New records and diet reconstruction using dental microwear analysis for *Neolicaphrium recens* Frenguelli, 1921 (Litopterna, Proterotheriidae)

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**ABSTRACT.** *Neolicaphrium recens* is the only survivor proterotherid in the Quaternary of South America, of which few fossil records from Argentina, Uruguay and probably Brazil are known. In this paper we describe new cranial remains with teeth series of *N. recens* from Artigas Department, Uruguay (Sopas Formation, Late Pleistocene), and previously published remains were reinterpreted and assigned to *N. recens*. We performed a dental microwear study of the seven most complete adult specimens from Argentina and Uruguay. The dental facets of interest were molded with silicone dental impression; the moulds were filled with epoxy resin and observed in a stereomicroscope. The paracone of M2 and the protoconid of m2 were photographed and the images digitally processed and studied. The results showed a high relative percentage of pits (scars of soft plants) on scratches (grooved pattern caused by silicophytoliths of grasses), indicating that the analyzed specimens were browsers, and their diet probably included fleshy leaves, buds and stems of trees and shrubs. These new data are consistent with previous results of carbon and oxygen isotopes studies and with inferences based on the associated fauna of *N. recens* in Uruguay, indicating for the Sopas Formation open to semi open grassland with forested areas, resembling a savanna. This study suggests some woodland environments for all the range of geographical distribution of the taxon.

**Keywords:** Feeding ecology, Paleoenvironment, Microwear, Quaternary, Sopas Formation, Uruguay.

**RESUMEN.** Nuevos registros y reconstrucción de dieta a través de análisis del microdesgaste dentario de *Neolicaphrium recens* Frenguelli, 1921 (Litopterna, Proterotheriidae). *Neolicaphrium recens* es el único proterotérido registrado en el Cuaternario de Sudamérica, del cual se conocen pocos ejemplares de Argentina, Uruguay y probablemente Brasil. En este trabajo se describen nuevos restos craneales con dentición de *N. recens* proveniente del departamento de Artigas, Uruguay (Formación Sopas, Pleistoceno Tardío), y restos fósiles previamente publicados fueron reinterpretados y asignados a *N. recens*. Se realizó un estudio del patrón de microdesgaste dentario de los siete especímenes adultos más completos de Argentina y Uruguay. Las facetas de interés se moldearon con silicona para impresión dental; los moldes se rellenaron con resina epoxi y se observaron en estereomicroscopio. El paracono de M2 y el protoconido de m2 fueron fotografiados y las imágenes estudiadas digitalmente. Los resultados mostraron un mayor porcentaje relativo de hoyos (cicatrices de vegetales blandos) sobre estrías (patrón de surcos causado por silicofitolitos de gramíneas), lo que indica que los ejemplares analizados fueron ramoneadores y su dieta probablemente incluyó hojas carnosas, yemas y tallos de árboles y arbustos. Estos nuevos datos son consistentes con los resultados previos de estudios de isótopos de carbono y oxígeno y con inferencias basadas en la fauna asociada a *N. recens* en Uruguay, indicando para la Formación Sopas, pastizales abiertos a semiabiertos, con áreas forestadas, semejante a una sabana. Este estudio permite sugerir ambientes con áreas boscosas para todo el rango de distribución geográfica del taxón.

**Palabras clave:** Ecología alimentaria, Paleoambiente, Microdesgaste, Cuaternario, Formación Sopas, Uruguay.
1. Introduction

The Litopterna Ameghino, 1889 is one of the native mammalian groups of South America with a wide distribution limited to this continent. They are diverse ungulates that appear in the Early Paleocene, showing a great diversification until the Pliocene when they dramatically decline. They got extinct during the Late Pleistocene/Early Holocene (Prado et al., 2015). The Proterotheriidae is the most diverse family of litopterns, represented by small to medium size cursorial ungulates, with a “horse-like” tendency to the monodactyly - long metapod III, digits II and IV reduced and digit I and V lost. Despite the fact that Frenguelli (1921) described Neolicaphrium recens found in Pleistocene sediments from Argentina, at that time this family was believed extinct in the Pliocene. New findings in Uruguay (Perea et al., 1995) suggested the presence of Neolicaphrium recens in the Quaternary, later confirmed by more collected and reviewed material from north center Argentina, northern Uruguay and probably in southern Brazil (Tauber, 2000; Bond et al., 2001; Scherer et al., 2009; Vezzosi et al., 2009; Ubilla et al., 2011; Luna et al., 2015; Schmidt et al., 2016; Vezzosi, 2016; Gaudioso et al., 2017).

