//). Information not written on labels (i.e., remarks by authors, depository institution, unique identifiers, and sex) is given in square brackets ([ ]) and the ellipsis (...) indicates that the missing information is the same as that in the preceding record.

DNA barcoding

One or two legs of selected specimens were used for DNA extraction. Besides specimens of the new species, we also newly sequenced specimens of other *Salpingogaster* species. The protocol for DNA primers, as well as extraction, amplification, purification,

sequencing protocols and edition are described in Żóralski et al. (2024) and Müller et al. (2024). All new sequences were submitted to GenBank (https://www.ncbi.nlm.nih.gov/genbank/) via BOLD (https://www.boldsystems.org). GenBank accession numbers (GB) are listed for each sequenced specimen in the examined material.

Public nucleotide sequences of *Salpingogaster* available at BOLD (with more than 500 bp and without contaminants; accessed on 15 December 2025) were downloaded. A distance-based Neighbour-Joining (NJ) analysis was carried out using the Jukes-Cantor Model as implemented in the software Geneious Prime 2025.1.2. The DNA barcode of Ocyptamus dimidiatus (Fabricius, 1781) (GB: MW473993) was constrained as the root for the NJ tree to facilitate the visualization. Bootstrap support values (BS) were estimated from 1000 replicates directly from Geneious Prime.

RAPD analysis

For the genetic studies using Random amplification of polymorphic DNA (RAPD) fifteen specimens of *S. nigra*, fifteen specimens of our new *Salpingogaster* species, and three specimens of *Allograpta exotica* (Wiedemann, 1830) as external control group were analyzed. Whole insects were subjected to DNA extraction. Four specimens from each group were used in the amplification test with RAPD primers to select the best option based on the sharpness and repeatability of the alleles. Six RAPD primers (Operon Technologies, Inc.) were used to amplify the DNA samples. The polymerase chain reaction cycling parameters consisted of an initial temperature of 94 °C for 5 min, followed by 45 cycles of 1 min at 94 °C, 1 min at 36 °C and 2 min at 72 °C, with a final extension for 10 min at 72 °C (Applied Biosystems 9700 Thermocycler, Foster City, CA, U.S.A.). The total volume of each reaction was 25 µL, containing 10 mM Tris-HCl, 50 mM KCl, 2.5 mM MgCl2, 0.1% Triton X-100, 0.4 µM primer, 0.1 mM each dNTP, 1 U Taq DNA polymerase, and 45 ng of genomic DNA. An agarose gel procedure was then carried out to separate the alleles and identify the variation among *Salpingogaster* specimens.

All reactions with the selected primers were carried out in duplicate. Only highly intense and reproducible fragments were included in the analysis, as recommended by Pérez et al. (1998). The RAPD amplification products were separated on a 2% agarose gel, stained with ethidium bromide and photographed under ultraviolet light. A binary matrix was constructed according to the presence (1) or absence (0) of alleles between the specimens. Data were analyzed using NTSYSpc software, version 2.02 (Rohlf, 1992). Similarities among

458 individuals were estimated using Jaccard coefficients similarity and the dendrogram was
459 produced according to unweighted pair-group method via arithmetic averages (UPGMA).
460
461 Results
462 Adults and larvae collected in the field were identified as an undescribed species of
463 *Salpingogaster*. This undescribed species was already known by the first author (XM) from
464 different entomological collections and previous studies, and we describe it here.
465
466 *Salpingogaster distincta* Mengual, Veríssimo & Auad sp. nov.
467 *Salpingogaster* sp.1 of Medeiros et al. (2019): Supplementary material.
468 *Salpingogaster* sp. of Medeiros et al. (2019): Figure 4.
469
470 urn:lsid:zoobank.org:act:xxx
471 Figs. 1, 2, 3e, 5, 6.
472
473 Differential diagnosis. *Salpingogaster distincta* sp. nov. keys out to *S. nigra* in the key
474 by Curran (1941). Both taxa, *S. nigra* and *S. distincta* sp. nov., differ from all other described
475 *Salpingogaster* species by the following combination of characteristics: yellow face with a
476 medial black vitta (Figs. 1b, 1f, 3c, 3d); frons (female) and frontal triangle (male) with an
477 anterior black macula that reaches the eye margins laterally; posterior half of the scutum
478 entirely black (Figs. 1a, 1e, 3a); scutellum black on the disc with anterior and posterior
479 margins yellow; wing membrane bicolor (hyaline on the posterior half and light brown to
480 black on the anterior margin); abdominal tergite 3 light to dark brown with two subbasal
481 narrow elongate yellow maculae (Figs. 1c, 1d, 3b); and pleuron mainly black (katatergum
482 always black), with or without yellow markings. Our new species differ from *S. nigra* by
483 having the wing cells br, bm and cua entirely microtrichose (largely bare in *S. nigra*); alula
484 narrow (Fig. 1a), as narrow as or narrower than cell c, linear and at least apically
485 microtrichose (in *S. nigra* the alula is triangular (Fig. 3a), much broader apically than basally,
486 bare and broad, as broad as cell bm in the middle point); notopleuron mostly black (Figs. 1c,
487 1d), with a small yellow macula at the transverse sulcus (notopleuron yellow in *S. nigra*; Figs.
488 3b, 3d); and posterior anepisternum and katepisternum with a narrow yellow fascia (Figs. 1c,
489 1d), narrower than half the width of the posterior anepisternum (in *S. nigra* the yellow fascia
490 on posterior anepisternum and katepisternum is broader (Figs. 3b, 3d), as broad as half the
491 width of the posterior anepisternum). Moreover, males of both species differ in the

morphology of sternite 4 (with two medial processes very close to each other in *S. distincta* sp. nov. (Fig. 3e) and two lateral processes well-separated in *S. nigra* (Fig. 3f)) and tergite 8 (rounded apically in *S. distincta* sp. nov. and pointed posteriorly in *S. nigra*).

Salpingogaster punctifrons Curran, 1929 occurs in Florida (U.S.A.) and Cuba. It is very similar to *S. nigra*, with minor differences in the body coloration and male genitalia (Thompson, 1981). *Salpingogaster distincta* sp. nov. differs from *S. punctifrons* by the same characteristics as it differs from *S. nigra* and the coloration of the abdomen and pleura: black in *S. distincta* sp. nov. and rusty red in *S. punctifrons*. Additionally, in *S. distincta* sp. nov. the frons (female) and frontal triangle (male) have an anterior black macula that reaches the eye margins laterally, while in *S. punctifrons* the anteromedial reddish brown to dark brown macula is isolated from the eyes by a yellow vitta along the eye margin (as an example, see https://www.inaturalist.org/observations/37358902).

