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# Occurrence of periodontal pathogens in ethnic groups from a native Brazilian reservation

Elerson Gaetti-Jardim Jr.<sup>a,\*</sup>, Maurício Fabiano Pereira<sup>a</sup>,  
Evanice Menezes Marçal Vieira<sup>a</sup>, Christiane Marie Schweitzer<sup>b</sup>,  
Ana Cláudia Okamoto<sup>a</sup>, Mario J. Ávila-Campos<sup>c</sup>

<sup>a</sup> Department of Pathology and Clinical Propaedeutics, School of Dentistry of Araçatuba, Univ Estadual Paulista – UNESP, Araçatuba, São Paulo, Brazil

<sup>b</sup> Department of Mathematics, Faculty of Engineering, Univ Estadual Paulista – UNESP, Ilha Solteira, São Paulo, Brazil

<sup>c</sup> Anaerobe Laboratory, Department of Microbiology, Institute of Biomedical Science, University of São Paulo, São Paulo, Brazil

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## ABSTRACT

**Objective:** The present study was designed to evaluate the occurrence of periodontal pathogens in the subgingival biofilm of 100 native Brazilians living at the Umutina Indian Reservation, Mato Grosso State, Brazil.

**Methods:** Periodontal clinical examinations were carried out prior to collection of subgingival biofilm, and the presence of 14 periodontal microorganisms was evaluated by polymerase chain reaction (PCR). The prevalence and risk analysis was performed using Cochran and Mantel–Haenszel statistics for dichotomous variables or Pearson's chi-squared test for analysis of proportions when variables had three or more categories. The interrelations between clinical and microbiological parameters were assessed using Fisher's exact test and the Mann–Whitney *U* test.

**Results:** Individuals with chronic periodontitis were frequently colonized by the association between *Porphyromonas gingivalis* and *Campylobacter rectus*, *P. gingivalis* and *Prevotella intermedia*, or *P. gingivalis* and *Tannerella forsythia*. Patients with chronic periodontitis were also colonized by *Porphyromonas gulae* and *P. intermedia* or by the association between *P. gulae* and *T. forsythia*. *P. gulae* was detected only in the subgingival samples from natives on a traditional diet. Gingival bleeding was associated with *Aggregatibacter actinomycetemcomitans*, *Fusobacterium nucleatum*, *T. forsythia*, *P. gingivalis*, *P. gulae*, *Porphyromonas endodontalis*, *P. intermedia*, and *Prevotella nigrescens*. *Treponema denticola* was uncommon.

**Conclusions:** Peculiar microbiota was demonstrated to be associated with different periodontal disease statuses in native Brazilians, with modest occurrence of certain pathogens, such as *T. denticola*, and the presence of *P. gulae* in natives with gingivitis or chronic periodontitis.

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\* Corresponding author at: R. José Bonifácio 1193, 16015-050 Araçatuba, São Paulo, Brazil. Tel.: +55 18 3636 2797; fax: +55 18 3636 4125.  
E-mail address: [egaettij@foa.unesp.br](mailto:egaettij@foa.unesp.br) (E. Gaetti-Jardim Jr.).

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## 1. Introduction

Estimates suggest that the Brazilian indigenous population was from three to five million natives during the period preceding the first contact with Europeans. At present, 300,000 still live in the Amazon region and in the vast central Brazilian highlands, distributed in 215 groups, whose communities have different integration statuses with the nonnative society, corresponding to 0.2% of the Brazilian population. Depending on contact with nonindigenous communities, the distribution of different types of oral diseases may change, reflecting changes in habits and nutrition, among other factors.<sup>1</sup>

Periodontitis is a highly prevalent infectious disease that leads to periodontal destruction and early tooth loss. The aetiology of these infections frequently involves members of the oral microbiota, particularly strict and facultative anaerobes of the genera *Aggregatibacter*, *Eikenella*, *Campylobacter porphyromonas*, *Prevotella*, *Fusobacterium*, *Tannerella*, and *Treponema* spp.<sup>2–5</sup> In addition, the microbiota associated with periodontitis shows important geographical particularities,<sup>6–8</sup> at times influenced by the ethnic/racial characteristics of the population.<sup>6,7,9</sup> However, little attention has been dedicated to the study of this microbiota in remote communities, such as the native population of the Umutina Indian reservation, where the way of life is strongly linked to their ancestral lifestyle with no history of racial mixing with nonnative communities.