Fossils attributed to N. recens are scarce and fragmented and most of them correspond to mandible remains. Ubilla et al. (2011) described the first cranial remains for the taxon, making progress in the cranial anatomy and expanding the diagnosis. Despite this, the knowledge of its paleobiology is poor. Indirect evidence from the faunal associations that N. recens integrated in Uruguay, indicate that the species was restricted to relative humid and forested environments linked to climatic conditions related to the Marine Isotopic Stage 3 (MIS-3, Late Pleistocene) (Ubilla et al., 2016). In this context, the tooth microwear pattern in N. recens provides the first and novel information in paleoenvironmental interpretations in Pleistocene endemic ungulates.

The aim of this study is to describe a new specimen of N. recens, improving the knowledge of this proterotherids. Furthermore, we analyze the dental microwear pattern of N. recens in order to recognize its dietary preferences. We analyze the congruence with results of previous carbon and oxygen isotopic studies (Morosi and Ubilla, 2017), but expanding the sample and with a greater coverage of the known geographical distribution for the taxon.

2. Geographical and geological settings

Neolicaphrium recens have been recorded for the first time in Córdoba Province, Argentina (Frenguelli, 1921), although the finding of this proterotherid in Quaternary deposits, which was believed extinct in the Pliocene, was not accepted definitely until the current century (Bond et al., 2001). Álvarez (1974) found a mandibular remain that is certainly similar to the specimen described by Frenguelli, which came from Pleistocene beds of the Toropí Creek, Corrientes Province, but she interpreted this fossil as removed within those deposits. Tauber (2000) described carpal and metacarpal bones of N. recens from Pleistocene deposits of Córdoba and Vezzosi et al. (2009) indicated evidence of an immature specimen of N. recens in Santa Fe Province (Tezanos Pinto Formation, Late Pleistocene-Early Holocene). Later, Luna et al. (2015) communicated the finding of dental remains in Córdoba but, unfortunately, there is no precise stratigraphic provenance. Schmidt et al. (2016) shown new materials from Pleistocene deposits of Córdoba, including postcranial remains, and Gaudioso et al. (2017) present the first record for Santiago del Estero Province, from layers of the same age. In southern Brazil (Río Grande do Sul State) there are some postcranial materials from Pleistocene deposits that probably correspond to N. recens, although more findings are necessary to confirm this hypothesis (Scherer et al., 2009).

The specimens from Uruguay (Bond et al., 2001; Ubilla et al., 2011; and the new material here described) have been found in Late Pleistocene sediments of the Sopas Formation (Anton, 1975) (Fig. 1). In recent years, the knowledge of the fossil content of this lithostratigraphic unit has been increased, as well as the mammal associations, and its value as biostratigraphic, paleoecological and paleoenvironmental source of information. The Sopas Formation crops out in northern Uruguay (Artigas, Salto, Tacuarembo, Paysandú and Río Negro Departments) alongside creeks and rivers (Ubilla et al., 2004). The deposits includes medium to coarse-grained sandy and conglomerate basal levels belonging to fluvial facies overlied by mudstones and siltstones linked to floodplains and paleosols. An update of the fossiliferous content of the Sopas Formation and the numerical ages obtained until now, linked to the stratigraphic columns of main localities, can be found in Ubilla and Martínez...
(2016) and Ubilla et al. (2016). The figure 2 shows a detail of the stratigraphic section from where the new *Neolicaphrium recens* material described in this work comes from. In summary, *Neolicaphrium recens* exhibit a distribution pattern in the Argentinian center-northern and northern Mesopotamia, northern Uruguayan and southern Brazilian Pampean area during the Pleistocene *sensu lato*.

