# Quantitative paleoparasitology applied to archaeological sediments

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*Three techniques to extract parasite remains from archaeological sediments were tested. The aim was to improve the sensibility of recommended paleoparasitological techniques applied in archaeological remains. Sediment collected from the pelvic girdle of a human body found in Cabo Vírgenes, Santa Cruz, Argentina, associated to a Spanish settlement founded in 1584 known as Nombre de Jesús, was used to search for parasites. Sediment close to the skull was used as control. The techniques recommended by Jones, Reinhard, and Dittmar and Teejen were used and compared with the modified technique presented here, developed to improve the sensibility to detect parasite remains. Positive results were obtained only with the modified technique, resulting in the finding of* Trichuris trichiura *eggs in the sediment.*

Key words: archaeological sediments - parasite remains - *Trichuris* eggs - archaeological sediment technique - paleoparasitology - Southern Patagonia

Life in the past can be reconstructed by applying different tools of science to understand ancient events. Combined, these tools offer well sustainable data to base and test hypothesis as they are obtained from different fields of science (Walker 1996). The study of bioanthropology in Southern Patagonia has the contribution of History, Archaeology, Anthropology, and Biology. Paleoparasitology emerged as a branch of Paleopathology and aims the study of parasites found in archaeological and paleontological material. Parasites have been found in coprolites, latrine sediments, mummified tissues, hairs, and many other organic remains (Bouchet et al. 2003a). Sediment associated with skeletons has been less explored than other organic remains in the search for parasites. Latrine and soil sediment recovered from latrines and soil occupied by ancient human populations are the most common examined and studied material (Pike 1968, Moore 1981, Bouchet 1995, Bouchet et al. 1999, 2002, Taek Han et al. 2003, Fernandes et al. 2005); shell middens (Bathurst 2005), artifacts (Harter et al. 2003), and skeleton remains (Aspöck et al. 1996, Bouchet et al. 2001, Dittmar & Teejen 2003) are less studied. Parasite remains are supposed to disperse and be lost in sediments. This is the case of Patagonian steppe sandy sediments submitted to hydric and thermal environmental conditions influencing parasite egg preservation in the soil.

Otherwise, sediment collected inside skeletons may offer opportunities to assay paleoepidemiological studies. Skeletons are more commonly found than coprolites, and sex and age can be more easily inferred. Therefore, associated with calibrated dating and well archaeologically and geographically located, skeletons allow obtaining data to associate parasite findings with the human host. Population studies may be approached as far as more skeletons are analyzed.

Sediments have been analyzed with paleoparasitological techniques, modified from Stoll technique (Jones 1988, Taek Han et al. 2003), spontaneous sedimentations (Faulkner et al. 2000, Fernandes et al. 2005), sedimentation-flotation (Bouchet 1995, Bouchet et al. 1999, 2001), and combining sedimentation and palynological technique (Reinhard et al. 1992, Dittmar & Teejen 2003). To understand and interpret correctly parasite finds in sediments of each locality of an archaeological site careful analysis is needed (Reinhard 1988). Even though, to better understand the meaning of parasite finds in archaeological sites frequencies must be achieved comparing parasite finds. Therefore, the study of parasites in archaeological sediments requires the use of quantitative techniques to compare the findings of parasites in different samples and controls.

We strongly reinforce that this kind of archaeological deposit is very important and the data obtained can be improved combining careful sediment in situ extraction method with quantitative paleoparasitological techniques adapted to this kind of material. We have tested techniques used and recommend a slight modification that improves significantly parasite analysis in organic sediments. All tests were performed with Southern Patagonian samples, which were submitted to characteristic environmental conditions.

## MATERIALS AND METHODS

The archaeological site Nombre de Jesús was recorded by Senatore and Guichón in 2005 (Guichón, pers. commun.). The burial site is under open sky. The site corresponds to the first Spanish attempts to colonize the

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Magellan Strait during the XVI century. In 1584 Sarmiento de Gamboa finally established the first settlings called Rey Felipe in the west coast, and Nombre de Jesús in the east, southern of Virgins Cape, Argentina (Fig. 1).

An Indian skeleton was found in 2004 dated of 475 ± 45 yr BP (Ua 22946). Another three skeletons in the same burial place were found during 2005 excavations. They were identified as of Europeans. Excavation was planned to recover burial samples following Jones (1988), Reinhard et al. (1992), and Berg (2002). Pelvic girdle and sacrococcygeal samples were collected. Control samples were collected from skull and the thighbone regions. Only results of the individual NJ-4 corresponding to the finds of the year 2005 are presented here (Fig. 2).

Sediments were rehydrated in trisodium phosphate 0.5% aqueous solution (Callen & Cameron 1960). Quantitative techniques applied in paleoparasitology were used. According to Jones (1982), 3 g of dry sediment were rehydrated in 42 ml of trisodium phosphate 0.5% aqueous solution. Five samples of the same material were made. A 50 µl drop of rehydrated sample was used to prepare ten slides to microscopic analysis. Reinhard et al. (1992) quantitative sedimentation technique was also tested, adding *Lycopodium* tablets containing known quantities of spores (12542 spores). Counting spores and parasite eggs allow calculating how many eggs are in each gram of feces [(eggs counted/spores counted)*12542/sediment weight = eggs parasites by g sediment].

