

## COMUNICACION

**Regeneración temprana de *Chusquea tomentosa*  
(Bambusoideae-Poaceae) en Talamanca, Costa Rica**

(Rec. 29-V-1995. Rev. 22-IX-95. Acep. 20-X-95)

Se estudió *Chusquea tomentosa* (Widmer & Clark 1991) en un robledal de la Cordillera de Talamanca, Costa Rica (a 2600-2900 msnm). Esta es una de las especies de bambúes que dominan el sotobosque de área descrita por Kappelle *et al.* (1989), y que florecieron y murieron masivamente en 1989-90 (Pohl 1991).

En enero de 1992 se censaron plántulas vivas y muertas (tres repeticiones) en a) claros por caída de árboles, y b) bosque primario inalterado, con diez parcelas de 0.25 m<sup>2</sup> por tipo de estrato: Hojarasca, Musgo y Troncos caídos. La densidad de plántulas fue mayor sobre musgos y menor sobre hojarasca y no hubo diferencias entre claro y bosque. Sobre troncos se encontró mas plántulas muertas por desecamiento. Las condiciones de menor humedad explicarían una menor germinación sobre hojarasca y la mayor mortalidad sobre troncos.

En noviembre de 1992 se hizo cinco repeticiones de cuatro coberturas: Claros, Bosque inalterado, Vegetación secundaria ("charrales")

y Bosques de *Alnus acuminata* ("jaulares") originados en deslizamientos de ladera (sin bambúes adultos en su interior), cada una con seis parcelas al azar de 4 m<sup>2</sup>. Sobre diez plántulas recolectadas al azar por repetición se midió área foliar y peso seco de la parte aérea. Dada la simultaneidad de la fructificación y la baja longevidad de las semillas (Rivera, obs. pers.) esta muestra debe reflejar el crecimiento y demografía de la cohorte que inició su germinación un año antes. La densidad fue muy inferior en los jaulares. El área foliar y peso seco por plántula fueron mayores en claros y jaulares. La biomasa por hectárea y el I.A.F. son mayores en claros, seguidos por charral, bosque y jaular (Cuadro 1). Disturbios menores como los claros combinarían las ventajas de menor estrés hídrico que el charral, y mas luz que el dosel cerrado dominado por robles. Dado el buen crecimiento en los jaulares, la baja densidad podría atribuirse a dificultades para la dispersión propia de los bambúes mas que a las condiciones microambientales.

CUADRO 1

*Características de plántulas de Chusquea tomentosa tras un año de regeneración*

Tipo de cobertura	Bosque	Claros	Charral	Jaular
Densidad (ind/ha)	b 42083	a 64083	a 77416	c 833
Área foliar (cm <sup>2</sup> /ind.)	b 3.15	a 10.50	b 3.70	a 14.30
Peso seco (g/ind.)	b 0.134	a 0.570	b 0.218	a 0.920
Biomasa aérea (g/ha)	b 522	a 3777	b 2107	c 30
I.A.F. (%)	b 0.120	a 0.674	a 0.305	c 0.006

Los números precedidos de igual letra dentro de la misma fila, no son significativamente diferentes (Kruskal-Wallis  $p < 0.05$  y comparaciones individuales por Duncan).

La densidad inicial fue de 1 172 000 plántulas/ha, disminuyendo a 50 000 (menos del 5%) en el primer año. En noviembre el I.A.F. fue de menos del 1% y la biomasa entre 0.5 y 3.7 kg/ha. Esto implica una productividad inferior al 0.1% y un I.A.F. inferior al 1% de lo observado para plantas adultas de *Chusquea* en otros ecosistemas montaños (Veblen *et al.* 1980, Toll & Cleef 1994). La lenta recuperación implicaría que los efectos ecológicos derivados de la ausencia temporal de la especie pueden prolongarse por varios años. La alta mortalidad, poca longevidad de semillas y baja eficiencia de dispersión hacen de este período del ciclo vital un momento de alta susceptibilidad en el que condiciones desfavorables como sequías o pastoreo pueden provocar desapariciones locales como las observadas por Pohl (1991) produciendo una importante alteración por décadas en el funcionamiento de ecosistemas donde *Chusquea* es importante.

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## REFERENCIAS

- Kappelle, M.; A.M. Cleef & A. Chaverri. 1989. Phytosociology of montane *Chusquea-Quercus* forests, Cordillera de Talamanca, Costa Rica. *Brenesia* 32: 73-105.
- Pohl, R.W. 1991. Blooming history of Costa Rican bamboos. *Rev. Biol. Trop.* 39: 111-124.
- Toll, G.J. & A.M. Cleef. 1994. Above ground biomass structure of *Chusquea tessellata* bamboo paramo, Chingaza National Park, Cordillera Oriental, Colombia. *Vegetatio* 115: 29-39.
- Veblen, T.T.; F. Schlegel & B. Escobar. 1980. Dry-matter production of two species of bamboo (*Chusquea culeou* and *Chusquea tenuifolia*) in south-central Chile. *J. Ecol.* 68: 397-404.

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## Microbiological quality of some powder spices of common use in Costa Rica

(Rec. 28-VIII - 95, Rev. 16-I-96, Acep. 22-11-96)

**Key words:** Spices, microbiological quality, *Salmonella*, *Escherichia coli*.

The microbiological contamination of spices has various sources, such as indigenous microflora of plants, microorganisms present in processing plants, post-harvest contamination from dust, use of contaminated water or other sources (Wirtanen & Sjöberg 1993). During cleaning and processing, there is a progressive reduction in the number and types of microorganisms: those remaining usually are aerobic spore-forming bacteria and common molds (Guarino 1973).

Even though few foodborne outbreaks have been traced to the consumption of contaminated spices, numerous isolations of pathogens, from a variety of spices, including oregano, black pepper and white pepper (whole and in powder) have been described (Wilson & Andrews 1976).

In Costa Rica, very little microbiological research has been done on spices. An analysis of black, white and green peppers showed that the total plate counts and fecal coliforms

exceeded the International Commission on Microbiological Specifications for Foods (ICMSF) standards, and that contamination was probably due to drying conditions and post-harvest treatment (Arce 1990). The aim of this work was to evaluate the microbiological quality of some of the powdered spices usually used in Costa Rican homes without any further thermic treatment.