This population encompasses a group of 480 native Brazilians of the Umutina, Paresi, Bororo, Bakairi, Kayabi, Irantxe, Nambikwara, and Terena ethnicities, who have occupied the same geographic area for six generations. This population has evidenced significant endogamy and presence in the oral cavity of some deviations from normality linked to genetic inheritance, such as ankyloglossia, which is much more frequent in the Umutina reservation than in non-Indian communities living around the reservation.<sup>1</sup>

The present investigation evaluated the distribution of major periodontal pathogens in the Umutina population, through polymerase chain reaction (PCR), correlating them with the periodontal status, lifestyle, tobacco and alcohol consumption, as well as the socioeconomic and cultural aspects of the native population.

## 2. Materials and methods

This study was approved by the Institutional Review Board of the School of Dentistry of Araçatuba, Univ Estadual Paulista – UNESP (2006-01417), Brazilian National Indian Foundation (FUNAI), and Mato Grosso School of Public Health (021/07 CEP/SES-MT), Brazil. The informed consent form was read and explained to all eligible individuals and the leaders of the communities, in Portuguese and in local languages. All subjects who agreed to participate were asked to sign the informed consent form. Anthropologists and social workers assisted in the application of a questionnaire to assess diet patterns, socioeconomic/cultural conditions, and tobacco and alcohol consumption.

Clinical examinations, collection, and sample processing were performed from December 2005 to December 2010.

Periodontal clinical examinations were carried out in 100 native Brazilians of the Umutina, Paresi, Bororo, Bakairi, Kayabi, Irantxe, Nambikwara, and Terena ethnic groups, living at the Umutina Indian Reservation (15°05'28.67" S, 57°06'27.10" W), Mato Grosso State. The inclusion criteria were as follows: age of at least 18 years (18–70 years; mean 36.2 ± 21.3 years) and the presence of 20 scorable teeth (not including the third molars). The exclusion criteria were systemic illnesses (diabetes mellitus, cancer, HIV, metabolic diseases, radiation, or immunosuppressive therapy), pregnancy or lactation, systemic antibiotics and/or anti-inflammatory drugs (within the previous 6 months), and dental or periodontal therapy in the last year. The demographic and periodontal status of the population studied is presented in Table 1.

### 2.1. Periodontal examinations

Periodontal clinical examinations were carried out by a single trained examiner. The clinical probing depth and gingival recession were measured and used to determine the clinical attachment level. These measures were obtained from six sites per tooth (mesio-vestibular, vestibular, disto-vestibular, mesiolingual, lingual, distolingual), excluding the third molars. The presence of supragingival biofilm and marginal

**Table 1 – Characteristics of the population examined.**

Characteristic	PHS <sup>a</sup>	GS <sup>b</sup>	CPS <sup>c</sup>
Age	18.04 ± 2.12	21.13 ± 1.09	33.01 ± 1.06
Gender			
Male N (%)	5 (35.7)	20 (41.7)	14 (36.8)
Female N (%)	9 (64.3)	28 (58.3)	24 (63.2)
Type of diet			
Traditional indigenous <sup>d</sup>	6 (42.9)	32 (66.7)	29 (76.3)
Industrialized <sup>e</sup>	8 (57.1)	16 (33.3)	9 (23.7)
Alcohol consumption (%) <sup>f</sup>	1 (7.1)	3 (6.3)	2 (5.2)
Tobacco consumption (%) <sup>g</sup>	0 (0.0)	4 (8.3)	2 (5.2)
Clinical probing depth (mm)	1.92 ± 0.23	3.09 ± 0.85	5.59 ± 1.17
% of sites with			
Visible plaque	22.45 ± 13.02	41.76 ± 11.75	48.22 ± 10.7
Bleeding on probing	12.44 ± 1.12	42.29 ± 2.36	38.88 ± 0.68
Bone loss	0.87 ± 0.21	4.22 ± 2.66	42.08 ± 2.06
Tooth mobility	1.02 ± 0.14	20.04 ± 1.62	27.56 ± 1.12
Food retention	3.02 ± 0.28	8.22 ± 1.44	21.04 ± 1.26
Gingival edema	8.33 ± 2.1	39.24 ± 2.44	36.42 ± 1.22

<sup>a</sup> Periodontally healthy subjects (N = 14).

<sup>b</sup> Subjects with gingivitis (N = 48).

<sup>c</sup> Subjects with chronic periodontitis (N = 38).