Salpingogaster distincta sp. nov. is most similar to *S. diana* Hull, 1943, as both taxa share a yellow face with a medial black vitta; frons (female) and frontal triangle (male) with an anterior black macula that reaches the eye margins laterally; posterior half of the scutum entirely black; scutellum black on the disc with anterior and posterior margins yellow; wing membrane bicolor (hyaline on the posterior half and light brown to black on the anterior margin); wing cells br, bm and cua entirely microtrichose; alula narrow, as narrow as or narrower than cell c, linear and apically microtrichose; notopleuron mostly black; and pleuron mainly black (katatergum always black), with or without yellow markings. Our new species differ from *S. diana* by having tergite 3 light brown on anterior margin with two subbasal narrow elongate yellow maculae and dark brown to black posterior to the yellow maculae, at least the posterior half of the tergite 3 is dark brown to black, not contrasting with the color of tergite 4 (Figs. 1a, 1c-e). In *S. diana*, tergite 3 is light brown, similar in color to tergite 2, with a yellow posterior margin, contrasting with a black tergite 4 (Figs. 4a-c). Moreover, in *S. distincta* sp. nov. the tergite 2 is almost parallel-sided, slightly broadening progressively towards the posterior margin (Figs. 1a, 1e); while in *S. diana*, tergite 2 has a bulge or protuberance on the posterior half (Figs. 4a-c). In addition, males of both species differ in the morphology of sternite 4: with two medial processes very close in *S. distincta* sp. nov. (Fig. 3e) and two lateral processes well separated in *S. diana* (Fig. 3g).

Description. Body length (n = 5): 18.2 mm (16.0–20.0 mm); wing length (n = 5): 12.3 mm (11.0–13.0 mm).

MALE.

Head (Figs. 1a, 1b). Face with a well-defined tubercle, not produced anteriorly nor ventrally. Face with yellow pile, yellow with a medial broad black vitta from antennal fossa to oral margin, which may become brown mesolaterally. Malar area darker, dark brown to black, from facial sulcus to oral margin. Gena yellow with brown areas. Frontal prominence present. Frontal triangle brown pilose, yellow along the eye margin, largely shiny black medially, with a black marking that reaches the eye margin at the lateral of the frontal prominence and extends a bit ventrally; frontal triangle slightly whitish pruinose posterior to transverse frontal sulcus. Lunule yellow with a medial isolated black macula. Head holoptic; eyes bare. Antenna yellow to brown, brown pilose; postpedicel paler apically, with a dorsal subbasal bare arista. Vertical triangle black pilose, black, black pruinose with some white to brown pruinosity posterolaterally. Occiput with pruinose, with whitish yellow pile on ventral 3/4 and black pile on dorsal 1/4. Occipital pile reduced to a single row dorsally.

Thorax (Figs. 1a, 1c). Scutum with short sparse brown and black pile, mostly black except for the yellow bare postpronotum and a narrow yellow fascia on notopleuron close to the transverse sulcus; postalar callus black with some paler areas. Scutum black or dark brown pruinose medially, with two submedial paler (whitish yellow) pruinose vittae that do not reach the posterior margin of the scutum; scutal pruinosity also a bit paler laterally. Scutellum with short black setulose pile, brown to black with the anterior and posterior margins yellow. Pleuron black with white pruinosity, except for a narrow (less than half the width of the posterior anepisternum) yellow vitta along the posterior margin of the posterior anepisternum and dorsal half of the katepisternum and a couple of paler areas (hard to see): a linear marking on the meron and a small marking on the metapleuron. Metasternum bare. Calypters reduced, yellow; plumule absent. Halter and spiracular fringes yellow. Legs: simple. Coxae black. Pro- and meso troachnter brown; metatrochanter black. Femora yellow with undefined brown areas, mostly ventrally; in the metafemur the brown coloration is more extense. Tibiae with orangish yellow and black pile; metafemur with short black setulae ventrally. Tibiae yellow pilose, yellow; metatibia with medial brown ring. Tarsomeres orange to light brown; apical tarsomeres paler. Wing: hyaline on the posterior 1/2, yellow to brown on the anterior 1/2. Entirely microtrichose. Alula narrow (Fig. 1a), as narrow as or narrower than wing cell c, linear and microtrichose.

Abdomen (Figs. 1a, 1c, 3e). Petiolate, unmargined. Tergite 1 produced laterally into a spina; tergite 2 cylindrical, approx. 8 times longer than wide; tergite 3 broadening posteriorly, with the posterior margin more than 3 times broader than the anterior margin. Tergite 1

brown, yellow laterally, with a central black macula. Tergite 2 light brown to brown, with two lateral elongate yellow maculae on the anterior margin. Tergite 3 light brown to brown on the anterior 1/3, up to the lateral oblique elongate yellow maculae, dark brown to black on posterior 2/3, with the posterior margin yellow medially (yellow color does not reach the lateral margin of the tergite on the posterior margin). Tergite 4 dark brown to black. Tergite 5 dark brown to black with two submedial yellow maculae on the posterior margin. Sternite 1 yellow with a medial black macula. Sternite 2 light brown to brown, partly yellow on posterior 1/3. Sternite 3 dark brown to black, yellow on anterior and posterior margins. Sternite 4 dark brown to black, the posterior margin with two medial processes very close to each with the apex pointing dorsally. Sternite 8 yellow with a brown fascia, rounded apically.
Variation. Holotype male is a bit paler, in general, than the paratype males, likely to the preparation process with a Critical-Point Dryer. Paratypes from Coronel Pacheco are a bit darker: black macula on the frontal triangle meets centrally the medial black macula on the lunule and joins ventrally the medial facial black vitta surrounding the antennal fossa; gena and mala more extensively black. Darker specimens have the antennal segments brown and the arista brown apically (almost entirely yellow in the holotype).

FEMALE (Figs. 1d-f). Similar to male except for normal sexual dimorphism and as follows: frontal prominence like in the male, with frons dorsal or posterior to the transverse frontal sulcus black medially, yellow along the eye margins with two yellow extensions towards the medial line at the transverse frontal sulcus and anterior to the anterior ocellus.

Etymology. The species name is derived from the Latin distinctus meaning separate, different (Brown, 1956: 274). Species epithet is to be treated as adjective.

Type locality. Brazil: Minas Gerais, Ijaci, Fazenda Exp. UFLA, CDTT, 21.16528° S 44.9178° W.

Examined material. Holotype male, pinned, with the two metalegs and the left mesoleg glued on the label, currently deposited in the ZFMK and labelled: “BRASIL, Minas Gerais, Ijaci, // Fazenda Exp. UFLA. // 21°09'55"S 44°55'04"W // 19-27.v.2016 / Malaise trap // Morales, M.N. (leg.)” “ZFMK DIP // 00083298” [QR code] “HOLOTYPE // *Salpingogaster* // *distincta* // Mengual et al. 2026” [red].