A total of 75 samples of five different powdered spices (onion, garlic, oregano, pepper), randomly acquired in supermarkets in the Metropolitan area of San José, were analyzed in the laboratory of Food Microbiology, University of Costa Rica, from July 1994 to June 1995.

Twenty five grams of each sample were added to flasks containing 225 ml of sterile peptone water 0.1%. These were homogenized for approximately 30 s. Duplicate serial dilutions were prepared with sterile peptone water.

The total viable counts of bacteria were determined using Standard agar with TTC (triphenyl-tetrazolium chloride), 0.1% after an incubation at 37 °C for 48 hours.

Mould enumeration was done in Potato-Dextrose Acidified agar, incubated for five days at room temperature. Moulds were identified according to their macroscopical morphology.

The fecal coliforms and *Escherichia coli* enumeration were done by the Most Probable Number (MPN) technique, (Vanderzant & Splittstoesser 1992).

*Salmonella* spp. were isolated according to the Association of Official Analytical Chemists (AOAC 1975), modifying the preenrichment broth as described by Wilson & Andrews (1976): 25 g of each spice was diluted in 225 ml tripticase-soy broth with 0.5% Na<sub>2</sub>SO<sub>3</sub> and incubated for 18-24 h at 37 °C. Subsequent selective enrichment and plating were done in accordance with the official AOAC methodology

The ICMSF establishes a norm of standard plate count below 10<sup>6</sup> UFC/g, a mould count below 10<sup>4</sup> UFC/g, *E. coli* MPN below 10<sup>3</sup>/g and absence of *Salmonella* sp.

Considerable variations were observed in the total aerobic plate count, ranging from <100 UFC/g to more than 1x10<sup>7</sup> UFC/g. Garlic, probably due to the marked bactericidal effect of its essential oil (Frazier & Westhoff 1978), had the lowest percentage of samples over

ICMSF proposed norm (26.7%), followed by white pepper (37.5%), onion (53.3%), black pepper (64.3%) and oregano (73.3%).

The study of moulds in spices and herbs is significant because of the potential production of mycotoxins. Flannigan and Hui (1976) showed moulds associated with spices and herbs to be predominantly *Aspergillus* spp. of which 7 out of 24 strains produced aflatoxins *in vitro*.

Some spices stimulate microbial activity, others such as garlic, onion and oregano exhibit antibacterial action (Hitokoto *et al.* 1980, Llewellyn, Burkett & Eadie 1982). In this study, pepper and in less degree garlic, showed negative effect over the growth of moulds, but an important percent of the other spices (onion and oregano) presented counts over 10<sup>4</sup> UFC/g. The mould species most isolated was also *Aspergillus* spp. and even though no aflatoxin determination was done, they all are potential producers of it.

Ca. 20% of oregano, black and white pepper samples showed levels of fecal coliforms over 10<sup>3</sup> NMP/g, as has been reported by other authors (Baxter & Holzapfel 1982) and the presence of ca. 7% of *E. coli* in pepper indicates fecal contamination at some stage of its production.

*Salmonella* sp. was isolated in 6.7% (2/30) of pepper. This spice has been implicated as vehicle for the spread of *Salmonella weltevredis*, resulting in several cases of salmonellosis (WHO 1974).

Onion and garlic are inhibitory to *Salmonella* growth because of the sulfite anion they contain (Wilson & Andrews 1976), so the addition of 0.5% K<sub>2</sub>SO<sub>3</sub> or Na<sub>2</sub>SO<sub>3</sub> to the preenrichment media overcomes this problem. Oregano must be examined by diluting it beyond its toxic level until a mean to neutralize its toxicity can be found (Wilson & Andrews 1976). Both recommendations were followed, so the absence of *Salmonella* and *E. coli* in these spices is not an artifact of the toxicity of their natural oils.

Even though *E. coli* and *Salmonella* spp. were not isolated in big numbers from this samples, the high microbial contamination found demonstrates inadequate hygiene conditions. The effect of this is not well known in the population, since there is an enormous sub-register in the country in this field. Increased

efforts shall be directed to improve the microbiological quality of spices and to develop and optimize pasteurization processes in order to offer safe spices to the consumers.

## REFERENCES

- Arce, U.P. 1990. Diagnóstico de la calidad de la pimienta (*Piper nigrum L.*) que se produce en tres zonas de Costa Rica. Tesis de Licenciatura en Tecnología de Alimentos. Universidad de Costa Rica, San José, Costa Rica.
- Association of Official Analytical Chemists. 1975. Official methods of analysis Assoc. Offic. Anal. Chem., Washington, D.C.
- Baxter, R. & W.H. Holzapfel. 1982. A microbial investigation of selected spices, herbs and additives in South Africa. *J. Food Sci.* 47: 570-574.
- Flannigan, B & S.C. Hui. 1976. The occurrence of aflatoxin producing strains of *Aspergillus flavus* in the mould floras of ground spices. *J. Appl. Bact.* 41: 411.
- Frazier, W.C. & D.C. Westhoff. 1978. Food Microbiology. McGraw Hill, New York. 370p.
- Guarino, P.A. & H. J. Pepler. 1976. Spices and condiments. In M.L. Speck (ed.). Compendium of Methods for the Microbiological Examination of Foods. American Public Health Association, Washington, D.C.
- Hitokoto, H., S. Morozumi, T. Wauka, S. Sakai & H. Kurata. 1980. Inhibitory effects of spices on growth and toxin production in toxigenic fungi. *Appl. Environ. Microbiol.* 39: 818-822.
- Llewellyn, G.C., M.L. Burkett & T. Eadie. 1981. Potential mold growth, aflatoxin and antimycotic activity of selected natural spices and herbs. *J. Assoc. Off. Anal. Chem.* 64: 955-960.
- Vanderzant, M. & V. Splittstoesser. 1992. Compendium of methods for the microbiological examination of foods. American Public Health Association, Washington, D.C.
- Wilson, C. R. & W. Andrews. 1976. Sulfite compounds as neutralizers of spice toxicity for *Salmonella*. *J. Milk Food Technol.* 39: 464-466.
- Wirtanen, G. & A. Sjöberg. 1993. Microbiological screening method for indication of irradiation of spices and herbs: a BCR collaborative study. *J. AOAC Intern.* 76: 674-681.
- World Health Organization. 1974. Salmonella surveillance. Weekly epidemiological record: 42: 351-352. World Health Organization, Geneva. 68 p.

