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# Health Risk From Ingesting Thasus Gigas (Hemiptera: Coreidae) ¨Xamues¨

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## ABSTRACT

*Thasus gigas is a species of the Coreidae family. It lives in the mesquite (Prosopis spp.), where it completes its biological cycle. It is consumed for supposed therapeutic and nutritional effects, which are under investigation, although they could represent health risks derived from the presence of bacteria in its intestinal tract.*
***Aim.*** *To determine the microbiota of medical importance for humans associated with this insect, since the limitation of knowledge determines that the risk conditions are largely unknown.*
***Methodology.*** *An observational, descriptive cross-sectional study was carried out during the years 2022-2023 in Tezontepec de Aldama in the State of Hidalgo, Mexico. Eighty insects were collected and transported to the Molecular Microbiology and Environmental Biotechnology Laboratory of the Autonomous University of Guerrero, where they were morphologically identified with the help of a stereoscopic microscope and taxonomic keys. Thirty specimens of Thasus gigas were selected, with the largest intestinal content visible, to be analyzed using microbiological techniques.*
***Results.*** *The analysis showed growth of the following bacteria: Serratia marcescens, Escherichia coli, Acinetobacter spp, Pasteurella stomatis, Pseudomonas aeruginosa, Bacillus subtilis, Corynebacterium spp, Enterococcus faecalis, and the fungus Candida albicans, all with more than >100,000 CFU (Colony Forming Units).*
***Conclusion:.*** *The ingestion of Thasus gigas represents a risk to human health due to the microbiota detected.*

**Keywords:** *bacteria, insect, intestinal microbiota, health risk, Thasus gigas.*

## Introduction

Heteroptera (Order Hemiptera) are insects commonly known as bugs, most of which feed on plant sap, although there are some families whose species are hematophagous and are therefore considered insects of public health importance. Here is documented evidence of the health relevance of phytophagous heteropteran taxa, many of which belong to the families Alydidae, Aradidae, Coreidae, Cydnidae, Lygaeidae, Miridae, Pentatomidae, Pyrrhocoridae, Rhopalidae, Rhyparochromidae, and Tingidae[1].

*Thasus gigas* (Klug, 1835) is a species belonging to the Coreidae family. It has five stages of development and is mainly found on mesquite trees (*Prosopis spp.*), where it feeds on the pods by sucking out their contents and resting on the foliage. The biological cycle is established on the same plant, it is consumed as food and has therapeutic uses in some parts of Mexico.

The concept of anthropoentomophagy was introduced by Costa Neto and Ramos Elorduy in 2006 [3] and it is used to refer to the consumption of insects by humans, a practice that is carried out around the world. In Mexico, it is mainly carried out in states such as Oaxaca, Hidalgo, Chiapas, Guerrero, and the State of Mexico. In Hidalgo, sampling is lacking in some localities, so the record of edible species and the risks they may pose to humans is not fully known.

Insects are good nutritional alternatives, with protein being the main nutritional component, along with amino acids and vitamins. The nutritional content depends on the species, stage of development, and diet, as it differs depending on whether they are farm-raised or wild insects. [4]

Edible insects are not only capable of meeting the energy demands of human populations, but also play an important role in aspects such as health, since insects, like plants, have been used since ancient times for therapeutic purposes. However, they are also important agents as vectors of disease in different species [5].

*Thasus gigas.* It is a bug that is consumed in the state of Hidalgo, particularly in towns in the Mezquital Valley. It can be eaten in a wide variety of traditional dishes [6], and it has been reported that its consumption, in addition to its nutritional value, is associated with therapeutic benefits. A study conducted in the state of Hidalgo reports the consumption of *Thasus gigas* as an alternative food for the treatment of type II diabetes mellitus [7].