3. Materials and methods

3.1. Theoretical framework

The microwear of tooth enamel is the abrasion produced during the mastication by the different food items, generating wear surfaces (Teaford and Oyen, 1989; Solounias and Semprebon, 2002).
The microwear analysis consists in the observation and direct counting on the dental surface or a 2D image obtained by scanning electron microscope (SEM) or stereomicroscope, of scars (typically pits and scratches). In herbivores, a high proportion of scratches on pits indicate the consumption of foods with higher content of silica or sand. These scars are caused by the high content of silicophytoliths present in the walls of grasses, which are the main source of food for grazers (Solounias and Moelleken, 1993; Solounias and Semprebon, 2002). On the other side, a higher frequency of pits on scratches indicates more fragile or delicate food consumption and potential seeds, which characterizes a browser diet. Unlike processing monocotyledons (grass), dicots have few silicophytoliths, whereby the browsers have proportionally few striations. The mixed-feeder regimen displays an intermediate pattern between these two extremes. It is important to point out that since the turnover rate of the abrasion scars is of few days or even daily, the microwear pattern gives us information about food eaten only a few days or hours before the death of the organism (Teaford and Oyen, 1989). A briefly background on microwear studies and the justification in this framework can be seen in the Appendix.

3.2. Studied materials

The studied remains correspond to seven adult specimens, all of them with accurate stratigraphic origin and housed at institutional collections of Uruguay and Argentina: Colección Paleontológica de Vertebrados de la Facultad de Ciencias (FC-DPV); Museo de Arqueología y Ciencias Naturales de Salto (MACN-S); Museo Histórico Departamental de Artigas (MHD-P); Museo La Plata (MLP); Museo Argentino de Ciencias Naturales “Bernardino Rivadavia” (MACN-PV) and Museo de Ciencias Naturales de la Región de Ansenuza “Aníbal Montes”, Miramar (MAMM-PV).

FC-DPV 776, right and left partial dentary; Sopas Creek, Salto, OSL ages: 43,500±3,600 and 30,600±5,400 yrs (Ubilla et al., 2016); described by Bond et al. (2001).

MACN-S 92, partial skull; Arapey Grande River, Salto. Late Pleistocene. See complete description in Ubilla et al. (2011).

MHD-P 509 (new specimen here described, Fig. 3) right and left partial upper dental series and basal
portion of zygomatic; Cuareim River, Artigas, OSL age: 36,100±6,200 yrs (Ubilla et al., 2016).

MAMM-PV 098, isolated right m2; Mar Chiquita Lagoon, Córdoba. Late Pleistocene. Described by Schmidt et al. (2016).

MACN-PV 16603b, isolated right M3; Coronda River, Sante Fé. Late Middle to Late Pleistocene (Iriondo and Kröhling, 2009; Vezzosi et al., in press). Described by Ubilla et al. (2011) as M1-2? of Neolicaphrium sp., designed here as N. recens.

MLP 34-V-22-12, holotype of N. recens, partial left dentary with p3-m3; Cuarto River, Altos de San Vicente, Córdoba. Middle to Late Pleistocene. See Frenguelli (1921).

3.3. Methodology

In this work, we follow the methodology described by Merceron et al. (2004a, 2005). The fossils of Neolicaphrium removed from original stratigraphic position, very fragmented, or immature individuals were not considered. Before beginning the preparation of the dental pieces, it was verified that they did not suffer any type of diagenetic alteration (King et al., 1999), fact that was discarded and allowed to continue without affecting the sample size. The analysis was performed on the second molars (Appendix, Fig. 1), except for one specimen (MACN-PV 16603b) for which we use M3, with the aim of increase the number of studied specimens. For detailed procedures see the Appendix. To avoid errors all the protocol (identification, counting and treatment of images) was carried out by a single operator (AC). The following microwear variables were recorded: total number of scratches, total number of pits, large pits and gouges per 0.09 mm². We distinguished pit as a scar that have a maximum/minimum diameter ratio less than four. More elongate features were considered scratches (Gordon, 1982; Merceron et al., 2004a; Mihlbachler et al., 2012). Large pits are deeper than pits, less refractive (always dark), with irregular outlines. Gouges are big scars, with irregular edges, deeper and larger than large pits (Solounias and Semprebon, 2002).