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## Fine structure of the capsule of *Cryptococcus neoformans* (Cryptococcales: Cryptococcaceae)

(Rec. 16-VI-1985. Rev. 17-X-1995. Acep. 31-I-1996)

**Key words:** *Cryptococcus*, ultrastructure, ruthenium red, capsule.

*Cryptococcus neoformans* is the only encapsulated fungi to infect humans. Its most prominent characteristic is its carbohydrate capsule, which, together with the capacity to grow at 37 °C and its production of melanin is considered a virulence factor (Kwon-Chung *et al.* 1982, Kwon-Chung & Rhodes 1986).

Infection occurs by inhalation of yeast and most of cases are asymptomatic or develop

weak pulmonary manifestations. However, normal individuals inhaling high doses of yeast can develop systemic infections; this condition is associated with people who have contact with dry pigeon feces, where the fungi grow abundantly. Additionally, *Cryptococcus neoformans* is an opportunistic pathogen and in immunosuppressed patients, such as those with AIDS or other debilitating conditions

(leukemia, lymphomas, sarcoidosis); the agent may disseminate systemically and usually induces meningitis (Sugar 1991, Warren & Shadomy 1991).

There are some ultrastructural reports of this fungi ( Tsukahara 1963, Iyo 1966 Mochisuki 1987); but in general, they describe the capsule briefly. This report is an ultrastructural description of the capsule.

*C. neoformans* (strain C-74, Faculty of Microbiology, UCR) was grown in Sabouraud agar at room temperature for 48 h. Yeast was harvested and sequentially fixed with 2.5 % glutaraldehyde and 1 % osmium tetroxide in cacodilate buffer (0.2 M, pH 7.2), with 0.75 % ruthenium red. A suspension of yeast was embedded in melted 3 % agar at 50 °C. When the agar solidified, it was divided in small blocks (1 mm<sup>3</sup>), fixed again with osmium, dehydrated, embedded in Spurr resin, and thin sectioned (ca. 90 nm thickness). Sections were caught on copper grids, stained with uranyl acetate-lead citrate and analyzed under a transmission electron microscope.

Intracellular structures such as mitochondria, endoplasmic reticulum, vacuoles and round to oval nucleus included in a granular cytoplasm (Figs. 1-2), matched previous descriptions (Tsukahara 1963, Iyo 1966, Mochisuki 1987). The cytoplasmic membrane appeared wavy, separated from the cell wall by a space of 5 to 15 nm. The wall was the most electrondense structure and was ca. 125 nm thick (Fig. 3). The capsule was an evident, threadlike structures, finer near the cell and thicker in the distal (Figs. 1 and 4).

Ultrastructural findings suggest that the capsule is a very hydrated envelope composed mainly of polysaccharides that were not fixed with the standard fixation procedures, and for that reason collapsed during dehydration with ethanol. Likewise, the same properties explain the frequent collapse of this yeast during critical point drying.

In this report the capsule was evident due to the reaction of polysaccharides with ruthenium red. This is, to the authors knowledge, the first time that this structure has been described with transmission electron microscopy.

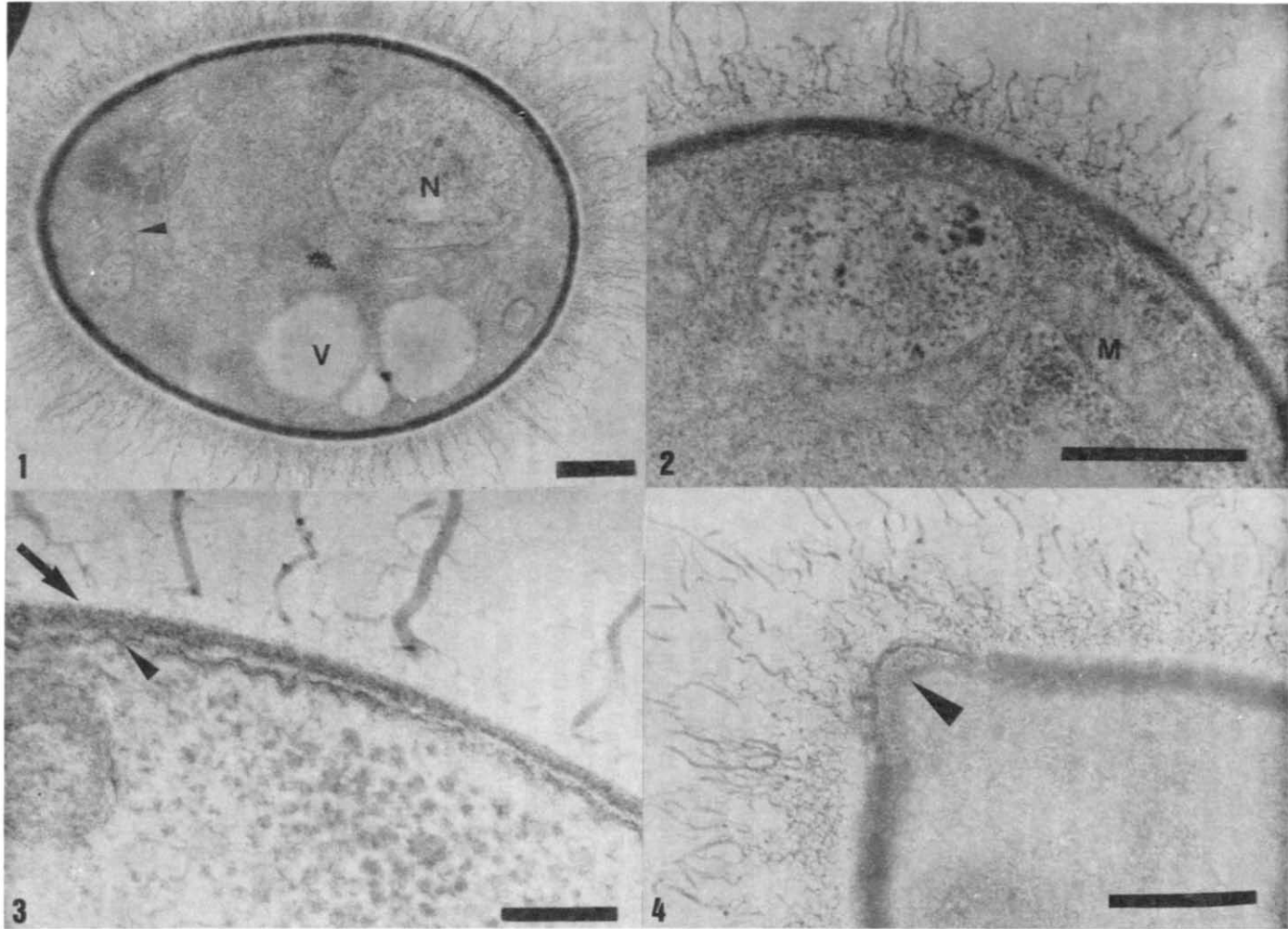
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## REFERENCES

