Paracoccidioidomycosis ceti (Lacaziosis/ Lobomycosis) in Dolphins

9

Raquel Vilela and Leonel Mendoza

Abstract

Infections caused by the fungal pathogen *Lacazia loboi* were first reported in 1931 by Jorge de Oliveira Lobo in a human with granulomatous skin lesions in Pernambuco, Brazil. Early histopathological and serological analyses found morphological similarities and cross-reactive antigens with *Paracoccidioides brasiliensis*. In 1971, veterinarians working with dolphins in Florida, USA, reported granulomatous skin lesions in a dolphin, similar to that in human lacaziosis. Based on histopathological findings, *L. loboi* was initially believed to be also the etiologic agent of cutaneous disease in dolphins. Ever since, cutaneous granulomas have been reported in different dolphin species around the coast of Asia, Europe, and North and South America. Recently, using molecular biology approaches, some investigators stated that the DNA sequences extracted from cases of cutaneous granulomas in dolphins were closely related to those of *P. brasiliensis*. This chapter deals with the history, taxonomy, and other features of *L. loboi* in humans and the unculturable *P. brasiliensis* var. *ceti* type affecting the skin of dolphins.

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9.1 History of *Lacazia loboi* in Humans and *P. brasiliensis* var. *ceti* in Dolphins

The first case of lacaziosis (lobomycosis) in humans was reported by Jorge de Oliveira Lobo in a male patient from the Amazon basin with chronic (19 years) nodular lesions on his sacral anatomical area, in Recife, Pernambuco, Brazil (Lobo 1931). Forty years later, Migaki et al. (1971) reported skin granulomas in dolphins (*Tursiops truncatus*) with the development of yeast-like cells similar to those initially reported by Jorge Lobo.

In the following years, it was evident that, despite the numerous yeast-like cells present in the infected tissue of humans with lacaziosis, *L. loboi* resisted culture on most mycological media (Almeida and Lacaz 1948–49; Borelli 1968; Fonseca and Lacaz 1971; Furtado et al. 1967; Lacaz 1996). This unique feature of the pathogen led to false claims on its isolation in pure culture (Fonseca and Area 1940; Lacaz et al. 1986). However, those claims were challenged, and their isolates later identified as contaminating fungi (Fonseca and Lacaz 1971; Lacaz 1996; Vilela et al. 2007). Even today, allegations on the isolation of contaminating fungi from cases of human lacaziosis persist (Costa 2015).

The pathogen was known under names such as *Blastomyces brasiliensis*, *B. loboi*, *Glenosporella loboi*, *G. amazonica*, *Lobomyces loboi*, *Loboa loboi*, and *Paracoccidioides loboi* (Camargo et al. 1998; Fonseca and Lacaz 1971; Furtado et al. 1967; Lacaz 1996). Due to many taxonomic uncertainties surrounding the etiologic agent of cutaneous granulomas in humans, Taborda et al. (1999a, b) proposed the genus *Lacazia* (genus name dedicated to Dr. Carlos da Silva Lacaz, for his contribution on *L. loboi*), ending 70 years of taxonomic uncertainties. Likewise, the disease name periodically changed according to the proposed taxonomy. Examples of these are lobomycosis (*Lobomyces*, Borelli 1968) and lacaziosis (*Lacazia*, Vilela et al. 2005). In addition, the following regional disease names were also used for both human or dolphin keloid blastomycosis (Lobo 1931), Jorge Lobo disease (Lacaz et al. 1986), blastomycosis Jorge Lobo type (Almeida and Lacaz 1948–49), and others (Almeida and Lacaz 1948–49; Arju et al. 2014; Azulay et al. 1976; Baruzzi et al. 1973; Lacaz et al. 1986; Xavier et al. 2008).