Jones modified technique consists in increasing six times sediment concentration due to the high quantity of sand in the sediment. Five preparations were made for each sample, each one of 5 g of the sediment in 10 ml of 0.5% trisodium phosphate solution. Fifty slides were prepared of each sample using 50 µl drops of shacked and mixed solution and observed at the microscope. Macroscopic hard concretions were observed in one sample.

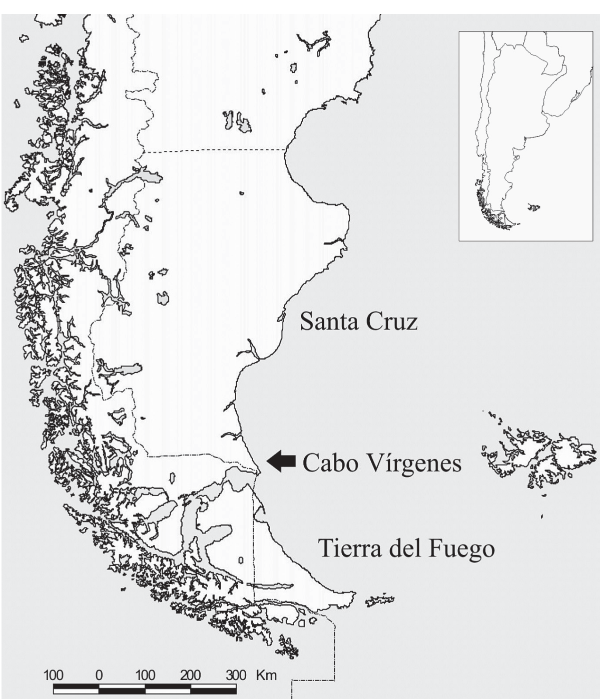


Fig. 1: geographical localization of Nombre de Jesús archaeological site.

Fig. 2: burial human remains of Nombre de Jesús archaeological site, Cabo Vírgenes, Santa Cruz, Argentina.

## RESULTS

Using recommended techniques no parasite eggs were found. Positive results were only obtained in the sediment collected in the pelvic girdle of the skeleton using Jones modified technique. *Trichuris* sp. (Fig. 3) eggs were recovered, with average measurements of 53.25 * 27.87 µm (range 46.25-62.5 * 25-32.5 µm), with a concentration of 5.1 eggs/g/feces.

Poorly preserved parasite eggs were also found (Fig. 4) but could not be identified. Free-living adult nematodes and abundant fungal structures were recovered using Jones modified technique, which was used to examine both the sediment collected from the pelvic girdle, sacrococcygeal, and the control (skull). Positive results contrasted with the control sediment where no parasites were found. Low concentration of fungal spores was also found in the skull sediment.

## DISCUSSION

First paleoparasitological sediment analysis was performed by spontaneous sedimentation in two Indian skeletons in the same archaeological region of Southern Patagonia, Argentina (Fugassa et al. 2004). Both were negative for parasites. Further tests are needed, but results

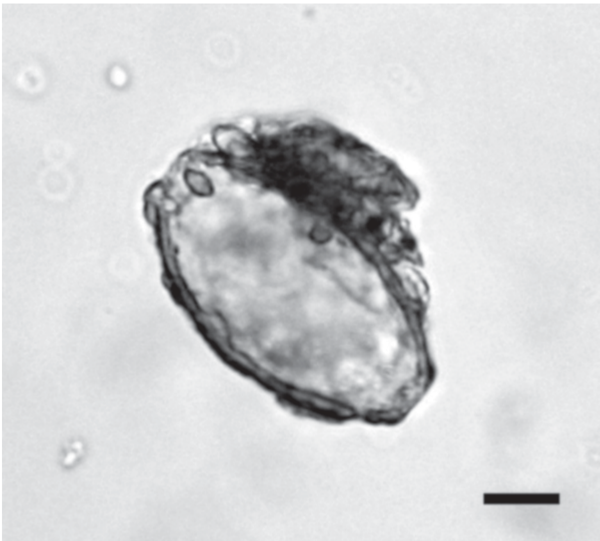

Fig. 3: *Trichuris* sp. egg found in the skeleton pelvic girdle of the archaeological site of Cabo Vírgenes, Santa Cruz, Argentina. Bar: 10 µ.

with the modified technique presented here are promising, especially for sediments submitted to high percolation, thermal and chemical stress, and high sand concentration.

Bouchet et al. (2003a) have advised that the technique developed by Jones is sensible for egg concentration higher than 400 eggs/g/feces. For this reason, increased dilution concentration was tried resulting in positive parasite findings.

Morphometric parameters of *Trichuris* eggs found correspond to those of *T. trichura* (Thienpont et al. 1978). Other *Trichuris* species found in hosts of the region have different egg dimensions (Table). Only *T. pampeana* has close egg measurements to *T. trichiura*. However, the rodent host, *Ctenomys azarae*, is found exclusively in the extreme northern part of Patagonia (Olrog & Lucero 1981). Rodent feces are also easily identified (Chame 2003).