Paratypes. BRAZIL: same locality as the holotype, 31.iii–7.iv.2016, ... [1♀, ZFMK; ZFMK-DIP-00083287]; same locality as the holotype, 6–12.v.2016, ... [1♀, ZFMK; ZFMK-DIP-00083285]; Minas Gerais, Coronel Pacheco, experimental field of Embrapa Gado de Leite, 21.5495° S 43.2665° W, 417 m., 15.xii.2022, leg. B. Veríssimo [2♂ 2♀, ZFMK; ZFMK-DIP-00084903, ZFMK-DIP-00107338, ZFMK-DIP-00084902, ZFMK-DIP-00107339]; ..., 28.xii.2022, ... [2♀, ZFMK; ZFMK-DIP-00084904, ZFMK-DIP-00107340]; ..., v.2017, ... [1♂ 2♀, ZFMK; ZFMK-DIP-00107341, ZFMK-DIP-00107342, ZFMK-DIP-00107343]; São Paulo, Espírito Santo do Pinhal, Pinhalzinho site, forest edge, 900 m., 22.135° S 46.7103° W, 20–26.vi.2016, Malaise trap, leg. H.R. Medeiros [1♀, ZFKM; ZFMK-DIP-00114780]; ..., 19–25.vii.2016, ... [1♂, ZFMK, ZFMK-DIP-00114785]; ..., 23–29.ix.2016, ... [1♀, ZFKM; ZFMK-DIP-00114779]; ..., 23.ii–2.iii.2017, ... [1♀, ZFKM; ZFMK-DIP-00114781]; ..., 25–31.iii.2017, ... [1♂, ZFKM; ZFMK-DIP-00114787]; ..., forest, ..., 13–19.xii.2016, ... [1♀, ZFKM; ZFMK-DIP-00114786]; ..., coffee plantation, ..., 21–27.x.2016, ... [1♀, ZFKM; ZFMK-DIP-00114782]; São Paulo, Espírito Santo do Pinhal, Pinhal Lake, forest, 890 m., 22.14556° S 46.73167° W, 20–26.vi.2016, Malaise trap, leg. H.R. Medeiros [1♀, ZFMK; ZFMK-DIP-00114794]; São Paulo, Espírito Santo do Pinhal, Seu Luiz site, forest edge, 720 m., 22.16° S 46.83472° W, 22–28.viii.2016, Malaise trap, leg. H.R. Medeiros [1♂, ZFMK; ZFMK-DIP-00114795]; São Paulo, Espírito Santo do Pinhal, Nova Cintra site, coffee plantation, 840 m., 22.21472° S 46.765° W, 25–31.iii.2017, Malaise trap, leg. H.R. Medeiros [1♂ 1♀, ZFMK; ZFMK-DIP-00114792, ZFMK-DIP-00114793]; São Paulo, São João da Boa Vista, Laureano site, forest, 755 m., 22.0803° S 46.8156° W, 23–29.i.2017, Malaise trap, leg. H.R. Medeiros [1♀, ZFMK; ZFMK-DIP-00114783]; São Paulo, São João da Boa Vista, Refugio site, forest edge, 1020 m., 21.9925° S 46.69° W, 23–29.i.2017, Malaise trap, leg. H.R. Medeiros [1♀, ZFMK; ZFMK-DIP-00114789]; ..., coffee plantation, ... [1♀, ZFMK; ZFMK-DIP-00114791]; São Paulo, Águas da Prata, Quartel Site, forest, 1200 m., 21.9572° S 46.6883° W, 23–29.i.2017, attractive trap, leg. H.R. Medeiros [1♀, ZFMK; ZFMK-DIP-00114788]; São Paulo, Águas da Prata, Maique river, forest, 960 m., 21.95361° S 46.70639° W, 25–31.iii.2017, Malaise trap, leg. H.R. Medeiros [1♂, ZFMK; ZFMK-DIP-00114797]; São Paulo, Águas da Prata, Andre site, forest edge, 1160 m., 21.89611° S 46.71778° W, 23.ii–.iii.2017, Malaise trap, leg. H.R. Medeiros [1♀, ZFMK; ZFMK-DIP-00114798]; São Paulo, Águas da Prata, Maique yellow, coffee plantation, 1055 m., 21.9722° S 46.7022° W, 25–31.iii.2017, Malaise trap, leg. H.R. Medeiros [1♀, ZFMK; ZFMK-DIP-00114799]; ..., forest, ..., 22–28.vi.2017, ... [1♀, ZFMK; ZFMK-DIP-00114800]; São Paulo, Santo Antônio do Jardim, Luiz Carlos site, coffee plantation, 875 m., 22.1133° S 46.7067° W, 24–30.iv.2017,

Malaise trap, leg. H.R. Medeiros [1♀, ZFMK; ZFMK-DIP-00114801]; ..., forest edge, ..., 25–31.iii.2017, ... [1♀, ZFMK; ZFMK-DIP-00114802]; Santa Catarina, Nova Teutônia, iii.1967, leg. F. Plaumann [1♀, NMB; ZFMK-DIP-00112385]; ..., 300-500 m., 27.18333° S 52.38333° W, vi.1969, ... [1♂ 1♀, CNC; Jeff_Skevington_Specimen 34130, Jeff_Skevington_Specimen 34135];..., 21.iv.1960, ... [1♂, CNC; Jeff_Skevington_Specimen 34132]; ..., iv.196[?], ... [1♂, CNC; Jeff_Skevington_Specimen 34133] [last digit of date not written in]; ..., i.1970, ... [1♂, CNC; Jeff_Skevington_Specimen 34131]; ..., ii.1970,... [1♀, CNC; Jeff_Skevington_Specimen 34134]; ..., xi.1970, ... [2♂, NCB].

Distribution. Species known from Brazil (Minas Gerais, Santa Catarina, and São Paulo).

Biology. Adults and second and third instar larvae were collected in the monoculture fields of *Cenchrus purpureus*, feeding on *Mahanarva spectabilis*. Captivity rearing was not successful using *M. spectabilis* as prey, and the entire development of the species from egg to adult was not observed due to the high mortality rate.

In the collections of the Universidade Federal do Paraná (Curitiba, Brazil) the first author found more specimens of *S. distincta* sp. nov. with the information "preying on *Aeneolamia postica*", now considered a junior synonym of *Aeneolamia contigua* (Walker, 1851) (Carvalho and Webb, 2005: 50).

Medeiros et al. (2019) collected this species in several localities from Sao Paulo at the forest edge, inside the forest, and in coffee plantations.

At the experimental field of Embrapa Gado de Leite (Minas Gerais), two other species of *Salpingogaster* were collected during our survey, *S. nigra* and *S. cothurnata* Bigot, 1884.

DNA Barcoding. Six specimens of *S. distincta* sp. nov. were successfully sequenced. All six DNA barcodes are very similar, with a percentage of similarity (or identity as calculated in Geneious Prime) between 99.582 to 100%.

In addition, we sequenced five Ecuadorian specimens of *S. diana* and individuals of *S. nigra* from Colombia (2) and Ecuador (4). A total of 170 COI sequences of *Salpingogaster* were downloaded from BOLD and the final NJ analysis comprised 188 *Salpingogaster* sequences (Fig. 5). The closest COI sequence to the barcode cluster of *S. distincta* sp. nov. (similarity: 95.552–95.706%) belongs to an unidentified *Salpingogaster* species from Peru

(BOLD Process ID: CBG-A27516-D12). DNA barcodes of *S. nigra* differ more than 10% from those of *S. distincta* sp. nov. (similarity: 88.146–89.096%).

RAPD studies. A total of 105 alleles were detected using six RAPD markers. All of them were polymorphic among the 33 individuals studied, which indicates the high genetic variability of the specimens. Three markers were selected exclusively for *S. nigra* and *S. distincta* sp. nov. (Table 1).

The dendrogram in Fig. 6 shows the degree of closeness between the different species studied and revealed that there is no tendency to group the two *Salpingogaster* taxa together in terms of genetic similarity (9% similarity).