4. Systematic paleontology

Order Litopterna Ameghino, 1889
Family Proterotheriidae Ameghino, 1887
Neolicaphrium Frenguelli, 1921
Type Species: Neolicaphrium recens
Frenguelli, 1921
Neolicaphrium recens Frenguelli, 1921
Figure 3, Table 1, Appendix Fig. 2.

Referred specimens. MHD-P 509, a partial right and left cheek dentition (P4-M3) with the jugal portion...
of the zygomatic arch and a little part of the lacrimal bone preserved (Fig. 3, table 1). MACN-PV 16603b, isolated right M3 with scarce wear (M1-2? sensu Ubilla et al., 2011) (Appendix Fig. 2).

**Locality and age.** MHD-P 509: Cuareim River (30°30’44.95”S; 56°20’32.33”W), Artigas, Uruguay, Sopas Formation, Late Pleistocene. MACN-PV 16603b: Coronda River, Bajo Hondo, Santa Fe, Argentina, late Middle to Late Pleistocene (sensu Vezzosi, 2016).

### 4.1. Description

The teeth of MHD-P 509 are similar in form and size to those described by Ubilla et al. (2011). The series P4-M3 is 45 mm in length, with all teeth wider than length. The anterior lingual cingulum is developed in P4-M3 and filled with cementum. P4 and M1 have the highest degree of wearing, being the cusps rounded to blunt in shape. The inner cementum is lost and the dentine is exposed. M1-M3 show variable development of the paracone and metacone folds. M2 is the largest tooth, subquadrangular, with rounded to sharp cusps. The cementum is coating the molars externally, especially M2 and M3 still preserving the inner cementum. In these teeth, the paracone and metacone are the most developed cusps, the *postcingulum* is sharp and the hypocone is especially reduced in M3. As in the specimen MACN-S 92 the M1-M3 metaconules are reduced as a slight crest. The quantitative comparison of MHD-P 509 with the specimens MACN-S 92 and FC-DPV 749 show that they are closely similar, being the M1 and M2 the less variable teeth, respect to size.

The following set of anatomical features allow assigning this material to *N. recens*: infraorbital foramen located at the P4 level; P4-M3 with a very well developed anterolinguinal cingulae; cementum covering the lateral walls of P4-M3, central valleys, and part of the occlusal surface; metaconule reduced to a small transverse crest connecting the hypocone to the metacone (Fig. 3).

MACN-PV 16603b has been completely described by Ubilla et al. (2011) as *Neolicapriephyrium* sp. These authors attribute the differences observed between this specimen and the Uruguayan specimens of *N. recens* (*e.g.*, size, less worn, thick cementum covering many crown features) to age differences, being MACN-PV 16603b a younger specimen. Moreover, there are other features to emphasize: 1) The way in which the paraconule, protocone and hypocone are arranged, with the protocone very lingually projected, forming a linguo-distal face of semicircular edge and not so straight as it happens in M1-M2; 2) the complexity of the enamel bands delimiting the mesio-distally elongated central fossa. These characters plus those enunciated by Ubilla et al. (2011) are exclusive of the M3 of *N. recens*, which allows assigning this molar to that taxon.

### 5. Results and discussion

#### 5.1. Feeding habits

All microwear data collected from *N. recens* are available in table 2. The predominant scars observed were small pits (Fig. 4). Some gouges, large pits and fine scratches were also present (Fig. 5). According to Solounias and Semprebon (2002) scratches are more discriminating than pits because there is no overlap in the scratch averages between browsers and grazers. Furthermore, the range of individual scratches is 0 to

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**TABLE 1. DENTAL MEASUREMENTS OF THE SPECIMEN MHD-P 509.**

<table>
<thead>
<tr>
<th>MHD-P 509 right side</th>
<th>P4-M3</th>
<th>P4</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
</tr>
</thead>
<tbody>
<tr>
<td>length</td>
<td>43.9</td>
<td>10.8</td>
<td>10.1</td>
<td>12.9</td>
<td>9.8</td>
</tr>
<tr>
<td>width</td>
<td>13.7</td>
<td>14.8</td>
<td>15.5</td>
<td>13.4</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MHD-P 509 left side</th>
<th>P4-M3</th>
<th>P4</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
</tr>
</thead>
<tbody>
<tr>
<td>length</td>
<td>45</td>
<td>10.8</td>
<td>9.7</td>
<td>12.2</td>
<td>12</td>
</tr>
<tr>
<td>width</td>
<td>13.7</td>
<td>14.5</td>
<td>16.3</td>
<td>14.6</td>
<td></td>
</tr>
</tbody>
</table>