Fragmented structures resembling *Ascaris* sp. eggs were found. Although no definitive diagnosis could be achieved, the association *A. lumbricoides*/*T. trichura* was very common for the same period in Europe (Bouchet et al. 2003b, Fernandes et al. 2005). The relation *A. lum-*

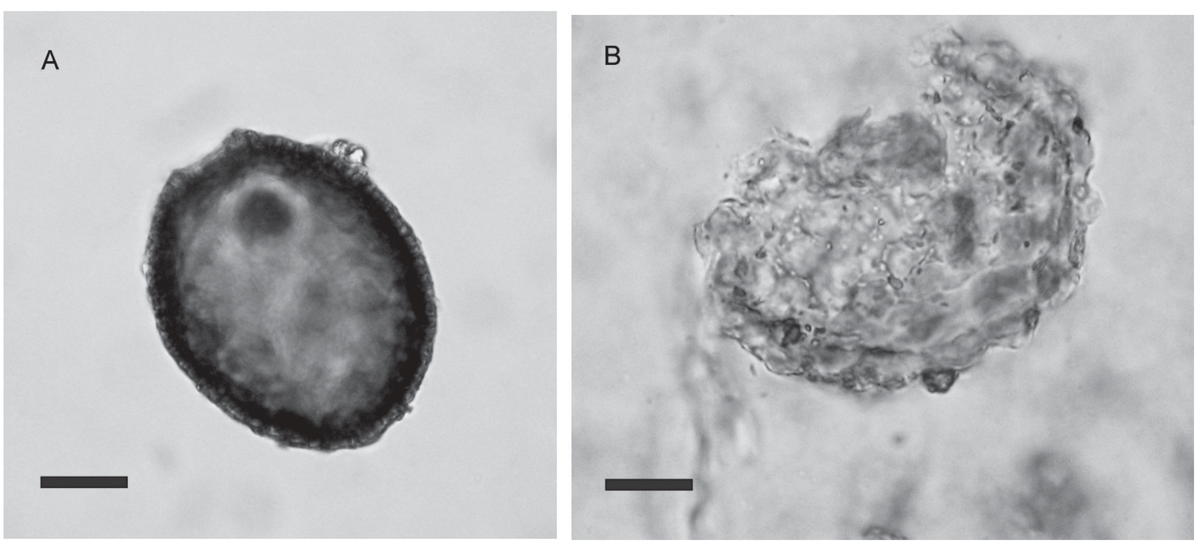


Fig. 4: parasite organic organic remains with no confirmed diagnosis. A: operculated egg; B: *Ascaris* sp. egg-like. Bars: 20 µ.

TABLE

*Trichuris* species recorded in Patagonian rodent and camelid species

| Host | Species | Lenght (m) | Width (m) | Reference |
|---|---|---|---|---|
| *Lama guanicoe* | *Trichuris* sp. | 79 | 32 | Beldomenico et al. (2003) |
| | *T. tenuis* [a] | 46-50 | 28-30 | |
| *Octodon* sp. | *T. bradleyi* | 57-65 | 29-34 | Babero et al. (1975) |
| *Ctenomys* sp. | *T. fulvis* | 65-72 | 28-31 | Babero & Murua (1987) |
| | *T. robusti* | 57-65 | 29-36 | Babero & Murua (1990) |
| | *T. bursacaudata* | 60-70 | 20-30 | Suriano & Navone (1994) |
| | *T. pampeana* | 50-60 | 20-30 | |
| *Akodon* sp. | *T. chilensis* | 60-67 | 32-34 | Babero et al. 1976 |
| *Myocastor coipus* | *T. myocastoris* | 53-60 | 30-34 | Barós et al. (1975) |
| *Dolichotis patagonum* | *T. dolichotis* | 75 | 45 | Morini et al. (1955) |

*a*: reported measurement by Chandler 1930.

*bricoides* and *T. trichiura* recorded for the European sites was 1:3 (Moore 1981) and 1:6 (Jones 1982). Therefore, if these proportions are correct, and taken into account that a density of 5.1 eggs/g/feces was obtained for *T. trichiura*, the probability of finding *A. lumbricoides* eggs is very low, using optical microscopy and the methodology described here. However, human roundworm and whipworm association is so common that *A. lumbricoides/T. trichiura* eggs is the probable diagnosis in this case.

All samples were positive for *T. trichiura*. The reduced number of fungi hyphae and spores in the control sample, in contrast with the pelvic sediment with no parasite eggs evidence the expected decomposition of intestinal region of the body until partially preservation of organic remains was completed. Controls add comparative critic information to know the origin of the material studied (Reinhard et al. 1992).

To conclude, combining systematic bioarchaeological and adapted paleoparasitological techniques allow interesting results regarding parasite findings in the less expected organic remains where parasites are recorded. It is important to note that parasitism is a natural phenomenon, and some parasite species accompany humans throughout the continents since the species arises in African landscapes (Araújo et al. 2003, Montenegro et al. 2006).

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