Discussion

Based in our integrative approach, combining molecular and morphological datasets, a new species of the genus *Salpingogaster* is described and characterized, *S. distincta* sp. nov. There are enough morphological characters to distinguish our new species from the common and widespread *S. nigra*, and we give enough detailed information to distinguish it from all other described species of this genus. Moreover, the two molecular analyses clearly supported this new taxon, and we presented RAPD markers that can be used as auxiliary tools for species identification.

Describing a single new species in a genus with several undescribed species might not be the best approach. The preference or suitability of taxonomic revisions of a supraspecific group is known, and many scientific journals prompt authors to write comprehensive monographic works. We think that the description of *S. distincta* sp. nov. is justified by the urgent need to name this taxon and explore its capabilities in the biological control of spittlebugs.

The first sampled specimen studied in our survey dates from 1960 and *S. distincta* sp. nov. was identified as a new taxon to science approximately 20 years ago. Since then, many undescribed species have been discovered, but the monographic revision has been procrastinated because of the amount of newly collected and studied specimens. We honestly think that, in this particular case, the description of a single species is justified accelerate the knowledge about the biology and pest control capabilities of *S. distincta* sp. nov. and the likely confusion or misidentification between *S. nigra* and our new species.

Spittlebugs are the main pest insects of pastures in Latin America (Schöbel and Carvalho, 2019), causing degraded pastures in Brazil (Cavalcanti et al., 2021) and an

important decrease in herd productivity: up to 74 % reduction in beef productivity and between 31 and 43 % in forage production (Congio et al., 2020). Cercopidae are also well-known pest for sugarcane and their combined damage on sugarcane and pasture, only in the Neotropical Region, could imply annual losses of 840 to 2100 million US dollars (Thompson, 2004). Not many syrphid species preying on spittlebugs are known. The reason might be the capacity of the cercopids to produce a cover of frothed-up plant sap that protects them against natural enemies (Nachappa et al., 2006). Besides *Salpingogaster* species, only Asarkina ericetorum (Fabricius, 1781) has been cited preying on spittlebugs nymphs (Rojo et al. 2003).

So far, the potential of the genus *Salpingogaster* as biological control agent has been studied only with *S. nigra* (e.g., Castro et al., 2005; Espitia et al., 2022), without mentioning the species diversity of this genus and the prey specificity of each species. As mentioned earlier, the study of *Salpingogaster* as control agent of pests is hampered by the poor results of the captive breeding and its parasitoids. Consequently, the discovery and description of a new *Salpingogaster* species that feed on *M. spectabilis* deserves dissemination, especially when *S. distincta* sp. nov. was collected mostly on agricultural landscapes, i.e., Fazenda Experminetal Universidade Federal de Lavras (UFLA), experimental field of Embrapa Gado de Leite, and several coffee plantations and forest edges in São Paulo. Although limited biological data are available for *S. distincta* sp. nov., the fact that the larvae of this predator were found feeding on spittlebug nymphs highlights the potential of this new predatory species as biological control agent of insect pest in forage crops.

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Contributors (CRediT)

Ximo Mengual: Conceptualization, Data curation, Formal analysis, Investigation, Resources, Visualization, Writing – original draft, Writing – review & editing. Bruno Antônio Veríssimo: Data curation, Formal analysis, Investigation, Resources, Visualization, Writing – original draft, Writing – review & editing. Milena Duarte: Writing – review & editing. Rafaella Lima Oliveira de Magalhães: Writing – review & editing. Ana Luiza Sousa Azevedo: Writing – review & editing. Alexander Machado Auad: Conceptualization, Funding acquisition, Investigation, Resources, Supervision, Writing – original draft, Writing – review & editing.

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Table 1. Oligonucleotide identification, nucleotide sequences, number of alleles analyzed and the exclusive markers for identifying *Salpingogaster nigra* and *Salpingogaster distincta* sp. nov. and their respective fragment sizes.

Primer name	Primer sequence	Number of alleles identified	Exclusive alleles	
			Species identified	Allele size (base pairs)
OPA-13	CAGCACCCAC	18	*Salpingogaster nigra*	1200
OPD-08	GTGTGCCCCA	23	*Salpingogaster nigra*	820
OPF-01	ACGGATCCTG	18	*Salpingogaster nigra*	1150
OPC-13	AAGCCTCGTC	10	*Salpingogaster distincta* sp. nov.	1100
OPE-11	GAGTCTCAGG	19	*Salpingogaster distincta* sp. nov.	270
OPB-01	GTTTCGCTCC	16	*Salpingogaster distincta* sp. nov.	590