The first report of a common bottlenose dolphin (*Tursiops truncatus*) displaying cutaneous lesions similar to human lacaziosis took place in the coastal areas of Florida, USA (Migaki et al. 1971). The following year, Woodard (1972) reported the second case on the same dolphin species and again in Florida. In 1973, De Vries and Laarman reported cutaneous lesions in a Guiana dolphin (*Sotalia guianensis*). As many others, the latter authors isolated fungal contaminants (*Scedosporium apiospermum* and *Candida haemulonis*) that they believed were the etiologic agents of the granulomas in dolphins. These cases were soon followed by reports of severe skin infections in several dolphin species. Most of them diagnosed around the US coastal areas, South America, and in other oceans (Bermudez et al. 2009; Dudok van Heel 1977; Kiszka et al. 2009; Lane et al. 2014; Symmers 1983; Tajima et al. 2015; Van Bressem et al. 2009). More recently, Minakawa et al. (2016) reported the infection in a new dolphin species (the Pacific white-sided dolphin, *Lagenorhynchus*

Table 9.1 Names applied to uncultivated types of humans and dolphins during the past 70 years

Nomenclature and synonyms	Human	Dolphin
Glenosporella loboi (1940)	√	
Blastomyces brasiliensis (1941)	√	
Glenosporopsis amazonica (1943)	√	
Paracoccidioides loboi (1948)	√	√
Blastomyces loboi (1952)	√	
Loboa loboi (1956)	√	
Lobomyces loboi (1958)	√	√
Lacazia loboi (1999)	+	√
Paracoccidioides brasiliensis var. *ceti* (2017)		+

√ = misapplied or synonymous name; + = correct name

obliquidens) suggesting that other cetacean species should also be investigated. Reports of infections are consistently diagnosed every year in different geographical areas, confirming the importance of the disease in this protected species (Table 9.1).

There are several reports of the unculturable nature of *L. loboi* in humans (Azulay et al. 1976; Borelli 1962; Fonseca and Area 1940; Furtado et al. 1967; Lacaz et al. 1986), but only two in dolphins. The first study indicated culture failure from an infected dolphin (Caldwell et al. 1975); the second is a recent well-documented study confirming its unculturable nature (Schaefer et al. 2016). In that study, Schaefer et al. (2016) tested fresh samples from common bottlenose dolphins (*T. truncatus*) collected at the Indian River Lagoon, Florida, USA. This study concluded that the etiologic agent was an unculturable version of *P. brasiliensis.* Based on phylogenetic analyses, in the interim, we use the name *P. brasiliensis* var. *ceti* to identify properly this unique type within the culturable *P. brasiliensis* as proposed by Vilela et al. (2016).

9.2 Taxonomy of *Lacazia loboi* and *Paracoccidioides brasiliensis* var. *ceti*

Paracoccidioides brasiliensis (Splendore) de Almeida **var**. *ceti* Vilela, St. Leger, Bossart, and Mendoza, **var. nov**.

Holotype B92-932, H&E histopathological slide collected from a US dolphin, deposited at the Michigan State University Herbarium, East Lansing.

Etymology To differentiate this novel uncultivated variety of *P. brasiliensis* affecting dolphins from the cultivated type causing human systemic paracoccidioidomycosis, the variety *ceti* (Cetacea) is proposed.

Description Uncultivated fungus causing cutaneous disease in dolphins. In vivo, numerous branching chains of globose to subglobose, yeast-like cells (5–10 μm) present, some connected by slender bridges (2–3 μm). Adjacent older cells detach by increasing cell wall thickness.

Disease nomenclature We propose the name "paracoccidioidomycosis ceti" to differentiate this cutaneous disease of dolphins from human systemic paracoccidioidomycosis (Vilela et al. 2016).

Due to the unculturable nature of *L. loboi* and *P. brasiliensis* var. *ceti*, the taxonomy of these two etiologic agents of cutaneous granulomas has been contentious (see above). *Lacazia loboi* from humans and *P. brasiliensis* var. *ceti* from dolphins share several features: (1) they resist culture (Lacaz et al. 1986; Schaefer et al. 2016); (2) they are restricted to cutaneous granulomas in mammalian hosts (Baruzzi et al. 1973; Bossart et al. 2015); (3) they develop similar yeast-like cells in chains (Haubold et al. 2000; Vilela et al. 2016); and (4) they possess cross-reactive antigens (Mendoza et al. 2008). Using traditional approaches, these two mammalian pathogens were believed to be the same organism. Only recently, with the use of molecular methodologies, their true position in the tree of life was unveiled (Herr et al. 2001; Rotstein et al. 2009; Vilela et al. 2016).