All measurements in millimeters.
TABLE 2. SUMMARIZE OF MICROWEAR VARIABLES.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Np</th>
<th>Ns</th>
<th>TN (p+s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FC-DPV 776</td>
<td>10</td>
<td>1</td>
<td>11</td>
</tr>
<tr>
<td>FC-DPV 749</td>
<td>18</td>
<td>0</td>
<td>18</td>
</tr>
<tr>
<td>MHD-P 509</td>
<td>22</td>
<td>10</td>
<td>32</td>
</tr>
<tr>
<td>MACN-S 92</td>
<td>20</td>
<td>3</td>
<td>23</td>
</tr>
<tr>
<td>MACN-PV 16603 b</td>
<td>10</td>
<td>0</td>
<td>10</td>
</tr>
<tr>
<td>MLP 34-V-22-12</td>
<td>12</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td>MAMM-PV 098</td>
<td>11</td>
<td>2</td>
<td>13</td>
</tr>
</tbody>
</table>

Np: number of pits; Ns: number of scratches; TN: total number of scars per 0.09 mm².

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17.5 for browsers and 17.5 to 29 for grazers (Solounias and Semprebon, 2002; Merceron et al., 2004a). The average number of pits overlaps between browsers and grazers, especially at less than 20 pits, so this number alone cannot be taken into account for discriminate browsing from grazing ungulates. The low number of scratches and high proportion of pits observed in the studied specimens (Table 2) are consistent with a browser diet for Neolicaphrium recens.

5.2. Dietary items

The microwear pattern recognized to N. recens possibly imply low intakes of silicophytoliths (no grass intake) and/or foliar strata with a low percentage of sediment (far from the ground). A diet with that features may probably include soft elements, such as fleshy leaves, buds and stems of trees and shrubs.

N. recens did not shows the typical pattern recognized for browser species that ingest heavy (hard) fruits or seeds, for example higher number of scratches than traditional browsers, higher percentages of coarsely or mixed types of scratches than found in traditional browsers or grazers, and very high percentages of large pits (Solounias and Semprebon, 2002).

5.3. Intraespecific variability

All the specimens here analyzed belong to the same biological entity but, even that, one of them (MHD-P 509) shows a coherent pattern of some deep pits and fine scratches on both right and left molars. This pattern matches with the evidence provided by the results of carbon and oxygen isotopic studies (Morosi and Ubilla, 2017) indicating a proportion of C3 plants consumption for the specimen MHD-P 509 lower than the other individuals of N. recens.

The observed differences in MHD-P 509 respect to the other specimens could have different explanations as the following:
1. The habitat where the specimen MHD-P 509 lived was a more open place respect from where the other specimens come.

FIG. 5. Detail of microwear of the specimen MHD-P 509 where pits (1), large pits (2) and fine scratches (3) are shown. Scale bar 500 micrometers.
2. Some seasonal changes in the environment could be possible as was demonstrated in the pioneering work of Walker et al. (1978). The change of feeding preferences in response to seasonal changes has been widely documented in extant ungulates. For example, Novello et al. (2010) pointed that the small antelope Tragelaphus scriptus adopts different feeding strategies during the year from young grasses and non-graminoid herbaceous in the wet season, to dicots leaves the rest of the year. There are evidence that support seasonal environment in the deposits of the Sopas Formation, such as the presence of the migrant bird Chloephaga picta and the record of worm aestivation chambers (Castrichnus incolumis) (Ubilla et al., 2016).

3. It is even possible to think that the different wear pattern may be showing simple individual variation. Solounias and Semprebon (2002) stated that the number of scratches can fluctuate widely within the same species: e.g., 0-36; 2.5-51.5 (p. 18).