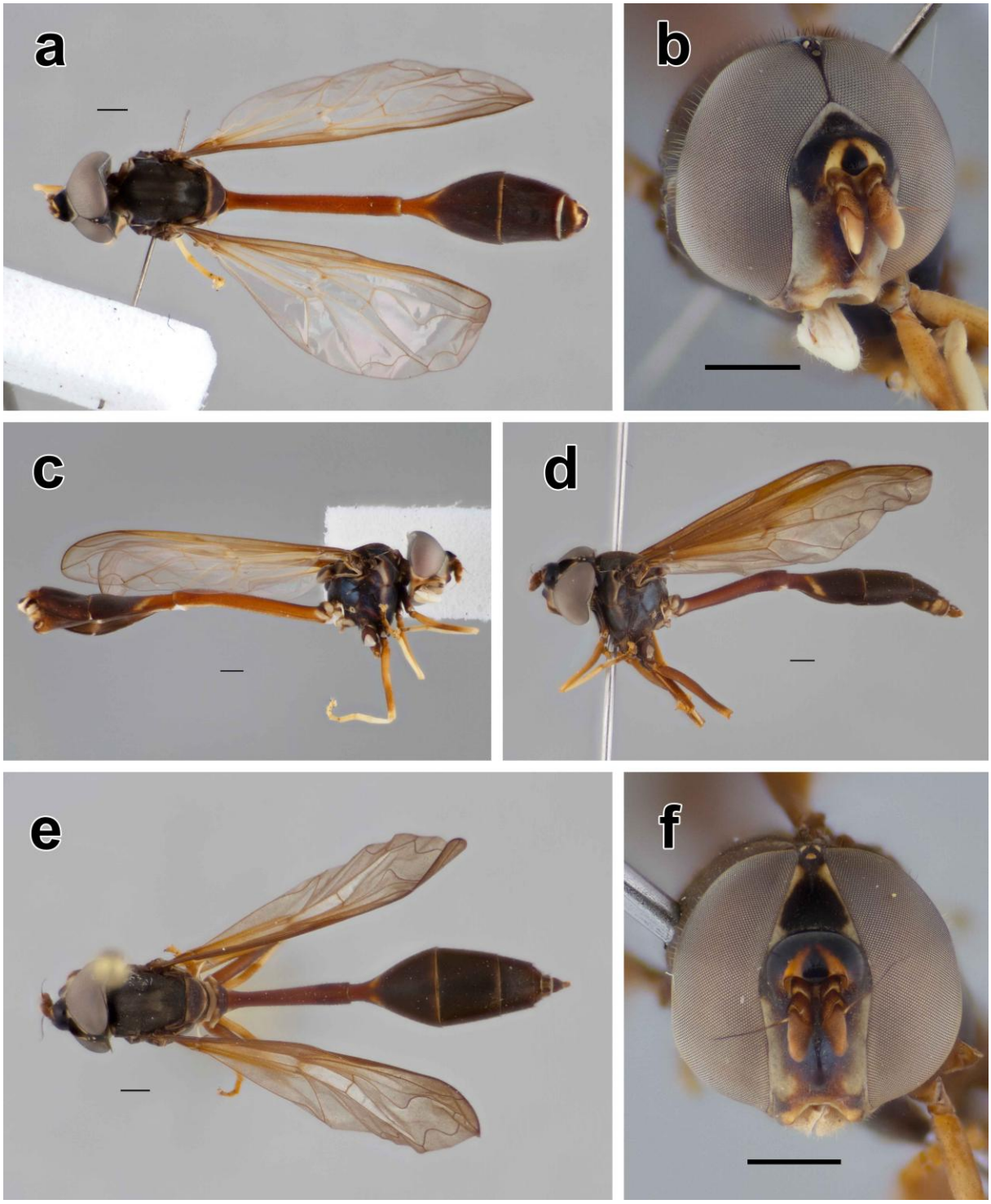


Figure 1. *Salpingogaster distincta* sp. nov., holotype male, ZFMK-DIP-00083298. (a) Habitus, dorsal view; (b) Head, frontal view; (c) Habitus, lateral view. *Salpingogaster distincta* sp. nov., paratype female, ZFMK-DIP-00114779. (d) Habitus, lateral view; (e) Habitus, dorsal view; (f) Head, frontal view. Scales = 1 mm.

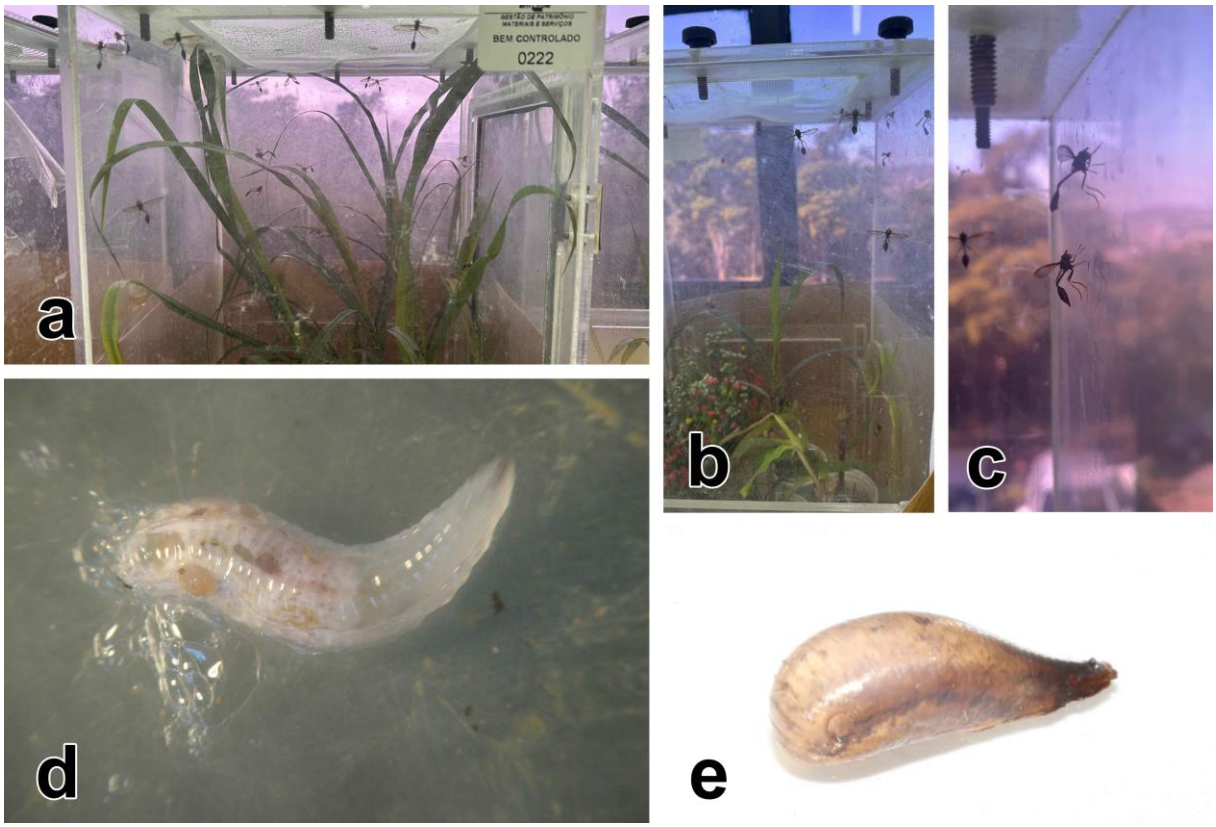


Figure 2. *Salpingogaster distincta* sp. nov. (a) Cage with adults for lab rearing; (b) and (c) Detail of the adults in captivity; (d) Larva; (e) Pupa.