- Iyo, S. 1966. Fine structure of *Cryptococcus neoformans* - an electron microscopy study. *Japanese J. Dermatol.* 76: 65-85.
- Kwon-Chung, K.J., I. Polachec & T.J. Popkin. 1982. Melanin- lacking mutants of *Cryptococcus neoformans* and their virulence for mice. *J. Bacteriol.* 150: 1212-1220.
- Kwon-Chung, K.J J.C. Rhodes. 1986. Encapsulation and melanin formation as indicators of virulence in *Cryptococcus neoformans*. *Infect. Immun.* 51:218-233.
- Mochizuki, T., S. Tanaka Watanabe. 1987. Ultrastructure of the mitotic apparatus in *Cryptococcus neoformans*. *J. Med. Vet. Mycol.* 25:223-233.
- Sugar, A.M. 1991. Overview: Cryptococcosis in the patient with AIDS. *Mycopathology* 114: 153-157.
- Tsukahara, T. 1963. Cytological structure of *Cryptococcus neoformans*. *Japan J. Microbiol.* 7: 55-60.
- Warren, N. G H.J. Shadomy. 1991. Yeasts of medical importance, p. 597-693 *In* A. Balows, W.J. Hausler Jr., K.L. Herrmann, H.D. Isenberg, H.J. Shadomy (eds). *Manual of clinical microbiology*. American Society of Microbiology, Washington.

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Figs. 1-2. Cellular organelles: nucleus (N), endoplasmic reticulum (arrowhead), vacuoles (V), mitochondria (M).

Fig. 3. Detail of the cell wall (Arrow). The cytoplasmic membrane appears wavy (Arrowhead).

Fig. 4. Yeast showing the extracellular moiety, such as fibers. The arrowhead indicates the altered wall at the place of budding. Bar=0.5 $\mu$ m in all figures.

## Inhibitory substance residues in unpasteurized and pasteurized milk in a tropical city

(Rec. 16-VI-95. Rev. 29-IX-95. Accep. 28-XI-95)

**Key words:** Milk, antibiotics, inhibitory substances.

The use of antimicrobials, especially  $\beta$ -Lactams and sulfonamides, has reduced monetary losses caused by bovine mastitis (Jones & Seymour 1988). Nevertheless, the absence of appropriate clinical management of mastitis results in the contamination of milk with these substances (Vautier & Postigo 1986). This contamination represents a serious problem for public health and the dairy industry.

The presence of antimicrobial substances has been reported to lead to an increased bacterial resistance to antibiotics (Wemer 1986), and to the development of asthma (Jones & Seymour 1988), dermatitis (Anonymous 1963), and anaphylaxis (Froppaolo 1984). In an effort to reduce these public health problems, the World Health Organization (WHO) established maximum permissible concentrations of penicillin G in milk for human consumption at 0.006  $\mu\text{g/ml}$  (Booth 1986).

In the dairy industry, the presence of inhibitory substances represents a serious economic loss (Jones & Seymour 1988), because of its negative impact on starter cultures used for dairy products production (Albrigh *et al.* 1961).

Because of limited efforts to investigate this important public health problem in Costa Rica, this study was undertaken to determine the incidence of Inhibitory Substances Residues (ISR) in pasteurized and unpasteurized milk sold in Costa Rica.

A total of 1154 milk samples were tested for the presence of ISR from January 1992 through December 1993, 659 (57%) of these samples came from unpasteurized milk sold by 37 milk deliverers (door to door vendors), 295 (26%) were samples of unpasteurized milk used by public food services (open market restaurants) and 200 (17%) were samples of pasteurized milk served by the food services of the ten largest hospitals in Costa Rica. All samples were analyzed according to methodology described for

three different commercial tests: Arla Micro test  $\text{\textcircled{R}}$ , Delvo test  $\text{\textcircled{R}}$  and Valio test 101  $\text{\textcircled{R}}$ .

Milk sold by milk deliverers was examined using *Bacillus subtilis* (ATCC-6633) growth inhibition test (Arla Micro-test  $\text{\textcircled{R}}$ ). The positive and uncertain samples were retested for *Bacillus stearothermophilus* var. *calidolactis* (ATCC-10149) growth inhibition test (Delvo-test  $\text{\textcircled{R}}$ ).

Milk samples from hospitals and public food services were also analyzed using *B. stearothermophilus* growth inhibition test. In this case, the positive and uncertain samples were retested for *Streptococcus thermophilus* (T-101) growth inhibition (Valio-test 101  $\text{\textcircled{R}}$ ).

ISR were detected in 26% (301 samples) of the total samples tested. 24% (158 samples) of the milk samples collected from milk deliverers, 39% (116 samples) from public food services and 13.5% (27 samples) from hospital food services tested positive for ISR.

In countries such as New Zealand, United States, Ireland and Sweden, the incidence of ISR is as low as 1% (Booth 1986, Carlsson & Bjork 1989, Eagan & Meaney 1985), but in Costa Rica it is found to be as high as 26%.