Initially Herr et al. (2001) extracted total *L. loboi* DNA from a biopsied tissue collected from a Brazilian man with cutaneous lacaziosis. They found that ITS, chitin synthase 4 (*CHS4*), and 18S SSU rDNA sequences placed this pathogen as a sister group to *P. brasiliensis*, the etiologic agent of systemic paracoccidioidomycosis. The authors argued that their phylogenetic data confirmed Lacaz (1996) position on the similarities shared by these two pathogens. The main problem studying the phylogenetics of *L. loboi*, however, was that the total DNA recovered from biopsied tissues contained DNA from the host and from normal skin and environmental microbiota. Vilela et al. (2005) proposed a molecular model to study this unculturable pathogen using specific primers. They argued that due to their phylogenetic proximity, *L. loboi* might share common DNA sequences with *P. brasiliensis*. Using this approach, they were able to amplify the *gp43*-like gene in *L. loboi* using well-known DNA sequences of this gene in *P. brasiliensis*. This approach was later validated when Vilela et al. (2009) amplified the ITS rDNA and chitin synthase 4, ADP-ribosylation factor, and *gp43* coding genes. The phylogenetic data showed that indeed *L. loboi* clustered with strong support in its own genus, confirming previous analysis (Vilela et al. 2009). These analyses showed that *P. brasiliensis* and *P. lutzii* shared the same ancestor with *L. loboi* and *P. brasiliensis* var. *ceti*; thus the latter unculturable microbes may possess putative dimorphic capabilities.

When Vilela et al. (2009) concluded their studies, *L. loboi* was still considered the same etiology affecting both humans and dolphins. However, Rotstein et al. (2009) found that the DNA sequences (26S LSrDNA) recovered from an offshore dolphin

(*T. truncatus*) with cutaneous granulomas displaying yeast-like cells shared 97% identity with *P. brasiliensis*. Unfortunately, their DNA sequences are not available. Subsequently, two teams in Japan (Minakawa et al. 2016; Ueda et al. 2013) and one in Spain (Esperon et al. 2012) reported that the DNA sequences, using at least two types of sequences (ITS and *gp43*), placed the etiologic agent of dolphin granulomas within the DNA sequences of the culturable *P. brasiliensis* isolates causing human systemic paracoccidioidomycosis. These findings came as a surprise since the clinical morphological features of the yeast-like cells in the dolphin-infected tissues look similar to that in human lacaziosis (Haubold et al. 2000; Lacaz et al. 1986). To validate these findings, Vilela et al. (2016) amplified the coding *kex* gene from six dolphins (*T. truncatus*) with cutaneous granulomas collected and two additional *CHS4* DNA sequences from different dolphins at the Indian River Lagoon, Florida, USA (Fig. 9.1). They confirmed previous studies (Esperon et al. 2012; Minakawa et al. 2016; Rotstein et al. 2009; Ueda et al. 2013) and concluded that an unculturable type of *P. brasiliensis*, different from the culturable type causing human systemic infections, is the etiologic agent of cutaneous granulomas in dolphins. They named the disease paracoccidioidomycosis ceti, epithet used throughout this chapter.