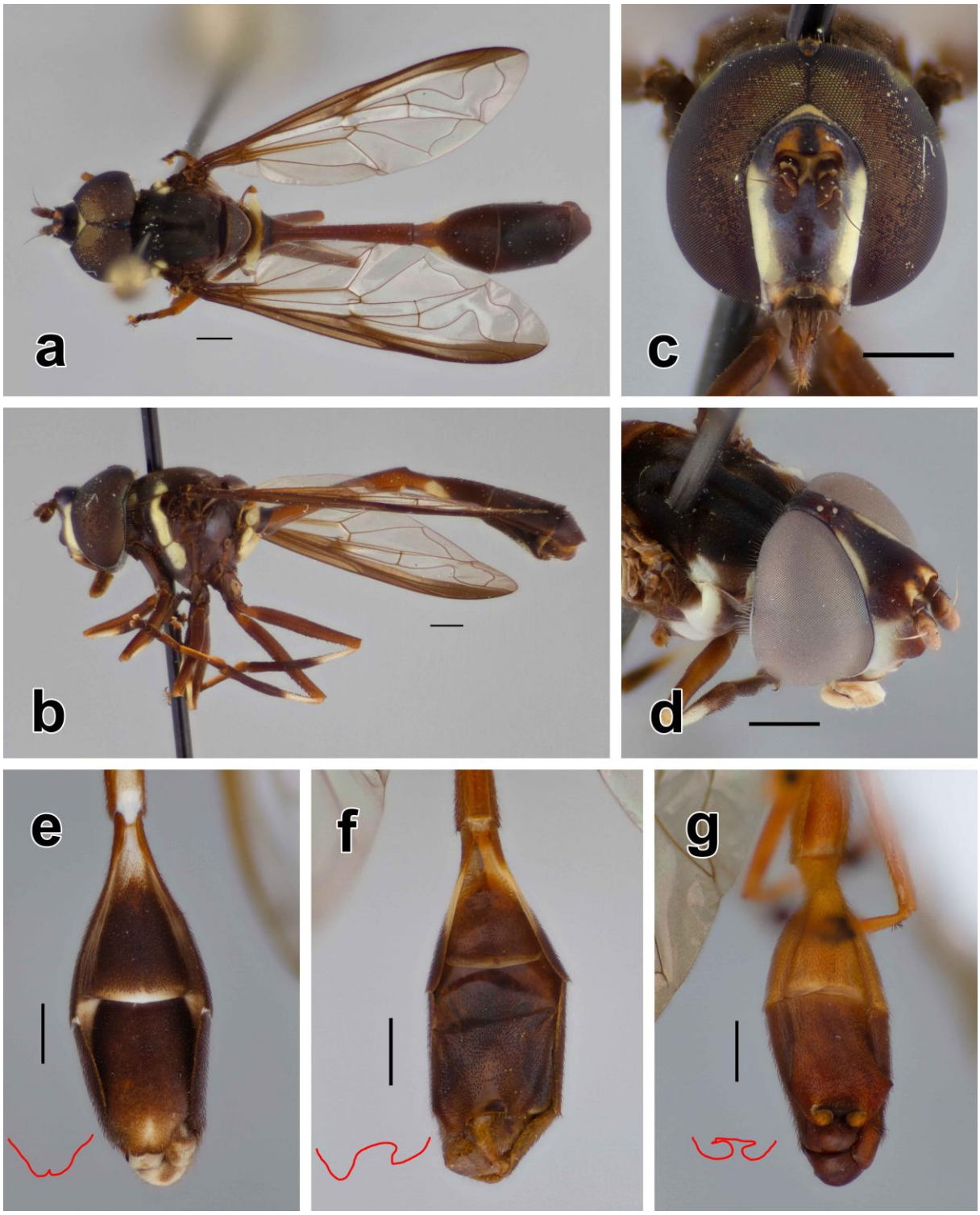
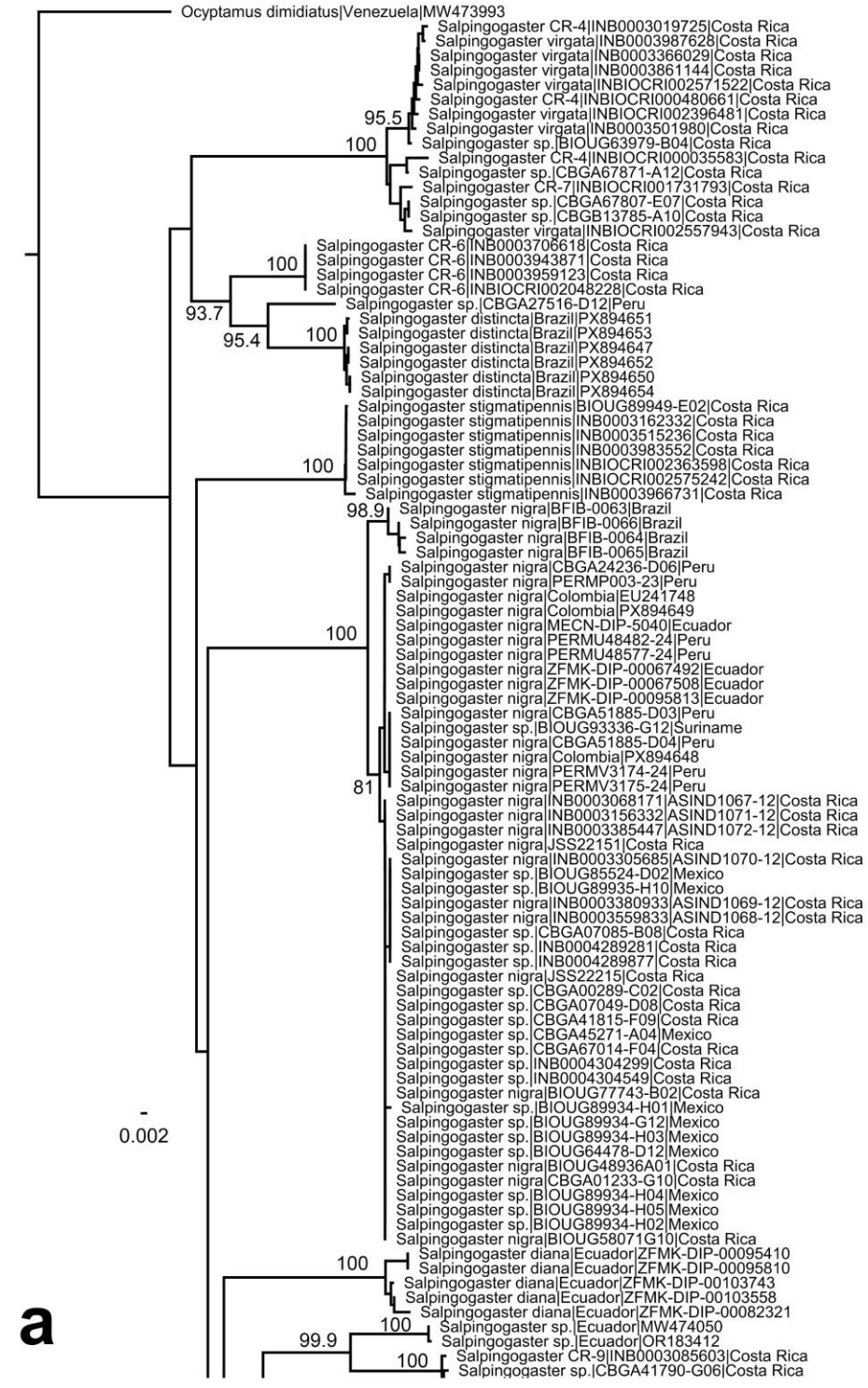


Figure 3. *Salpingogaster nigra* Schiner, 1868, male from Ecuador, ZFMK-DIP-00112389. (a) Habitus, dorsal view; (b) Habitus, lateral view; (c) Head, frontal view. *Salpingogaster nigra*, female from Brazil, ZFMK-DIP-00112386. (d) Head and thorax, frontolateral view. Abdominal sternites 3 and 4 and postabdomen; in red the profile of the posterior margin of the sternite 4. (e) *Salpingogaster distincta* sp. nov., holotype male, ZFMK-DIP-00083298; (f) *S. nigra*, male from Ecuador, ZFMK-DIP-00112387; (g) *S. diana* Hull, 1943, male from Ecuador, ZFMK-DIP-00095810. Scales = 1 mm.

Figure 4. *Salpingogaster diana* Hull, 1943, female from Ecuador, ZFMK-DIP-00103558. (a) Habitus, dorsal view; (b) Habitus, lateral view. *Salpingogaster diana*, male from Ecuador, ZFMK-DIP-00095810 (c) Habitus, dorsal view. Scales = 1 mm.