The Costa Rican problem is meaningful and persistent. Meaningful since our results indicate that incidence of IRS in unpasteurized milk reaches up to 55% in some areas and in pasteurized milk up to 35% in some lactic industries. Persistent because even though the problem was detected in 1987 by Arias *et al.*, it continues, although a large number of lactic industries have begun the systematic determination of those substances and have established punishment measures against those producers that sell milk with concentrations of penicillin G greater than 0.006  $\mu\text{g/ml}$ .

The quality of milk in Costa Rica presents an important problem, and the situation is even worse when one considers that ISR pre-

sent a high resistance to heat (Carlsson & Bjorck 1987, Vautier & Postigo 1986); so that thermal treatments such as boiling, pasteurization and ultrapasteurization can not solve the problem.

Our results indicate that governmental surveillance of milk quality needs to be implemented soon.

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## REFERENCES

- Albright, V., S. Tuckey, & G. Woods. 1961. Antibiotics in milk: A Review. *J. Dairy Sci.* 44: 770-807.
- Anonymous. 1963. Normas para el examen de los productos lácteos. Organización Mundial de la Salud. Washington, D.C. 137p.
- Arias, M. L., F. Antillón & Z. Cubillo. 1988. Residuos de penicilina en leche bovina en Costa Rica. *Rev. Cost. Cienc. Med.* 9: 125-129.
- Booth, J. 1986. Intramammary antibiotic preparations and their withholding times. *Vet. Rec.* 118: 34-35.
- Carlsson, A. & L. Bjorck. 1987. The effect of some indigestible antibiotics in milk on growth of *Bacillus stercorophilus* var. *calidolactis*. *Milchwissenschaft* 42: 282-283.
- Carlsson, A. & L. Bjorck. 1989. Detection of antibiotic residues in herd and tanker milk. A study of the Charm Test II. *Milchwissenschaft* 44: 7-10.
- Carlsson, A. & Z. Bjorck. 1991. Charm Test II for confirmation of inhibitory substances detected by different microbial assays in hard milk. *J. Food Prot.* 54: 32-36.
- Eagan, J. & L. Meaney 1985. The persistence of detectable residues of penicillin and cloxacillin in normal and mastitic quarters following intramammary infusion. *Vet Rec.* 116: 436-438.
- Frappalo, P. 1984. Current research and regulatory status on antibiotics in animal feeds. *J. Am. Vet. Med. Assoc.* 185: 28-30.
- Jones, G. & E. Seymour. 1988. Cowside antibiotic residue testing. *J. Dairy Sci.* 71: 1691-1699.
- Vautier, H. & C. Postigo. 1986. Mastitis bovina y residuos de antibióticos en la leche, riesgos para la salud pública. *Rev. Mund. Zootec.* 60: 111-113.
- Werner, E. 1986. Tropical aspects of the use of antibiotics in cattle. *Monatshfte Veterinaermedizin* 41: 685-690.
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## Coral colony fragmentation by whitetip reef sharks at Coiba Island National Park, Panamá

(Rec. 20-V-95. Rev. II-X-95. Acep. 1-XII-95)

**Key words:** Coral fragmentation, asexual reproduction, sharks, Pocillopora, Eastern Pacific, Costa Rica, Panamá.

Breakage and detachment of branching corals from the substrate are caused by a variety of agents that range from boring organisms to hurricanes. Fragmentation can be a mean of asexual reproduction when the fragments are large enough to survive and eventually reproduce (Highsmith 1982,

Szmant 1986). Large fragments of all species seldom fail to survive after the breakage (Highsmith *et al.* 1980). The fate of the fragments is also associated with their position on the reef and the environmental conditions such as surge or currents (Kay & Liddle 1989).



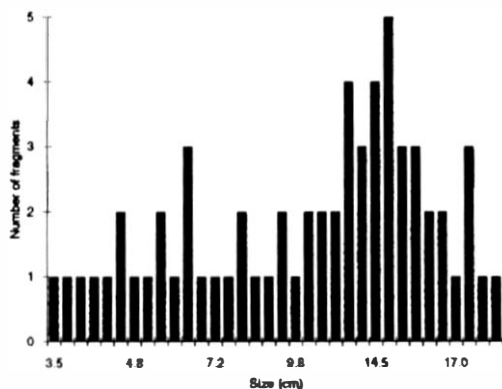


Fig. 1. Size distribution of coral fragments detached by hunting whitetip sharks, Coiba Island National Park, Panamá.

Coral colony fragmentation by fishes in the Eastern Pacific Reefs is known for several corallivorous species (see Guzmán & Cortés 1993 for review) that feed primarily on corals or on the burrowing organisms living within the coral skeleton, while others overturn corals and rubble in the process of capturing crustaceans (Glynn 1982). On one opportunity (II-18-95) at Coiba Island National Park, Panamá, while snorkeling on a reef framework composed by the branching corals *Pocillopora damicornis* (Linnaeus 1758) and *P. elegans* (Dana 1846) at a particular small inlet known as Granito de Oro, three whitetip reef sharks *Triaenodon obesus* (Rüppell 1837) were observed breaking coral colonies. The sharks (1.50-1.70 m in length) were swimming above the framework during the low tide (2 m depth, 10:00 hr.), and circling consistently at a particular spot on the reef where there were two juveniles of the orangeside triggerfish *Sufflamen verres* (Gilbert & Starks 1904) hidden among the coral. The sharks pushed their heads into the coral branches twisting and shaking their bodies, producing the breakage and detachment of several colonies. As the trigger fish escaped the sharks repeated the maneuver, increasing the breakage.

During the observation hour the proximity of the snorkeler did not seem to affect the sharks. Other fish such as the king angelfish *Holocanthus passer* (Valenciennes 1846) were also hunted unsuccessfully, with the same results.

Four colonies of *P. elegans* and one of *P. damicornis* (>20 and 15 cm maximum diameter respectively) were detached by the sharks. The mean length of 63 fragments (Fig. 1) was 11.24 cm (4.62 SD.; range: 3.5-18.2 cm).