<sup>d</sup> Percentage of natives with diet based on traditional indigenous food products (products from hunting, fishing and small community gardens).

<sup>e</sup> Percentage of natives with diet based on industrial or nonnative food products.

<sup>f</sup> Percentage of natives who consume alcohol.

<sup>g</sup> Percentage of natives who smoke.

bleeding were recorded with the visible plaque index (yes/no) and the gingival plaque index, respectively.

## 2.2. Collection of clinical samples

Samples from the three deepest periodontal sites and gingival bleeding were collected from patients with gingivitis or periodontitis. In healthy subjects, subgingival samples were collected from the mesio-vestibular, vestibular, and disto-vestibular periodontal sites of the right or left upper first molar. The sites were isolated and dried with cotton rolls before sampling. After removing the supragingival biofilm and calculus with curettes and sterile cotton pellets, subgingival biofilm samples were obtained by inserting three sterile paper points into the bottom of the sulcus or pocket. After 60 s, the paper points were removed, pooled, and transferred to ultrapure Milli-Q water and kept at  $-196^{\circ}\text{C}$ . DNA was extracted using a QIAamp DNA kit (QIAGEN, Hilden, Germany), according to the manufacturer's instructions. Concentrations of bacterial DNA were determined in the spectrophotometer by reading the absorbance at 260 nm.

## 2.3. Detection of periodontal microorganisms

The presence of *Aggregatibacter actinomycetemcomitans*, *Campylobacter rectus*, *Dialister pneumosintes*, *Eikenella corrodens*, *Fusobacterium nucleatum*, *Parvimonas micra*, *Porphyromonas endodontalis*, *Porphyromonas gingivalis*, *Porphyromonas gulae*, *Prevotella intermedia*, *Prevotella nigrescens*, *Selenomonas sputigena*, *Tannerella forsythia*, and *Treponema denticola*<sup>10,11</sup> was assessed by PCR.

DNA amplification was performed in volumes of 25  $\mu\text{L}$ , containing 2.5  $\mu\text{L}$  of 10 $\times$  PCR buffer, 1.25  $\mu\text{L}$  of  $\text{MgCl}_2$  (50 mM), 2.0  $\mu\text{L}$  of deoxyribonucleotide (dNTP) (10 mM), 0.25  $\mu\text{L}$  of Taq DNA polymerase (0.5 U), 1.0  $\mu\text{L}$  of each primer (0.4  $\mu\text{M}$ ), 7  $\mu\text{L}$  of ultrapure Milli-Q water, and 10  $\mu\text{L}$  of DNA (ng), in a thermocycler (Perkin Elmer, GeneAmp PCR System 2400) programmed for an initial cycle of  $94^{\circ}\text{C}$  (5 min), 30–36 cycles of  $94^{\circ}\text{C}$  (30 s to 1 min), with an annealing temperature of each primer (30 s to 1 min) of  $72^{\circ}\text{C}$  (30 s to 1 min), and a final cycle of  $72^{\circ}\text{C}$  (5 min) for complete extension of DNA amplicons. In all reactions, DNA samples for reference of the microorganisms studied were used as positive control. PCR amplification products and 1-kb DNA ladder (Gibco, São Paulo, Brazil) were submitted to electrophoresis in 1% agarose gel, stained with ethidium bromide (0.5  $\mu\text{g}/\text{mL}$ ), and photographed on a UV transilluminator.

## 2.4. Statistical analysis

Data were plotted and analysed using SPSS software. The prevalence and risk analysis were performed using Cochran and Mantel-Haenszel statistics for dichotomous variables or Pearson's chi-squared test for analysis of proportions when variables had three or more categories. The interrelations between clinical and microbiological parameters were assessed by Fisher's exact test and Mann-Whitney U test. Statistical tests were carried out using Bonferroni correction with  $p$ -value adjusted from 0.05 to 0.00357, due to the detection of 14 microbial species.