Ocyptamus dimidiatus|Venezuela|MW473993
Salpingogaster CR-4|INB0003019725|Costa Rica
Salpingogaster virgata|INB0003987628|Costa Rica
Salpingogaster virgata|INB0003366029|Costa Rica
Salpingogaster virgata|INB0003861144|Costa Rica
Salpingogaster virgata|INBIOCRI002571522|Costa Rica
Salpingogaster CR-4|INBIOCRI000480661|Costa Rica
95.5
Salpingogaster virgata|INBIOCRI002396481|Costa Rica
Salpingogaster virgata|INB0003501980|Costa Rica
100
Salpingogaster sp.|BIOUG63979-B04|Costa Rica
Salpingogaster CR-4|INBIOCRI000035583|Costa Rica
Salpingogaster sp.|CBGA67871-A12|Costa Rica
Salpingogaster CR-7|INBIOCRI001731793|Costa Rica
Salpingogaster sp.|CBGA67807-E07|Costa Rica
Salpingogaster sp.|CBGB13785-A10|Costa Rica
Salpingogaster virgata|INBIOCRI002557943|Costa Rica
Salpingogaster CR-6|INB0003706618|Costa Rica
100
Salpingogaster CR-6|INB0003943871|Costa Rica
Salpingogaster CR-6|INB0003959123|Costa Rica
Salpingogaster CR-6|INBIOCRI002048228|Costa Rica
93.7
Salpingogaster sp.|CBGA27516-D12|Peru
95.4
100
Salpingogaster distincta|Brazil|PX894651
Salpingogaster distincta|Brazil|PX894653
Salpingogaster distincta|Brazil|PX894647
Salpingogaster distincta|Brazil|PX894652
Salpingogaster distincta|Brazil|PX894650
Salpingogaster distincta|Brazil|PX894654
Salpingogaster stigmatipennis|BIOUG89949-E02|Costa Rica
Salpingogaster stigmatipennis|INB0003162332|Costa Rica
Salpingogaster stigmatipennis|INB0003515236|Costa Rica
Salpingogaster stigmatipennis|INB0003983552|Costa Rica
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Salpingogaster stigmatipennis|INBIOCRI002363598|Costa Rica
Salpingogaster stigmatipennis|INBIOCRI002575242|Costa Rica
Salpingogaster stigmatipennis|INB0003966731|Costa Rica
98.9
Salpingogaster nigra|BFIB-0063|Brazil
Salpingogaster nigra|BFIB-0066|Brazil
Salpingogaster nigra|BFIB-0064|Brazil
Salpingogaster nigra|BFIB-0065|Brazil
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Salpingogaster nigra|PERMP003-23|Peru
Salpingogaster nigra|Colombia|EU241748
Salpingogaster nigra|Colombia|PX894649
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Salpingogaster nigra|MECN-DIP-5040|Ecuador
Salpingogaster nigra|PERMU48482-24|Peru
Salpingogaster nigra|PERMU48577-24|Peru
Salpingogaster nigra|ZFMK-DIP-00067492|Ecuador
Salpingogaster nigra|ZFMK-DIP-00067508|Ecuador
Salpingogaster nigra|ZFMK-DIP-00095813|Ecuador
Salpingogaster nigra|CBGA51885-D03|Peru
Salpingogaster sp.|BIOUG93336-G12|Suriname
Salpingogaster nigra|CBGA51885-D04|Peru
Salpingogaster nigra|Colombia|PX894648
Salpingogaster nigra|PERMV3174-24|Peru
81
Salpingogaster nigra|PERMV3175-24|Peru
Salpingogaster nigra|INB0003068171|ASIND1067-12|Costa Rica
Salpingogaster nigra|INB0003156332|ASIND1071-12|Costa Rica
Salpingogaster nigra|INB0003385447|ASIND1072-12|Costa Rica
Salpingogaster nigra|JSS22151|Costa Rica
Salpingogaster nigra|INB0003305685|ASIND1070-12|Costa Rica
Salpingogaster sp.|BIOUG85524-D02|Mexico
Salpingogaster sp.|BIOUG89935-H10|Mexico
Salpingogaster nigra|INB0003380933|ASIND1069-12|Costa Rica
Salpingogaster nigra|INB0003559833|ASIND1068-12|Costa Rica
Salpingogaster sp.|CBGA07085-B08|Costa Rica
Salpingogaster sp.|INB0004289281|Costa Rica
Salpingogaster sp.|INB0004289877|Costa Rica
Salpingogaster nigra|JSS22215|Costa Rica
Salpingogaster sp.|CBGA00289-C02|Costa Rica
Salpingogaster sp.|CBGA07049-D08|Costa Rica
Salpingogaster sp.|CBGA41815-F09|Costa Rica
Salpingogaster sp.|CBGA45271-A04|Mexico
Salpingogaster sp.|CBGA67014-F04|Costa Rica
Salpingogaster sp.|INB0004304299|Costa Rica
Salpingogaster sp.|INB0004304549|Costa Rica
Salpingogaster nigra|BIOUG77743-B02|Costa Rica
Salpingogaster sp.|BIOUG89934-H01|Mexico
Salpingogaster sp.|BIOUG89934-G12|Mexico
Salpingogaster sp.|BIOUG89934-H03|Mexico
Salpingogaster sp.|BIOUG64478-D12|Mexico
Salpingogaster nigra|BIOUG48936A01|Costa Rica
Salpingogaster nigra|CBGA01233-G10|Costa Rica
Salpingogaster sp.|BIOUG89934-H04|Mexico
Salpingogaster sp.|BIOUG89934-H05|Mexico
Salpingogaster sp.|BIOUG89934-H02|Mexico
Salpingogaster nigra|BIOUG58071G10|Costa Rica
100
Salpingogaster diana|Ecuador|ZFMK-DIP-00095410
Salpingogaster diana|Ecuador|ZFMK-DIP-00095810
Salpingogaster diana|Ecuador|ZFMK-DIP-00103743
Salpingogaster diana|Ecuador|ZFMK-DIP-00103558
Salpingogaster diana|Ecuador|ZFMK-DIP-00082321
100
Salpingogaster sp.|Ecuador|MW474050
99.9
Salpingogaster sp.|Ecuador|OR183412
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-
0.002
a