The observed survival rate for *Pocillopora* spp. fragments, 4-7 cm in size, after three years of monitoring at Caño Island, Costa Rica (Guzmán 1991), was about 80 %. Because the majority of the fragments (93 %) produced by the sharks were longer than four centimeters (Fig. 1), a high rate of survival and eventual attachment to the reef framework are expected. These data combined with the fast growth rates of *P. damicornis* and *P. elegans* -3.46 and 3.48 cm yr<sup>-1</sup> on average respectively (Guzmán & Cortés 1989)- suggest that the dominance of the species in parts of the reef results from asexual reproduction by fragmentation together with attachment and growth capacity. This is also true of other pocilloporid corals in the Eastern Pacific (Glynn & Wellington 1983). Therefore an occasional event, such as excavations by foraging fish (Glynn 1982) or the shark's behavior reported here, can favor reproduction, dispersal and dominance. The latter is supported by observations made by divers at Cocos Island National Park (Costa Rica) of similar whitetip shark's behavior (M. Arroyo, pers. comm. 1994). The observed whitetip shark behavior could be considered then, as an unusual but potential mean for asexual reproduction of pocilloporid corals in the Eastern Pacific.

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## REFERENCES

- Glynn, P. 1982. Coral communities and their modifications relative to past and prospective Central American seaways. *Adv. Mar. Biol.* 19: 91-132.
- Glynn, P. & G. Wellington. 1983. Corals and coral reefs of the Galápagos Islands. (With annotated list of the scleractinian corals of Galápagos by J.W. Wells) University of California, Berkeley. 330 p.

Guzmán, H. 1991. Restoration of coral reefs in Pacific Costa Rica. *Conserv. Biol.* 5: 189-195.

Guzmán, H. & J. Cortés. 1989. Growth rates of eight species of scleractinean corals in the Eastern Pacific (Costa Rica). *Bull. Mar. Sci.* 44: 1186-1194.

Guzmán, H. & J. Cortés. 1993. Arrecifes coralinos del Pacífico Oriental tropical: revisión y perspectivas. *Rev. Biol. Trop.* 41:523-557.

Highsmith, R. 1982. Reproduction by fragmentation in corals. *Mar. Ecol. Prog. Ser.* 7: 207-226.

Highsmith, R., A. Riggs, C. D'Antonio. 1980. Survival of hurricane-generated coral fragments and a disturbance model of reef calcification/growth rates. *Oecologia* 46: 322-329.

Kay, A. & M. Liddle. 1989. Impact of Human trampling in different zones of a coral reef flat. *Environm. Manag.* 13:509-520.

Szmant, A. 1986. Reproductive ecology of Caribbean reef corals. *Coral Reefs* 5:43-54.

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### Nest density of *Trigona spinipes* (Hymenoptera Apidae) in cerrado vegetation of central Brazil

(Rec. I-IX-95. Rev. 30-XI-95. Acep. 28-VI-96)

**Abstract:** *Trigona spinipes* nest density was recorded in a area of cerrado (neotropical savanna) in Central Brazil and ranged from 0.2 - 0.3 nests/ha. Natural history data are presented on nest persistence and plant support to nest. The richness and abundance of *Trigona* species in Cerrado are lower than in tropical rain forests. It is suggested that the absence of larger trees could limit the richness and abundance of *Trigona* species in cerrado

**Key words:** stingless bees, colony density, savanna, *Trigona almathea*, *Trigona branneri*.

Several studies have show that species of stingless bees of the genus *Trigona* are abundant in the forests of tropical and subtropical regions (Michener 1946, Wille and Michener 1973, Hubbell and Johnson 1977). In contrast there is a lacking of studies in the tropical savannas (Darchen 1972). The following notes presents the results of some observations on *Trigona* nest density and natural history, which were recorded during a vegetation survey for plant ecology study in the cerrado (neotropical savanna) of Central Brazil.

The study areas are located in Reserva Ecológica do IBGE (RECOR) at 15° 57'S and 47° 53' WGr, 16 km SW of Brasília (D.F.) and Fazenda Água Limpa (FAL) the field station of the Universidade de Brasília at 15° 56'S and 47°56' WGr., 18 km SW from Brasília (D.F.). The climate and vegetation of the areas are typical of the cerrado region. There is a marked

dry season the average annual rainfall is 1456 mm falling from November to February. The mean annual temperature is 21.3° C. The sampled area of \* 300 ha in Reserva Ecológica do IBGE is covered by cerrado vegetation an open tree-scrub woodland averaging 6 m tall and with 40-60% cover. This area has been protected from fire since 1974. Two suveys (July 1991 and May 1993) were conducted in 4 parallel transect 150 m apart (two of 20 m x 250 m, one of 20 m x 110 m, and a fourth of 20 m x 140 m). The surfaces of all plants in the sampled transects were carefully examined in order to record the presence of conspicuous nests of *Trigona* bees. Holes in termite nests and tree trunks were also investigated. This detailed examination ensured the discovery of all the nests. Additional observations were made outside transects in the cerrado of RECOR and FAL

The only nests of *Trigona spinipes* (F.) were registered inside and outside transects. Three nests were recorded inside and ten outside the transects. The number of active nests at the same time of sampling in 1991 was two and one in 1993. Colony densities computed from these data were 0.3 nests/ha (1991) and 0.2 nests/ha (1993). The active nests of 1991 were inactive in 1993, indicating a colony persistence of < 22 months.