5.4 Tooth wear versus gross morphology

Dental microwear analysis provides insights into the diet of extinct species trough comparison with extant species, being possible to relate dental morphology, the environment and the food composition. However, some contradictions may occur if results are compared with additional evidences. Fraser and Theodor (2011) performed a discriminant analysis to find how well the most commonly used dietary proxies classify ungulates into the main dietary categories (browsers, grazers, and mixed feeders). They found that mesowear and microwear perform better than morphological dietary proxies, though none are strong proxies in isolation.

Traditionally, high-crowned (hypsodont) teeth that are widely found among mammalian herbivores have been assumed as a mechanism for endure wear tooth (due to a higher intake of abrasive food, e.g., grass). In the opposite extreme are the brachyodont or low-crowned teeth. Therefore, a consistent pattern of microwear would be expected: a higher percentage of scratches in relation to pits (grazer) in a hypsodont tooth and a higher percentage of pits on scratches (browser) in a brachyodont one. Nevertheless, Solounias and Semprebon (2002) indicated that some brachyodont species was a mixed feeder-grazer while other hypsodont species was browser (see a full updated discussion in Damuth and Janis, 2011).

The teeth of Neolicaphrium recens are mesodont (HI following Janis, 1988=2.00-2.50) and the molariforms are covered with cementum and accordingly is expecting a higher percentage of abrasive scars and a more mixed diet. Bond et al. (2001) suggested that the relatively high values of mesodonty recorded for Neolicaphrium recens, along with the presence of cementum, are indicators of an adaptation to forested, savanna-like habitats. Actually, this observation is in agreement with the information provided by the associated fauna (Ubilla et al., 2004) and the results of carbon and oxygen isotopic studies (Morosi and Ubilla, 2017).

5.5 Behavioral plasticity and scale

Organisms show high behavioral plasticity in diets, which is independent of tooth hypsodonty and must be recognized as a long term adaptation, being related to a different time scale level: microwear is the result of a single organism in his environment and hypsodonty is a condition reached by adaptation during the evolution of the species. In fact, there are many documented inconsistencies in different organisms because different proxies may predict different diets for the same species (see comprehensive review in Davis and Pineda, 2016). Incongruence can be caused by proxies recording diet over vastly different timescales. There are few previous studies of microwear pattern in South American mammals (Notoungulata: Townsend and Croft, 2008; Billet et al., 2009. Gomphotheriidae: Acevedo et al., 2012; Avilla et al., 2014. Cervidae: Rotti et al., 2018). Townsend and Croft (2008) reached the same contradictory results between diet previously inferred by hypsodonty and those based on microwear.

Each proxy records a slightly different aspect of diet (Davis and Pineda, 2016), so we have to think if our results are congruent with the question we made at the beginning of the research, in this case: which were the food preferences of these analised specimens of Neolicaphrium recens? We consider that this questioning has been successfully answered taking into account the number of specimens available and the data obtained from them. This is the first time that a microwear analysis is performed on litopterns, we therefore took a conservative attitude. Even that, there is enough objective evidence to continue increasing the use of this method to the study of these ungulates.
6. Final remarks

The new specimen (MHD-P 509) of Neolicaphrium recens here described correspond to one of the most complete upper dentition materials and with an intermediate tooth wear, which allowed to identify diagnostic characters. They also offered a comparative framework to confirm that the molariform (MACN-PV 16603b) primarily assigned to Neolicaphrium sp, corresponds to a M3 of N. recens. Both specimens represent new localities where the taxon is recorded, expanding its range of geographical distribution.

The data obtained in our study revealing the feeding habits of seven specimens of Neolicaphrium recens, indicating a browser diet. It must be stated that in the microwear pattern we are only seeing the scars produced by food items ingested just a few days before death. Therefore, this result is very specific and is not indicating a pattern of food preferences over a lifetime of the organism or the result of an adaptive process of the species. This data only show the preferences of the studied organisms confined to a restricted environmental context.