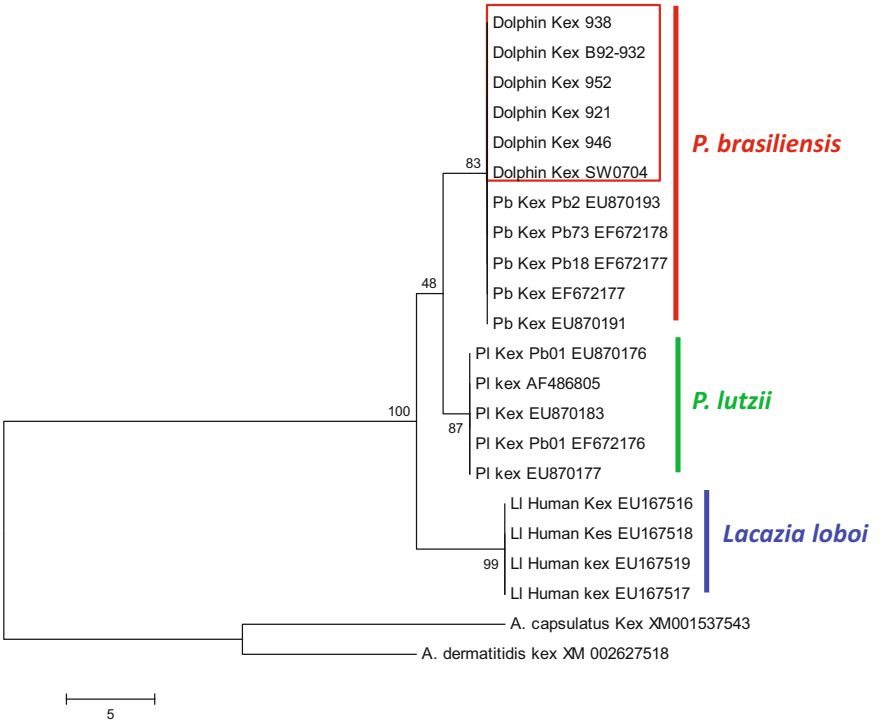

Fig. 9.1 Maximum parsimony tree of the exon partial *kex* DNA sequences PCR amplified from six US dolphins. The phylogenetic tree depicts the dolphin *kex* DNA sequences clustered among the culturable *P. brasiliensis* homologs. The species *P. lutzii* grouped as the sister taxon to *Lacazia loboi* (Vilela et al. 2016). Note the low bootstrap support placing *L. loboi* as an independent genus. The scale bar indicates nucleotide substitutions per site

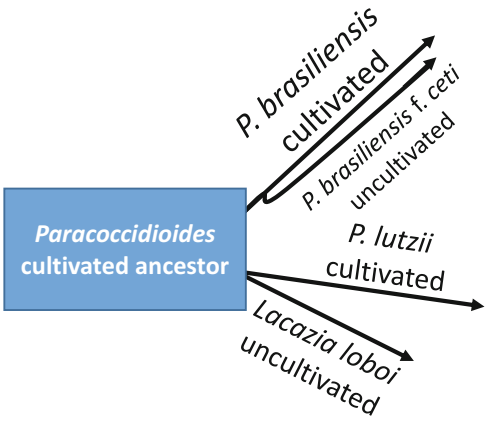

Fig. 9.2 The putative evolutionary paths of the culturable *P. brasiliensis* and *P. lutzii* recovered from humans with systemic paracoccidioidomycosis and the unculturable *P. brasiliensis* var. *ceti* and *Lacazia loboi*, restricted to subcutaneous tissues in dolphins and humans, respectively. The diagram shows the phylogenetic placement of these pathogens according to current phylogenetic analyses with one or more genes (Vilela et al. 2009, 2016) (Fig. 9.1)

One of the striking findings of the latter study (Vilela et al. 2016) was that after the addition of *P. brasiliensis* var. *ceti*, the DNA sequences of *L. loboi* showed lower bootstrap support to be in its own genus. This was best illustrated using the partial DNA sequences of the exons *CHS4*, *gp43*-like, and *kex* (Fig. 9.1) (Vilela et al. 2016). In previous analysis (Vilela et al. 2009), these exons placed the DNA sequences of human *L. loboi* with strong support as the sister taxon of two *Paracoccidioides* species (98–100% bootstrap support). However, with the addition of the dolphin DNA sequences, the bootstrap values plummeted to 83 (*CHS4*) and 48 (*kex*) (Fig. 9.1), and the *gp43*-like exon formed a strong supported sister taxon with *P. lutzii*, but did not affect bootstrap support using ITS DNA sequences. These analyses suggested that *L. loboi* maybe just another species in the genus *Paracoccidioides* (Vilela et al. 2016). The placement of the unculturable *P. brasiliensis* var. *ceti* among well-known DNA sequences of culturable isolates from human is intriguing. However, the fact that *L. loboi* and *P. brasiliensis* var. *ceti* shared many features strongly supports their common origin (Fig. 9.2).