The nests detected were observed on the following tree species (number of nests): *Eriotheca pubescens* (3), *Didymopanax macrocarpum* (1), *Qualea parviflora* (3), *Vochysia thyrsoidea* (1), *Symplocos lanceolata* var. *ramnifolia* (2). Two nests were observed upon the same tree of *E. pubescens*. The nests were observed from 1 to 4 m above the ground in tree with > 10 cm diameter on the ground. Although three species of *Trigona* have been recorded in Brasília region (A. Raw, personal communication) only one species (*T. spinipes*) were observed nesting in cerrado vegetation during this study. The densities estimated for *T. spinipes* in cerrado are lower than 2.5 nests/ha recorded for Lamto savanna (Darchen 1972), and 1.4 nests/ha for 5 species pooled (range per species 0.1-0.4 nests/ha) calculated for dry forests in Guanacaste in Costa Rica (Hubbell and Johnson 1977). There is evidence that nesting in larger bodied forests *Trigona* species is limited by size of trees for large colony size (Hubbell and Johnson 1977). In cerrado *Trigona spinipes* have body length ( $X = 6.9\text{mm}$ ;  $N = 10$ ), which is of similar size of *T. branneri* ( $X = 6.8$ ;  $N = 10$ ) both smaller than *T. almathea* ( $X=9.9\text{mm}$ ;  $N = 10$ ). These last two species build larger nests recorded only in gallery forest (A. Raw, pers. comm.) In Darchen's (1972) study the species of *Trigona*

recorded in the Lamto savanna are smaller than the forest species (Hubbell and Johnson 1977). I suggest that the low abundance of larger trees in cerrado precludes the colonization of cerrado vegetation by gallery forest species of *Trigona*. In Costa Rica the minimum diameter size for five *Trigona* species to nest in trees was 20 cm dbh (Hubbell and Johnson 1977). In the four cerrado transects of this study only 6 trees had a diameter > 20 cm diameter, in a total of 3710 plants recorded.

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## REFERENCES

- Darchen, R. 1972. Ecologie de quelques trigone (*Trigona* sp) de la savanne de Lamto (Cote d'Ivoire). *Apidologie* 3: 341-367.
- Henriques, R. P. B., Rocha, I. R. D. & Kitayama, K. 1992. Nest density of some social wasps species in cerrado vegetation of Central Brazil (Hymenoptera: Vespidae). *Entomol. Gener.* 17: 265-268.
- Hubbell, S. P. & Johnson, L. K. 1977. competition and nest spacing in a tropical stingless bee community. *Ecology* 58: 949-963.
- Michener, C. D. 1946. Notes on the habitats of some Panamanian stingless bees (Hymenoptera, Apidae). *J. N. Y. Entomol. Soc.* 54: 179-197.
- Wille, A. & Michener, C. D. 1973. The nest architecture of stingless bees with special reference to those of Costa Rica (Hymenoptera, Apidae). *Rev. Biol. Trop.* 21: 1-278.

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## Hábitos alimentarios de *Bufo marinus* (Anura Bufonidae) en Costa Rica

(Rec. 19-V-94. Rev. 29-VI-95. Acep. 13-X-95)

**Key words:** *Bufo marinus*, feeding habits, diet, Costa Rica.

El sapo gigante, sapo de la caña o sapo de América Central (*Bufo marinus*), se distribuye de los 27° N, noroeste de México a los 10° S, zona central de Brasil (Zug 1991). Esta especie ha ampliado su ámbito geográfico, ya que fue introducida como control biológico de plagas agrícolas en el Caribe, sur de E.U.A., Asia y Oceanía (Easteal 1981), pues es un voraz depredador (Honnegger 1970).

En Costa Rica *B. marinus* se encuentra en la mayoría de los hábitats abiertos y semiabiertos hasta una altitud de 2000 msnm; sin embargo nunca se le encuentra en bosques de dosel cerrado (Zug 1991). Los adultos alcanzan tallas que fluctúan entre 90 y 200 mm de longitud total y un peso entre 80 a 1200 g. Es una especie carnívora oportunista indiscriminada que consume invertebrados y pequeños vertebrados (Strüssman *et al.* 1984 y Freeland y Kerin 1988).

Se trabajó con 23 machos y 32 hembras, recolectados en el Refugio Nacional de Vida Silvestre de Golfito, Puntarenas, Costa Rica (83° 10' N y 8° 37' W) de mayo de 1986 a junio de 1987. Se midió longitud total (Lt) con una precisión de 0.01 mm. Los organismos se agruparon en tres ámbitos de tallas (44.66 a 77.39; 77.40 a 108.19 y 108.20 a 138.99 mm de Lt) para el estudio de los hábitos alimenticios (Strüssman *et al.* 1984).

El análisis del contenido estomacal, siguió la metodología sugerida por Freeland *et al.* (1986) y la clasificación de los organismos se hizo a nivel de órdenes (Valerio 1970)

No hubo diferencias significativas en los porcentajes promedios de ítemes ingeridos por sexo (t-student,  $p > 0.05$ ), por lo que este dato se agrupó (Fig. 1). Los porcentajes promedio de los ítemes ingeridos variaron según la talla. El análisis del contenido estomacal muestra que sus principales alimentos son himenópteros, coleópteros, miriápodos, hemípteros y otros (gastropodos, isópodos, homópteros y neurópteros); lo que confirma su característica

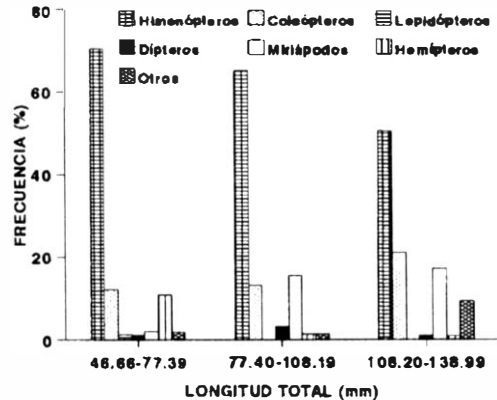


Fig. 1. Frecuencia (%) de los diferentes ítemes encontrados en el contenido estomacal de *Bufo marinus* por ámbito de tallas para la población total.

de especie carnívora oportunista. El alto consumo de himenópteros coincide con lo que señalan para la misma especie Strüssman *et al.* (1984) en Brasil, Freeland y Kerin (1988) en Australia y Toft (1981) en Panamá, sin embargo, su dieta cambia con la talla de los ejemplares, coincidiendo con Strüssman *et al.* (1984). Durante el estudio se capturó un macho de 132.5 mm de Lt que presentaba el estómago lleno de trozos de plástico; se reporta por primera vez ese contenido estomacal en *B. marinus*, lo que indica que esta especie puede ser dañada por la contaminación humana.