## 3. Results

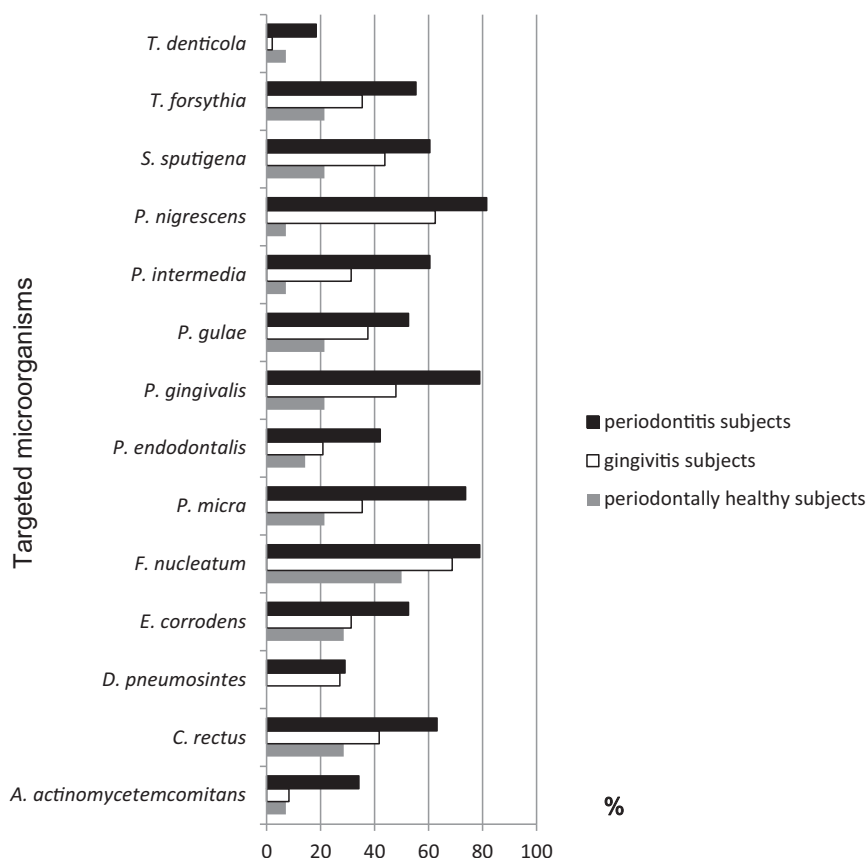
Clinical data related to periodontal status (Table 1) demonstrated that only 14% of the population was periodontally healthy (probing depth  $<3$  mm and no clinical evidence of gingival bleeding on probing), 48% had gingivitis (probing depth  $<3$  mm and at least 30% of periodontal sites with bleeding on probing), and 38% had chronic periodontitis (at least three periodontal sites presenting pocket depth  $>5$  mm and  $\geq 30\%$  of periodontal sites with bleeding on probing). Aggressive and necrotizing periodontitis was not detected. Age was associated with gingival oedema (chi-squared test,  $p < 0.001$ ), food retention (chi-squared test,  $p = 0.001$ ), and clinical probing depth (Mann-Whitney test,  $p < 0.001$ ). The traditional diet was predominant (chi-squared test,  $p = 0.0026$ ), observed in 67% of the population; fishing and hunting were the most frequent sources of proteins, while tubercles and typical roots from the native cuisine as well as small subsistence farms complemented the food source of the population (Table 1). It was noted that, in the population examined, the tooth mobility was significantly higher among natives who consumed alcohol and had abandoned the traditional diet (chi-squared test,  $p < 0.001$ ) or who presented with chronic periodontitis (chi-squared test,  $p < 0.001$ ).

Individuals with chronic periodontitis were frequently colonized by the association between *P. gingivalis* and *C. rectus* ( $p = 0.0016$ ), *P. gingivalis* and *P. intermedia* ( $p = 0.0028$ ), and *P. gingivalis* and *T. forsythia* ( $p < 0.001$ ). Patients with chronic periodontitis were also colonized by *P. gulae* and *P. intermedia* ( $p = 0.002$ ) and *P. gulae* and *T. forsythia* ( $p = 0.0019$ ). *P. gulae* was detected only in the subgingival samples from natives on a traditional diet ( $p < 0.001$ ). The data also evidenced a negative association between *P. gulae* and *P. gingivalis*. Associations between different species of black-pigmented anaerobic rods were commonly observed, particularly between *P. gingivalis* and *P. intermedia* (chi-squared test,  $p < 0.001$ ), and between *P. gingivalis* and *P. nigrescens* ( $p = 0.001$ ).