9.3 Ecology and Epidemiology

Infections caused by *L. loboi* in humans are restricted to Mexico, Central America, and some countries in South America (Bermudez et al. 2009; Francesconi et al. 2014; Paniz-Mondolfi et al. 2012; Paniz-Mondolfi and Sander-Hoffmann 2009; Talhari and Talhari 2012; Vilela and Mendoza 2015). In these areas, the disease is more prevalent in dense forests with large rivers, geographical areas usually having elevated annual rainfall, high humidity, and hot temperatures. Most cases are diagnosed around the

Brazilian Amazon basin, but several cases are also reported in the rainforest areas of Colombia and Venezuela and less frequently in Bolivia, Ecuador, Guyana, Peru, Surinam, Central America (Costa Rica and Panama), and Mexico. In addition, imported cases outside these areas such as North America (Canada and the USA), Europe (France, Germany, Greece, the Netherlands), and South Africa have been reported (Arju et al. 2014; Burns et al. 2000; Elsayed et al. 2004; Fischer et al. 2002; Papadavid et al. 2012; Saint-Blancard et al. 2000; Symmers 1983; Vilela and Mendoza 2015). These cases were mainly diagnosed in individuals that had visited endemic areas in South America or were exposed, through direct contact with infected dolphins.

In contrast, the majority of reports in dolphins occurred in the coastal areas of Florida, USA (Bossart et al. 2015; Murdoch et al. 2010; Reif et al. 2006, 2009). The disease has been also diagnosed in other geographical areas such as Brazil (Daura-Jorge and Simões-Lopes 2011; Sacristan et al. 2016), Costa Rica (Bessesen et al. 2014), France (Symmers 1983), Spain (Esperon et al. 2012), Japan (Minakawa et al. 2016; Tajima et al. 2015; Ueda et al. 2013), Madagascar (*T. aduncus*, Kiszka et al. 2009), South Africa, Surinam (de Moura et al. 2014), and Venezuela (Bermudez et al. 2009).

Because human infections by *L. loboi* and the culturable *P. brasiliensis* are restricted to Central and South America, the finding of *P. brasiliensis* var. *ceti* affecting dolphins in Spain (Esperon et al. 2012), Japan (Minakawa et al. 2016; Tajima et al. 2015; Ueda et al. 2013), and the USA (Rotstein et al. 2009; Vilela et al. 2016) is significant. The phylogenetic data suggest that earlier in the life history of the pathogen, some dolphins swimming along South America coastal areas could have been infected around the river estuaries of the above endemic countries, with propagules from an ancestor of *P. brasiliensis*. Newly infected dolphins probably stayed around South America coastal areas, and others could have migrated to North America and other oceans. The fact that *P. brasiliensis* var. *ceti* cannot be cultured suggests that the pathogen muted from its original form or that this was indeed a unique strain derived from culturable *P. brasiliensis* ancestor. Two hypotheses are possible: (1) infected animals could transmit the pathogen by direct contact with non-infected dolphins or (2) dolphins are constantly in contact with propagules of the pathogen located around South America river estuaries. One clue supporting the first hypothesis is that the majority of bottlenose dolphins around the US coastal regions stay in those areas for long periods before developing skin granulomas (Bossart et al. 2015; Murdoch et al. 2010; Reif et al. 2006, 2009). More importantly, phylogenetic analysis showed that *P. brasiliensis* var. *ceti* affecting dolphins probably originated from an ancestral strain located in South America.