However, the health risks involved in consuming insects should not be overlooked, as it is not known for certain whether this risk is present. In 1994, Blum [8] discusses the toxicity of insects ingested by humans, noting that food safety, processing, and preservation are closely related. Insects, like many foods, are rich in nutrients and moisture, providing a favorable environment for microbial survival and growth [9]. They have a very diverse microbiota that is associated with their life habits, such as their diet and breeding and processing conditions (if they are farm-raised), which makes a difference between the risks posed by wild insects and farm-raised insects, in addition to accidental ingestion [10].

### Risks arising from consumption

Food safety can be defined as the set of conditions and measures necessary during the production, storage, distribution, and preparation of food to ensure that, once ingested, it does not pose an appreciable health risk [11].

Like all foods, edible insects can be associated with a number of food safety hazards. They can cause disease through various mechanisms, especially if they are in direct contact and if they are alive. They can behave as allergens, vectors, or transmitters of various microorganisms that are harmful to humans, especially in poorly controlled hygienic conditions. This must be prioritized when considering their handling and consumption [12].

Among the structures that can contain microorganisms is the digestive system. The digestive system of insects normally consists of a continuous tube that extends from the mouth to the anus [13]). It is a nutrient-rich ecological niche in which endosymbiotic microorganisms known as gut microbiota are found, which can be made up of bacteria, viruses, protozoa, fungi, and nematodes[13,15and15] (Aquí está doble la referencia)

### Bacterial pathogens

There is research on the microbiological content of edible insects that reports the presence of bacteria that are pathogenic to humans.

The presence of pathogenic bacteria in edible insects has been regularly demonstrated, with references to bacteria such as *Salmonella spp.*, *Campylobacter spp.*, *E. coli 0157:H7*, *Staphylococcus aureus*, and *Bacillus cereus*[16,17,18]. Certain insects are considered vectors of *Salmonella spp.* and *Campylobacter spp.* [16]. Spore-forming bacteria are considered a concern, as they are resistant to processing treatments generally applied to insects and are also capable of growing during storage [19].

Given the limited number of studies worldwide and nationally on the health risks associated with insect consumption, it was considered of interest to perform microbiological analyses on an edible insect from the State of Hidalgo where the practice of anthropoentomophagy is carried out, specifically *Thasus gigas* (Xamues), to determine the microbiota of medical importance to humans associated with it, since the limited knowledge means that the risk conditions are largely unknown.

## Methodology

### Study design

A quantitative study was conducted, due to its cross-sectional nature and observational variable management, during the years 2022-2023 in the municipality of Tezontepec de Aldama in the state of Hidalgo.

### Sample size and sampling technique

Entomological collections of *Thasus gigas* were carried out in May 2022 in Tezontepec de Aldama. The mesquite tree (*Prosopis spp.*) was located, as these insects are associated with it. Eighty insects were collected manually with the aid of a tool consisting of a stick and a bottle. They were placed in plastic tubes with a label containing identification data such as the date, location, and condition of the collected material (live) and transported to the Molecular Microbiology and Environmental Biotechnology Laboratory at the Autonomous University of Guerrero, where they were morphologically identified using a stereoscopic microscope and an identification key for Thasus species by Brailovsky et al. (1994)[20], finally, a non-probabilistic convenience sample of 30 specimens of *Thasus gigas* was obtained, selecting those with the highest visible intestinal content for analysis using microbiological techniques.

### Preparation of culture media

Four different culture media were prepared: Tryptic Soy Agar, MacConkey Agar, Blood Agar Slants, and Sabouraud Dextrose Agar, poured into 30 Petri dishes each, which were used for the microbiological inoculation of the intestinal contents of *Thasus gigas*.

### Preparation of intestinal sample

The intestinal sample was prepared by sacrificing the insects with 70% ethyl alcohol in a glass jar according to the manual “Techniques for collecting and Preserving Insects” by Márquez Luna (2005)[21], a dissection was performed with a previously sterilized scalpel on each specimen in the abdominal area with the aid of a stereoscopic microscope to extract the intestine with sterile forceps. The intestine was then placed in glass test tubes, 15 milliliters of 0.9% physiological saline solution was added, and it was homogenized with the aid of a vortex. and then diluted 1:10, 1:100, and 1:1000 to buffer and isolate the bacterial concentration. Finally, it was preserved in refrigeration for later use.