The occurrence of *P. gingivalis*, *P. gulae* (Mann-Whitney test,  $p < 0.001$ ), *P. nigrescens* (Mann-Whitney test,  $p = 0.001$ ) and *T. forsythia* (Mann-Whitney test,  $p = 0.002$ ) was higher in patients aged 31–50 years and 51–70 years when compared to younger subjects (Fig. 2). Significant differences were not observed in the occurrence of such selected microorganisms between patients aged 31–50 and 51–70 years (*P. gingivalis*,  $p = 0.005$ ; *P. gulae*,  $p = 0.004$ ; *P. nigrescens*,  $p = 0.35$ ; and *T. forsythia*,  $p = 0.26$ ).

The microorganisms most frequently associated with chronic periodontitis (Fig. 1) were *T. forsythia* (chi-squared test,  $p = 0.003$ ), *P. micra* (chi-squared test,  $p = 0.002$ ), *P. gingivalis* (chi-squared test,  $p < 0.001$ ), *P. intermedia* (chi-squared test,  $p = 0.002$ ), *P. nigrescens* (chi-squared test,  $p < 0.001$ ) and *S. sputigena* (chi-squared test,  $p = 0.002$ ). The anatomical sites with food retention were more frequently colonized by *P. gingivalis* (chi-squared test,  $p < 0.001$ ). *T. denticola* was rarely detected in all age and gender groups or periodontal status (Figs. 1 and 2).

The occurrence of *A. actinomycetemcomitans*, *D. pneumosintes*, *E. corrodens*, *F. nucleatum*, *P. micra*, *P. endodontalis*, *S. sputigena*, and *T. denticola* was not correlated with clinical or sociodemographic variables ( $p > 0.00357$ ).



**Fig. 1 – Occurrence of oral microorganisms in subgingival samples from native Brazilians living at the Umutina Indian Reservation.**

#### 4. Discussion

The periodontal conditions of native communities living at the Umutina reservation do not differ significantly from that reported by Ide et al.,<sup>12</sup> Dowsett et al.,<sup>13</sup> and Figueiredo et al.,<sup>14</sup> studying other native Americans, where the adult population presented with generalized chronic periodontitis or gingivitis. However, this study appears to be the first report on the influence of a traditional diet and other social and cultural factors on periodontal microbiota in South American Indians.

The distribution of bacterial species related to periodontitis presents particularities associated with ethnic groups and geographic locations.<sup>6,7</sup> In the American continent, due to the great diversity of influences associated with colonization, habits, and geographic conditions, these particularities are outstanding, even within one country.<sup>8</sup> However, little is known about the microbiota of native Americans, particularly in groups that maintain traditions established prior to European occupation of the territory.

The occurrence of periodontal pathogenic bacteria in native Brazilians living at the Umutina Indian Reservation was similar to that previously described for other populations worldwide, but with some peculiarities. In the present study, the most frequently detected microorganism was *F. nucleatum*, irrespective of clinical periodontal conditions or other assessed data. There are controversies regarding its importance in the onset

and progression of gingivitis and chronic periodontitis, given that some subspecies may behave as commensals.<sup>4,15,16</sup> However, the present study evidenced that *F. nucleatum* could be correlated with gingival bleeding and gingivitis, as also reported by Pradhan-Palikhe et al.<sup>17</sup>

*A. actinomycetemcomitans* is frequently related to aggressive episodes of periodontitis,<sup>6,8</sup> and its distribution is influenced by ethnic and geographic characteristics.<sup>6,9</sup> This bacterium has been detected in North American (35%), Brazilian (27–90%), and Mexican (86.4%) patients with periodontitis,<sup>3,15,16,18</sup> besides its high prevalence in North African<sup>9</sup> and Asian subjects.<sup>6</sup> However, other studies have demonstrated low frequency of this microorganism in nonnative American populations.<sup>4,7,19</sup> This rod was detected in 8.3% and 34.2% of the samples from natives with gingivitis and periodontitis, respectively (Fig. 1), not being observed in healthy individuals. In comparison to nonnative Brazilians,<sup>3,18</sup> the frequency of this microorganism in natives of the Umutina reservation was reduced. This heterogeneous distribution in different human populations is likely to be related to the virulence of its serotypes.<sup>9</sup>

The association between *P. gingivalis* and *T. forsythia* with connective attachment loss confirms data from several parts of the world.<sup>3,4,8,15</sup>

The occurrence of *P. gingivalis* was lesser in healthy natives (21.4%), compared to natives with gingivitis (47.9%) and periodontitis (79%). These frequencies were higher than those