Our results are consistent and complementary with the result of carbon and oxygen isotopic studies performed on N. recens specimens restricted to Late Pleistocene of northern Uruguay (Morosi and Ubilla 2016, 2017). The isotopic results show a browser regimen, which means that the carbon incorporated through diet came from C3 plants (predominantly trees and shrubs with a minor portion of grass). This matches with the proposals of environments reconstructed from the fauna associated with N. recens, indicating for the Sopas Formation open to semi open grassland with forested areas (tree and shrub vegetation), resembling a wooded savanna (Ubilla et al., 2016). Since our study encompasses a larger geographical area and a larger number of specimens, the results suggest that the taxon is potentially useful as environmental indicator, even though the samples come from different localities, not necessarily contemporaneous.

This is the first microwear analysis on the family Proterotheriidae shedding light to the paleobiology of a Pleistocene species and to the paleoecological and paleoenvironmental reconstruction.

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Appendix

Materials, methods and specimens used in this study

1. Methodological background and fundamentation

The studies of the microwear pattern began in the 70’s trying to find correlations between this microscopic pattern and the diet of extant organisms to use this tool as a proxy in fossils (Walker et al., 1978; Ryan, 1979a, b; Covert and Kay, 1981; Gordon, 1982, 1988; Teaford and Oyen, 1989). All these works, even more recent ones, use SEM to visualize the dental surface, a method that is expensive, time-consuming and very dependent of the expertise of the observer. Ungar (1995) developed a software that allow a semi-automated image analysis making more automated approaches feasible. Alternatives methods were developed by Solounias and Semprebon (2002) and Merceron et al. (2004a, b; 2005) which use a stereomicroscope and refracted light to visualize the scars at low magnification (LM: X35 and X30). Solounias and Semprebon (2002) make the counting directly on the tooth’s cast surface while Merceron et al. (2004a, b) do it on a high resolution micrograph in the computer, using specific software. These LM’s methods are low-cost and faster compared to SEM, allowing a larger number of specimens to be analyzed.

There are some works that have attempted to calculate the error rates associated with the SEM method (Grine et al., 2002; Galbany et al., 2005) and LM method (Semprebon et al., 2004; Mihlbachler et al., 2012). Semprebon et al. (2004) argue that it is possible (and statistically strong) to reproduce the method of identification and counting at LM (X35) by a single researcher as well as by several independent researchers. By the other hand, Mihlbachler et al. (2012) indicate that the inter observer differences are frequent and therefore the comparisons of independent data (meaning a different operator) obtained by the same technic are unreliable.

2. Specific techniques

The scars were recorded at the paracone and protoconid (as usually occur in ungulates), and even in the dentine or in the junction dentine-cementum immediately adjacent to that facets (Fig. 1). The teeth were cleaned with a soft brush and ethanol, preparing the occlusal surface under stereomicroscopy to ensure the elimination of any kind of glue or sediment particles. In order to preserve the integrity of the fossils (most of them in a delicate state of conservation), we generated moulds from each piece of interest. The moulds were prepared by double impression (first step: rigid putty; second step: light silicone) with polysiloxane (condensation silicone) impression material and were filled with transparent epoxy resin (60% resin and 40% catalyst) to make the casts (Fig. 1). The cast were prepared on a vibratory plate for eliminate bubbles. The polymerization of the resin was 100% complete after 24 hours. These casts were covered with an ultra thin coating of gold, observed in a Nikon SMZ800 stereomicroscope and photographed at 30X using a Nikon digital sight ds-f1 camera mounted to the stereomicroscopy. Then, the photographs were treated in the computer: a square of 300 µm x 300 µm (0.09 mm²) was defined for quantification, and it was replicate in two different regions of the facets and the mean number was recorded.
FIG. 1. A. Materials and instruments used in making the moulds; B. Metalized epoxy resin casts showing the analyzed areas (M2 paracone in upper dentition and m2 protoconid in lower dentition) enclosed in the red squares; C. One of the tooth surfaces (lower dentition) observed showing the area of 300 µm x 300 µm defined for counting the scars.
FIG. 2. Photographs of the specimens of *Neolicaphrium recens* used in this study. A. MACN-S 92; B. FC-DPV 776; C. MLP 34-V-22-12, holotype of *N. recens*; D. MAMM-PV 098; E. MACN-PV 16603b; F. FC-DPV 749. Scale bar: 1 centimeter.