### Microbiological culture

Microbiological culture was carried out in a total of 120 Petri dishes with a microbiological loop using the streak technique from a 1:1000 dilution of *Thasus gigas* intestine. They were sealed and incubated in an oven at 37°C for 48 hours.

### Gram Staining

The technique was performed based on the general biology laboratory manual by Ruvalcaba Ledezma et al. (2016)[22].

### Colony morphology

The morphology of a colony depends on various characteristics such as shape, edge, elevation, texture, pigmentation, odor, consistency, and optical behavior in light on the culture medium. This allows us to observe a group of bacteria macroscopically and characterize them, so these criteria were taken into account to identify the different bacteria.

### Colony-forming unit (CFU) count

This count is important to comply with the standards established under the regulations, as this data will provide us with information to determine whether the microorganisms will be capable of performing a harmful function. For the colony-forming unit count, a dark field colony counter, gridded glass plate, and stereoscopic microscope, as suggested in Mexican Official Standard NOM-092-SSA1-1994 Goods and Services. Method for counting aerobic bacteria on plates[23].

### Statistical plan

A database was created with the bacteria identified in the seeded samples, which were analyzed in Excel to obtain descriptive statistical data such as frequencies and percentages of the results obtained.

### Ethical aspects

The research project protocol was submitted for evaluation by the bioethics committee of the Institute of Health Sciences of the Autonomous University of the State of Hidalgo, which approved it with modifications, valid from May 31, 2023, to May 31, 2024.

The provisions described in Title Four, Chapter 1, Article 79 of the Regulations of the General Health Law on Health Research were taken into account, as biological material and pathogenic microorganisms were handled [24].

According to Article 79, the degree of infection risk of the research project falls within Risk Group II: Microorganisms that pose a moderate risk to individuals and a limited risk to the [24].

## Results

### Microscopic characterization

The colonies grown in the cultures were characterized microscopically using Gram staining, obtaining Gram-negative and Gram-positive samples.

### Macroscopic characterization

The presumptive or preliminary identification of a bacterium is based on colonial morphology, so this analysis was carried out on the cultures, obtaining the following data from the most representative samples, as well as applying biochemical tests that allowed for more reliable identification of the bacterial strains.

*Table 1: Colonial morphology of isolated microorganisms.*

| No. | Agar | Grth. | Color | Morph. | Elev. | Cons. | Size | Gram | Biochemistry | Morphological homology |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | MC | +++ | R | B | Co | 3 | 1-2 mm | - | -Oxidase negative | *Serratia Marcescens* |
| 2 | MC | +++ | P | B | Co | 3 | 2 mm | - | -Oxidase negative<br>-Catalase positive | *Escherichia coli* |
| 3 | Sa A | +++ | B | Y | Co | 3 | 2 mm | + | -Positive germline test | *Candida albicans* |
| 4 | TSA | +++ | B | CB | F | 1 | 3 mm | - | -Oxidase negative<br>-Catalase positive | *Acinetobacter spp.* |
| 5 | TSA | +++ | B | C | F | 3 | 1 mm | + | -Catalase negative | *Enterococcus spp.* |
| 6 | TSA | +++ | W | B | A | 2 | 3 mm | + | -Catalase positive | *Bacillus subtilis* |
| 7 | Sa A | +++ | G | B | F | 3 | 1-3 mm | + | -Catalase positive | *Corynebacterium spp.* |
| 8 | Sa A | +++ | G | B | F | 2 | 2-3 mm | - | -Catalase positive<br>-Oxidase positive<br>- β hemolysis | *Pseudomonas aeruginosa* |
| 9 | Sa A | +++ | W | CB | Co | 4 | 1-2 mm | +- | -Oxidase negative | *Pasteurella stomatis.* |
| | | | | | | | | | | |