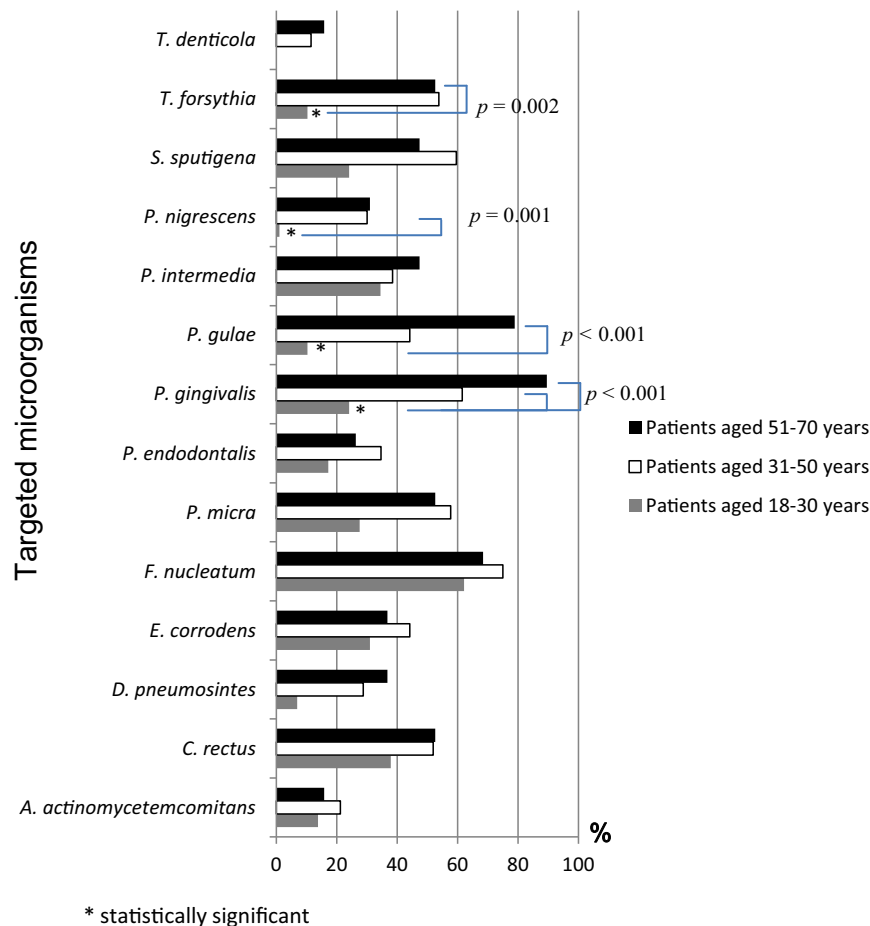


Fig. 2 – Occurrence of targeted microorganisms in subgingival samples from native Brazilians in relation to patients' age.

observed in native Brazilians from the Xingu Indian Reservation and Guatemalan Mayas,<sup>12,13</sup> and these frequencies were similar to nonnative populations.<sup>2–4</sup> In the literature, the occurrence of *T. forsythia* varies from 41.4% to 96% in patients with chronic periodontitis.<sup>4,6,15,17</sup> In the present investigation, the detection frequency of this anaerobe in patients with gingivitis and periodontitis was superior to that described for Brazilian natives from the Xingu Indian Reservation,<sup>12</sup> but inferior to the data from North American, Asian, and European patients with chronic or aggressive periodontitis.<sup>4,6,17</sup>

Some isolates of black-pigmented anaerobes of animal origin are genetically and phenotypically related to *P. gingivalis* of human origin. Generally, these catalase-producing strains are referred as *P. gulae*, and this species has been recovered from subgingival samples of different animals displaying periodontitis, particularly dogs,<sup>11</sup> but not from humans. In the present study, *P. gulae* was detected in 21.4% of periodontally healthy natives, 37.5% of native Brazilians with gingivitis, and in 52.6% of subjects with periodontitis. All subjects harbouring *P. gulae* subsisted on a traditional diet, and infrequently *P. gingivalis* and *P. gulae* were detected from the same patient.