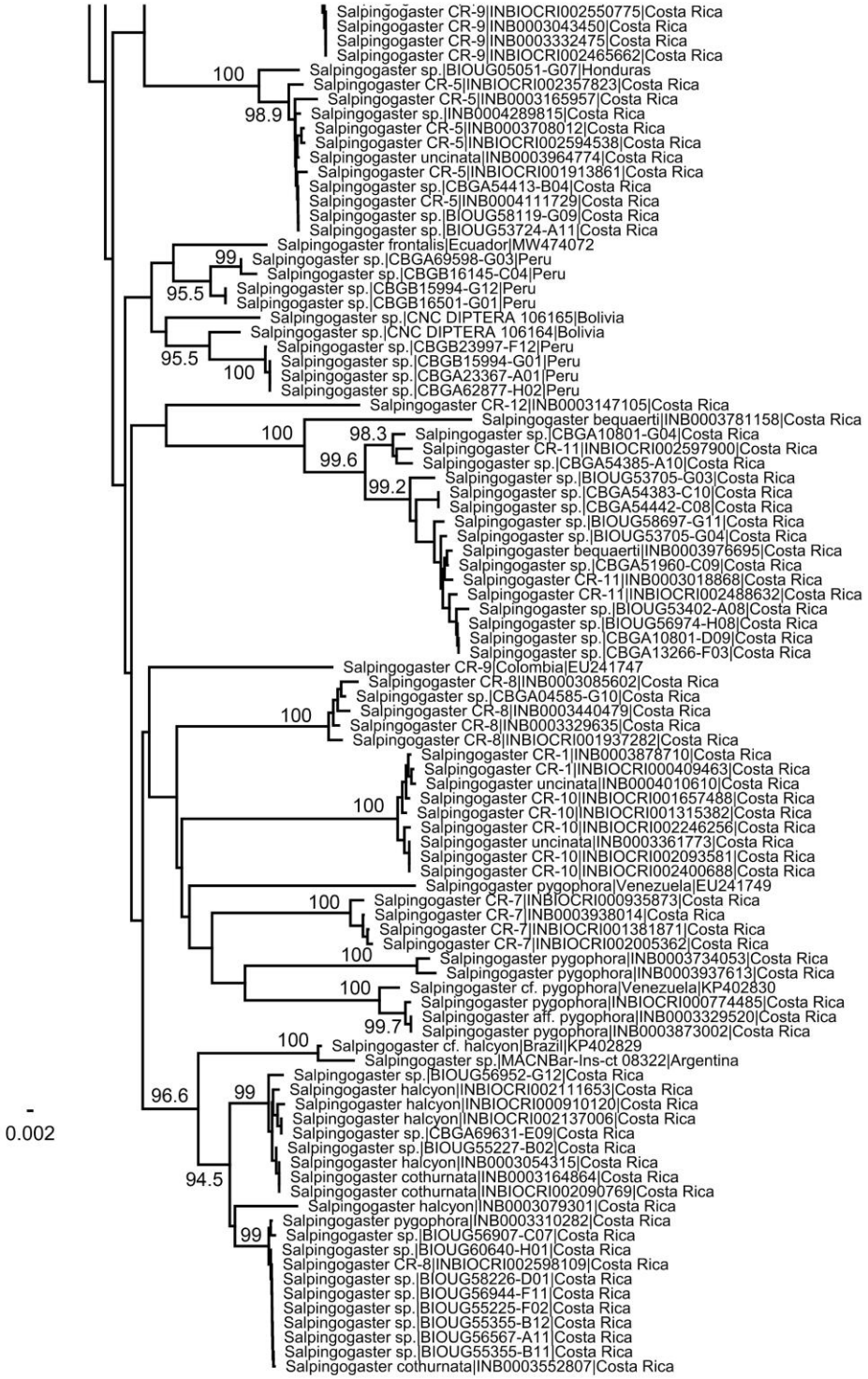

Figure 5. Neighbour-Joining tree using Jukes-Cantor model of the available DNA barcodes of *Salpingogaster* species, based on 658 bp-long sequences of the 5'-end of the mitochondrial cytochrome c oxidase subunit I gene (COI). The numbers at the branches are bootstrap values ≥ 90% based on 1000 replicates. For each sequence and separated by a |, the name of the species, the BOLD Process ID or Sample ID, the country of origin of the specimen, and the GenBank accession number are given.

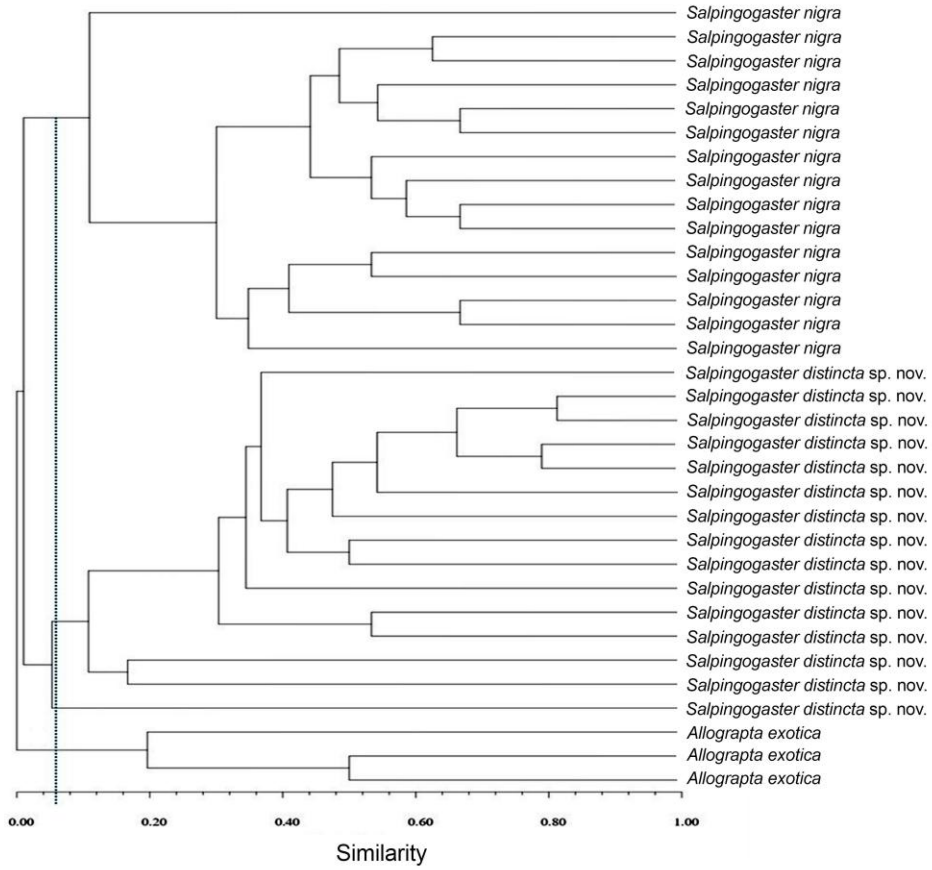

Figure 6. UPGMA dendrogram based on the Jaccard coefficient of similarity with the genetic relationship among the studied species. The vertical dashed line indicates the maximum similarity found between *Salpingogaster distincta* sp. nov. and *Salpingogaster nigra*.

Final Considerations

In general, the results show that, although it is possible to establish adequate parameters for the maintenance of *S. nigra* eggs and adults, such as a temperature range between 20 and 30 °C, daily hydration, and the use of an artificial diet based on yeast and honey, there are still significant obstacles to its complete rearing in the laboratory. The limitations observed in larval development, both in artificial and natural diets, indicate that the biological cycle of this species cannot, to date, be fully sustained under controlled conditions, restricting its large-scale production.

In this context, the practical use of *S. nigra* in biological control depends on alternative strategies, such as the collection of adults in the field and maintenance in the laboratory to obtain eggs, which can be used in inoculative releases. This approach allows exploring the predatory potential of the species even in the face of the limitations of mass rearing.

On the other hand, this thesis broadens the discussion by showing that the exclusive focus on *S. nigra* does not encompass the diversity and ecological potential of the genus *Salpingogaster*. The description of *S. distincta* sp. nov., associated with the predation of *M.*

spectabilis in agricultural environments. The record of this new species in agricultural landscapes and its direct association with the predation of spittlebug nymphs reinforces its potential as a control agent in forage crops.

In this way, the three chapters, together, indicate that the advancement in the use of *Salpingogaster* in biological control depends on a change of approach: in addition to deepening studies on *S. nigra* rearing techniques, it is essential to expand investigations to other species of the genus, considering their diversity, food specificity and adaptation to different agroecosystems. Thus, this thesis not only consolidates current knowledge about *S. nigra*, but also opens new perspectives by highlighting the potential of species that are still little studied, such as *S. distincta* sp. nov., contributing to the development of more effective and sustainable pest management strategies.