| Parameter | Description |
|---|---|
| Agar | (MC) MacConkey, (TSA) Triptic Soy Agar, (Sa A) Sabouraud Agar, (BA) Blood Agar. |
| Growth | (+) Light (++) Moderate, (+++) Abundant. |
| Color | (R) Red, (P) Pink, (B) Beige, (W) White, (G) Gray. |
| Morphology | B) Bacillus, (Y) Yeast, (CB) Coccobacillus, (C) Coccus. |
| Elevation | (F) Flat, (Co) Convex, (A) Acuminada. |
| Consistency | (1 ) Mucoide, (2) Seca, (3) Cremosa, (4) Húmeda. |
| Gram | (+) Positive, (-) Negative, (+-) Variable. |

### Colony-forming unit count.

The results obtained from the colony-forming unit count (Table 2) indicate that the bacteria present in the intestine of *Thasus gigas* are capable of causing a bacterial infection, as the CFU count is uncountable for all bacteria identified in the cultures.

*Table 2: Classification of Colony Forming Unit Count*

| Bacteria | Colony Forming Units (CFU) |
|---|---|
| Gram Negative | |
| *Serratia marcescens* | >100,000 UFC |
| *Escherichia coli* | >100,000 UFC |
| *Acinetobacter spp.* | >100,000 UFC |
| *Pasteurella stomatis.* | >100,000 UFC |
| *Pseudomonas aeruginosa* | >100,000 UFC |
| Gram Positive | |
| *Bacillus subtilis* | >100,000 UFC |
| *Corynebacterium spp.* | >100,000 UFC |
| *Enterococcus faecalis* | >100,000 UFC |
| *Candida albicans* | >100,000 UFC |

Table 2. Classification of colony-forming units (CFU) in Gram-negative and Gram-positive bacteria obtained from cultures in four types of agar from the intestinal sample of *Thasus gigas.*

### Statistical analysis

### Tryptic Soy Agar

Trypticase Soy Agar is a medium used for general purposes. It promotes the growth and isolation of a wide variety of aerobic facultative and strict anaerobic microorganisms, i.e., it promotes the growth of both demanding and non-demanding microorganisms. Table 3 shows the frequency of microorganisms isolated on Trypticase Soy Agar, with Acinetobacter spp. being the least frequent in 11 of the 30 boxes with a percentage of 36.6%, Enterococcus faecalis in 18 of the 30 boxes with a percentage of 60%, and Bacillus subtilis being the most frequent in 27 of the 30 boxes with a percentage of 80% of the samples.

*Table 3: Frequency of microorganisms isolated in Tryptic Soy*

| Bacteria | Frequency | Percentage |
|---|---|---|
| *Acinetobacter spp.* | 11 | 36.6% |
| *Enterococcus faecalis* | 18 | 60% |
| *Bacillus subtilis* | 27 | 80% |

### MacConkey Agar

MacConkey agar is a selective culture medium used for the isolation of Gram-negative bacilli, especially those of the Enterobacteriaceae family, including opportunistic and enteropathogenic species.

Table 4 shows the frequency of microorganisms isolated on MacConkey agar, with Acinetobacter spp. being the least frequent in 7 of the 30 boxes with a percentage of 23.3%, *Escherichia coli* was found in 8 of the 30 boxes, with a percentage of 26.6%, Pseudomonas aeruginosa in 15 of the 30 boxes, with a percentage of 50%, and *Serratia marcescens* in all 30 inoculated boxes, with a percentage of 100%.