In our laboratory, *P. gulae* was not detected in clinical specimens obtained from >2000 patients, from 2005 to 2012, but it was detected from subgingival samples from periodontally healthy (52.0%), gingivitis-affected (38.5%), or periodontitis-affected (46.2%) nonhuman primates (*Cebus apella* or capuchin

monkeys; data not shown). The colonization of the oral cavity by this anaerobe could be associated with the traditional diet of the natives, as these people use the flesh of nonhuman primates in the diet. In addition, the presence of monkeys as companion animals around indigenous villages is a feature well documented in Brazilian Amerindian populations, which could facilitate the transmission of this anaerobe. It is possible that the negative association between *P. gingivalis* and *P. gulae* reflects the competition for the same ecological niche, as these anaerobes were statistically associated with gingival bleeding and bone loss.

The results of this study evidenced that *P. intermedia* and *P. nigrescens* were associated with deep periodontal pockets and food retention. The occurrence of these gram-negative anaerobes showed a positive correlation with the presence of *P. gingivalis* and *T. forsythia* in the subgingival biofilm. These *Prevotella* species are likely to be associated with periodontitis in the population of the Umutina Indian Reservation (Fig. 1), similar to nonnative Brazilians<sup>6,18</sup> and other populations.<sup>4,8,17,19</sup> Among native Brazilians with periodontitis, data from Ide et al.<sup>12</sup> evidenced *P. intermedia* in 22% of the patients, while Dowsett et al.<sup>13</sup> reported its occurrence in 70% of Mayas, who are more exposed to nonnative populations than the native communities living at the Umutina Indian Reservation.

*C. rectus* can be implicated in inflammation in sites converting from periodontal health to disease, in association with *F. nucleatum* and *Prevotella* species,<sup>4</sup> and its association



with chronic<sup>8,20,21</sup> or aggressive periodontitis<sup>6,8</sup> has been established in different populations. In native Brazilians, Ide et al.<sup>12</sup> detected *C. rectus* in 31% of the subjects with chronic periodontitis, and Dowsett et al.<sup>13</sup> noted it in 50% of the Guatemalan Mayas. In the present investigation, the presence of *C. rectus* was found in 41.7% and 63.2% of natives with gingivitis and periodontitis, respectively, similar to data reported for nonnative Brazilians, Colombians, and Europeans.<sup>8,17,18</sup>

The participation of *P. micra* in the aetiology of periodontal disease remains controversial. Studies suggest that this gram-positive anaerobe constitutes a part of the resident microbiota in the oral cavity,<sup>16,20</sup> while others<sup>4,17,18</sup> suggested this species could contribute to the establishment and progression of periodontal inflammation and bone loss, as also observed in the present investigation (Fig. 1). *Selenomonas* spp. and *S. sputigena* are frequently detected in periodontal pockets and healthy gingival sulcus,<sup>5</sup> and their participation in the periodontitis microbiota has also been questioned. In natives of the Umutina Indian Reservation, it a positive correlation was evidenced between bone loss, gingival bleeding, and occurrence of *S. sputigena*, which is one of the most frequently occurring microorganisms in the microbiota of aggressive or chronic periodontitis.<sup>5,22</sup>

Since the 1960s, the participation of different oral spir-ochetes in the development of periodontal diseases has been discussed. *T. denticola* is related to bone loss.<sup>2,3,6,15,20</sup> However, in Native American populations, the distribution of this anaerobe seems to be more restricted, as it was observed from 5% of Guatemalan Mayas<sup>13</sup> and 17% of natives from the Xingu Indian Reservation.<sup>12</sup> The results presented in Fig. 1 are similar to those reported by Ide et al.,<sup>12</sup> and they suggest that this anaerobe is uncommon in the oral cavity of native Brazilians.

It is possible that the infrequent nature of interactions of the Umutina population with exogenous communities has preserved some peculiar characteristics of the oral microbiota of the American people of the pre-Columbian period. These native Brazilians are descendants of a small group who survived the first contact with settlers and had no great interchange with other nonnative communities geographically close to them.

## 5. Conclusion

This study revealed that the microbiota observed in natives from an Indian Brazilian reservation presents characteristics frequently associated with periodontopathies in nonnatives.

However, peculiarities were evidenced, including a modest occurrence of *T. denticola* and *A. actinomycetemcomitans* in subjects with periodontitis, as well the association of *P. gulae* with bone loss in natives subsisting on a traditional diet.

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## Competing interests

None declared.

## Ethical approval

This study was approved by the Institutional Review Board of the School of Dentistry of Araçatuba, Univ Estadual Paulista – UNESP (2006-01417), Brazilian National Indian Foundation (FUNAI) and Mato Grosso School of Public Health (021/07 CEP/SES-MT), Brazil.

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