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## REFERENCIAS

- Easteal, S. 1981. The history of introductions of *Bufo marinus* (Amphibia:Anura) a natural experiment in evolution. *Biol. J. Linn. Soc.* 16: 93-115.

- Freeland, W. L., B. L. Delvinquer & B. Bonnin. 1986. Food and parasitism of the cane toad *Bufo marinus*, in relation to time since colonization. *Aust. Wildl. Res.* 13: 489-499.
- Freeland, W. J. & S. H. Kerin. 1988. Within-habitat relationships between invading *Bufo marinus* and australian species of frog during the tropical dry season. *Aust. Wildl. Res.* 15: 293-305.
- Honnegger, R. E. 1970. Eine Kröte erobert die Welt. *Natur. Museum.* 100: 447-453.
- Striissmann, C., M. B. Ribeiro do Vale, M.H. Meneghini & W. E. Magnusson. 1984. Diet and foraging mode of *Bufo marinus* and *Leptodactylus ocellatus*. *J. Herpetol.* 18: 138-146.
- Toft, C. A. 1981. Feeding ecology of Panamanian litter anurans: patterns in diet and foraging mode. *J. Herpetol.* 15: 139-144.
- Valerio, C. E. 1970. Una clave artificial para clasificar insectos. *O'BIOS Revista de Ciencias Naturales, Universidad de Costa Rica, San José, Costa Rica* 11: 53- 54.
- Zug, G. 1991. *Bufo marinus* (Sapo Grande, Giant toad, Marine toad). In D. Hanzen (ed). *Historia Natural de Costa Rica. Universidad de Costa Rica. San José, Costa Rica.* 390-392.

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### A case of *lapsus calami* in Costa Rican scorpion nomenclature

(Rec. 1-V-1996. Rev. 30-V-1996. Accep. 13-VIII-1996)

**Key words:** *Chactas*, Chactidae, Costa Rica, *Lapsus calami*, nomenclature, scorpions.

In the *Conspectus Genericus Scorpionorum 1758-1982* Francke (1985) defined *lapsus calami* (significant error) as the case in which an original publication has clear evidence of unadverted mistake. This is supported more generally in the International Code of Zoological Nomenclature [VII, Art. 32 a (ii)]. The case of *lapsus calami* presented here is related with the only species of Chactidae Pocock (1893) known from Costa Rica and can be verified in the recent monograph by Francke & Stockwell (1987).

One species was originally described by Werner (1939) as *Iomachus exsul* (Ischnuridae) based on a single male specimen from Las Mercedes, Limón, Costa Rica (type locality). It was designed as the holotype in the Zoologisches Institut und Zoologisches Museum of Hamburg, Germany, where it was destroyed during World War II. Francke & Stockwell (1987) redescribed this species and designed as

neotype a female from El Valle, Panamá, collected in January 1947 by N.L.H Kraus and deposited in the United States National Museum, Smithsonian Institution, Washington, D.C. They also proposed a new combination (*combinatio nova*) for the species name: *Chactas (Chactas) exsul* (Werner, 1939) Francke & Stockwell, 1987.

This *lapsus calami* was made when Lourenço (1996) published the first description of the male of the species mentioned as *Chactas bonito* Francke & Stockwell, 1987 that according to the author was previously described from Costa Rica on the basis of female specimens. Lourenço (1996) described the male from an specimen collected in Santa Rita, Río Llano Sucio, Colon province, Panama, May 1971 by D. Quintero and deposited in the Muséum National d'Historie Naturelle, Paris (MNHN-RS-7928).

I conclude that *Chactas bonito* Francke & Stockwell, 1987 as mentioned by Lourenço (1996) is a *nomen non publicatum*. The name does not meet the requirements for publication (International Code of Zoological Nomenclature IV, Art. 11 a). This name can not be used or applied to any taxon.

The descriptions of male *Chactas bonito* by Lourenço (1996) and of female *Chactas (Chactas) exsul* by Francke & Stockwell (1987) are similar and probably represent the same species. The *lapsus calami* is here defined as follows: *Chactas bonito* Francke and Stockwell 1987, *lapsus calami* [*Chactas (Chactas) exsul* (Werner, 1939)] in the context of Lourenço (1996)

## REFERENCES

- Alvarado, R., Calogne, F.D. & J. Izco (eds.) 1976. Nomenclatura Biológica. Código Internacional de Nomenclatura Botánica. Código Internacional de Nomenclatura Zoológica. Madrid, Blume Ediciones, 353 p.
- Francke, O.F. 1983. Conspectus genericus scorpionorum 1785-1982 (Arachnida: Scorpiones). Occas. Pap. Texas Tech Univ. 98: 1-32.
- Francke, O.F. & S.A. Stockwell. 1987. Scorpions (Arachnida) from Costa Rica. Special Publication, The Museum Texas Tech University 25: 1-64.
- Lourenço, W.R. 1996. Additions to the scorpion fauna of Panama and Costa Rica. Revista de Biología Tropical 44(1): 177-181.
- Werner, F. 1939. Neu-Eingänge von Skorionen im Zoologischen Museum in Hamburg. Teil II Feitschrift Prof. Embrick Strand, Riga 5: 351-360.

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## Reply to Montoya about the use of the name *Chactas bonito*

(Accep. 30-VIII-1996)

Montoya's comments are correct. I used a proof version of Francke & Stockwell's paper for my bibliography, and in the final printed version they changed their decision about the description of *Chactas bonito* new species, and rather decided to accept the old Werner's species *Iomachus exsul*, in principle also described from Costa Rica. So, for what is of nomenclature Dr. Montoya is right; however, for what is of the validity of Werner's species I am not so sure. In fact the description given by Werner for *Iomachus exsul* is poor and the single illustration bad. Besides, the types were

destroyed in the war. In my opinion, Werner probably associated some juveniles of *Opisthacanthus* to his *Iomachus* species (which only exist in India and parts of Africa). However, young *Opisthacanthus* may look like *Iomachus*. He also cited one specimen of *Opisthacanthus elatus* (one female) in his paper, but this was an adult. In conclusion, my mistake had to be corrected, however, I do think that the *Chactas* present in Costa Rica and Panama should be named as a new species, as was originally intended by Francke & Stockwell.

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