*Table 4: Frequency of microorganisms isolated on MacConkey agar*

| Bacteria | Frequency | Percentage |
|---|---|---|
| *Acinetobacter spp.* | 7 | 23.3% |
| *Escherichia coli* | 8 | 26.6% |
| *Pseudomonas aeruginosa* | 15 | 50% |
| *Serratia marcescens* | 30 | 100% |

### Blood Agar Slants

Blood agar is a culture medium used for the isolation of numerous nutritionally demanding microorganisms. It allows the hemolytic capacity of microorganisms to be distinguished, enabling them to be differentiated from others. Table 5 shows the frequency of microorganisms isolated in blood agar, with *Corynebacterium spp.* being the least frequent in 3 of the 30 inoculated boxes, with a percentage of 10%, *Pasteurella stomatis* in 9 of the 30 boxes with a percentage of 30%, *Pseudomonas aeruginosa* in 16 of the 30 boxes with a percentage of 53.3%, and *Serratia marcescens* present in all 30 inoculated boxes with a percentage of 100%.

*Table 5: Frequency of microorganisms isolated in Blood Agar Slants*

| Bacteria | Frequency | Percentage |
|---|---|---|
| Corynebacterium spp. | 3 | 10% |
| Pasteurella stomatis | 9 | 30% |
| Pseudomonas aeruginosa | 16 | 53.3% |
| Serratia marcescens | 30 | 100% |

### Sabouraud Dextrose Agar

Sabouraud agar is a culture medium recommended for the isolation and growth of pathogenic or opportunistic fungi, so it was of interest to carry out this culture in order to determine whether there is fungal microbiota associated with *Thasus gigas*. Table 6 shows the frequency of microorganisms isolated in Sabouraud Agar, with *Candida albicans* being the only microorganism present in the culture with a frequency of 22 out of the 30 boxes inoculated, representing 73.3%.

*Table 6: Frequency of microorganisms isolated on Sabouraud Dextrose agar*

| Fungus | Frequency | Percentage |
|---|---|---|
| Candida albicans | 22 | 73.3% |

## Discussion

There is little research on the health risks associated with insect consumption, according to the FAO/WHO in 2013[25] anthropoentomophagy is a practice carried out around the world, mainly in Africa, Asia, and America, by more than 2 billion people. This is attributed to the fact that insects are rich in nutrients, making them good candidates for meeting the energy demands of populations. Like all foods, edible insects can be associated with a number of food safety hazards that can affect public health, so the risk they may pose to human health should not be overlooked.

Klunder (2012[9] mentions that insects are rich in nutrients and moisture, which provides a favorable environment for the growth and survival of microorganisms. It also mentions that insects carry a very diverse microbiota that is associated with their vital habits, such as their diet.

According to AECOSAN[10,] microorganisms associated with insects may be present in their digestive tract or on their external surface.

Belluco et al. in 2013[16], Dobermann et al. in 2017[17,] and Grabowski and Klein in 2017[18] demonstrate the presence of bacteria such as *Salmonella spp, Campilobacter spp, E. coli 0157:H7, Staphylococcus aureus, and Bacillus cereus* in edible insects. This study showed the presence of E. coli in 27% of the gut samples from *Thasus gigas*. However, no test was performed to identify strain 0157:H7, which is known to be a toxin-producing bacterium that causes intestinal disease in humans.

In studies conducted by EFSA in 2015[27], Garofalo, 2007[28], Dillon and Charnley in 2002[29,] and FAO in 2021[30] report other bacteria like *Streptococcus, Bacillus spp, Pseudomonas, Micrococcus, Lactobacillus, Erwinia, Clostridium,* and *Acinetobacter*, in the samples of intestine of *Thasus gigas.* Bacterial growth was obtained as *Bacillus subtilis* in 80%, *Pseudomonas* in 50%, and *Acinetobacter* in 23% of the samples of the intestine of *Thasus gigas*. Grabowski et al. in 2017[18] and Vandeweyer et al. in 2018[31] conducted studies on the microbiota of an edible cricket (*G. sigillatus*), obtaining similar results regarding the presence of certain bacteria, such as *Proteus, Serratia, Morganella,* and fungi like *Candida albicans*. In this study, *Thasus gigas* reported the growth of *Serratia* marcescens, which was present in 100% of the samples, and the fungus *Candida albicans* in 73% intestinal samples of *Thasus gigas*.

This study reports similar results in terms of bacterial growth to those reported in other studies, where we also found other bacteria such as *Enterococcus faecalis* in 60%, *Corynebacterium* in 10% and *Pasteurella stomatis* in 30% from the intestinal samples of *Thasus gigas*, the presence of these microorganisms allows us to deduce that *Thasus gigas*, in addition to being phytophagous, also exhibits coprophagous and scavenging habits, as mentioned by Faundez and Carbajal in 2011[32], since bacteria such as *Enterococcus faecalis* and *Pasteurella* stomatis are present in the fluids and feces of other animals such as dogs and cats.

Klunder in 2012[9] and the NMWA in 2014[19] report that there is a greater risk to food safety from ingesting the intestinal tract of insects, as its rupture leads to a significant increase and release of microorganisms that are potentially dangerous. *Thasus gigas* is consumed with the intestine in various forms in which it is ingested.

Stoops in 2017[33], Vandeweyer et al. 2017[34,] and by Wynants et al in 2018[35] mention that roasting for 10 minutes reduces the content of enterobacteria, so it must be verified that during the preparation of any edible insect, the appropriate temperatures and times are reached for the heat treatment to be effective, although there are spore-forming bacteria that can survive heat treatments.

As for insects that are consumed raw for other purposes, such as medicinal purposes, the risk is imminent because the insect's microbiota is consumed in its entirety, and, as reported in this study, all samples have a high count of more than 100,000 colony-forming units (CFU), which makes them infectious.

## Conclusions

The consumption of insects is a dietary practice that has advantages and disadvantages, depending on various factors such as the type of insect, its origin, its processing, and legislation. Edible insects are a good source of nutrients that can contribute to food security and human nutrition. However, insect consumption also involves health risks such as the presence of pathogenic microorganisms, allergens, heavy metals, or pesticide residues.

The biological risks associated with insect consumption are generally considered low as long as they comply with food safety standards. Therefore, those that are extracted from nature are of concern, as safety variables cannot be controlled as they are in industry, and there is no guarantee that they will receive the appropriate treatments to eliminate any microorganisms that may be relevant to public health, as is the case with *Thasus gigas* (Xamues), which is collected from the wild for food and therapeutic purposes in the municipality of Tezontepec de Aldama.

The treatments applied to *Thasus gigas* to eliminate microorganisms are unreliable. Pretreating water with salt is not safe, since the concentration of salt used will depend on each person and whether that amount is adequate to have a bactericidal effect. Nor does it ensure that microorganisms present in the insect's digestive tract, where a high bacterial content is known to exist, will be eliminated. Likewise, post-treatment contamination can occur during handling for preparation. Heat treatment may not be carried out properly because there are no time and temperature parameters established in legislation to ensure that the microorganisms present in the insect are eliminated correctly. This will also depend on the time that each person considers sufficient to cook the insect. Another important point is that the population is unaware that this insect carries bacteria that are medically important to humans.

For people who consume it alive or "raw," the risk is definitely high simply because of the presence of bacteria found in this insect. However, this will depend on other factors such as the individual's state of health, as well as their perception of the insect. It is common knowledge that *Thasus gigas* is used to treat diseases such as type II diabetes and hypo-or hypothyroidism. In the first case, there is a study currently being published that reports that consumption of this insect has an effect on individuals' glucose levels, leaving open the question: What properties or active ingredients does this insect have that achieve these effects? In the second case, its use in the treatment of the thyroid is based on the belief that this insect contains iodine and that when consumed alive or raw, it cures or improves the disease.

In this study, a simple iodine detection test was carried out, which is in the process of being published, so it would be of great importance and interest to continue conducting studies on the advantages and disadvantages of consuming this insect.

**Conflict of interest.**

The authors declare that there is no conflict of interest for the publication of this article.

**Artificial intelligence.**

The authors declare that no artificial intelligence tools were used in any section of this article.

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