Since *L. loboi* and *P. brasiliensis* var. *ceti* resist culture, the epidemiology of the infection caused by these two pathogens remains an enigma. In humans, however, it is believed that *L. loboi* is acquired after contact with environmental propagules. A support for this theory came after a tribe of Caiabi Indians in Brazil was relocated from the Tapajos River (a hyperendemic area of lacaziosis) to the Xingu National Park. Before relocation, numerous cases of the disease were annually diagnosed, whereas new cases have not been reported after relocation (Baruzzi et al. 1973;

Talhari and Talhari 2012; Lacaz et al. 1986). Since phylogenetic analysis placed *L. loboi* with the dimorphic *Onygenales*, it is quite possible that conidia (yet to be found) of this unique pathogen may be present in the environment. In humans, the disease is considered occupational or related to recreational activities. *Paracoccidioides brasiliensis* var. *ceti* affecting dolphins can be considered a zoonotic pathogen because transmission between dolphins and humans has been documented (Symmers 1983). However, the infection rate may be low (Norton 2006; Reif et al. 2013). Accidental laboratory transmission (Rosa et al. 2009) and successful experimental infections in animals (Belone et al. 2001; Madeira et al. 2000; Sampaio and Dias 1970) and humans (Borelli 1962; Lacaz et al. 1986) have been reported.

9.4 Host Response and Pathogenesis

In contrast with the epidemiological features of *P. brasiliensis* infecting their hosts by aerial propagules (Lacaz et al. 1986) (Chap. 6), *L. loboi* in humans and *P. brasiliensis* var. *ceti* in dolphins are restricted to the subcutaneous tissues. This fact suggests that environmental propagules of both pathogens are more likely introduced by traumatic implantation of such elements in the subcutaneous tissues of the infected hosts. Accounts of humans with lacaziosis after traumatic lesions involving contaminated plants, snake bites, insect bites, stingray trauma, and others support this hypothesis (Almeida and Lacaz 1948–49; Azulay et al. 1976; Baruzzi et al. 1973; Lacaz et al. 1986; Rodriguez-Toro 1993; Talhari and Talhari 2012). Once these environmental propagules reach the subcutaneous tissues, *L. loboi* and *P. brasiliensis* var. *ceti* switch to a yeast-like form and successfully establish in the host subcutaneous tissues.

According to many observations (Bossart et al. 2015; Daura-Jorge and Simões-Lopes 2011; De Vries and Laarman 1973; Silva and Brito 1994; Talhari and Talhari 2012; Woods et al. 2010), both pathogens increase in size very slowly and could take months or even years to produce large granulomatous parakeloidal lesions. The disease rarely disseminates to other organs (Azulay et al. 1976; Opromolla et al. 2003). It has been found that yeast-like cells of both pathogens actively proliferate inside inflammatory cells (macrophages, mainly giant cells) activating the release of transforming growth factor β1 (TGF-β1), a powerful cytokine involved in immunosuppressive events (Francesconi et al. 2014; Reif et al. 2009; Vilani-Moreno et al. 2004a; Xavier et al. 2008), and can block the release of nitric oxide in giant cells and macrophages, also inhibiting the production of interferon gamma (IFN-γ) and thus locking the immune response in a Th2 subset, a typical reaction observed in both infected humans and dolphins (Goihman-Yahr et al. 1989; Vilani-Moreno et al. 2004b, 2011). The subcutaneous granulomas in infected humans and dolphins showed numerous branching yeast-like cells uniform in size and arranged in chains, surrounded by inflammatory cells and heavy fibrosis (Francesconi et al. 2014; Goihman-Yahr et al. 1989; Pecher et al. 1979; Reif et al. 2009; Vilani-Moreno and Opromolla 1997). It is believed that the proliferation of CD8 T cells promoted by TGF-β1 is responsible also for the production of immunoglobulins and other factors

that favor the process of fibrosis giving the external parakeloidal appearance of the cutaneous lesions.

The presence of IL-10, IL-4 and IL-6 has been found in infected humans, an evidence that the immune response is locked into a Th2 subset (Lacaz et al. 1986; Vilani-Moreno et al. 2007). It is likely that the Th2 events are triggered by metabolites released by the fungus during its in vivo reproduction, but those factors are yet to be investigated. Mendoza et al. (2008) detected several antigens using sera from experimentally inoculated mice and infected humans and dolphins. The study found a ~193kD *gp43*-like antigen in infected humans and dolphins. The presence of melanin in the cell wall of *L. loboi* and *P. brasiliensis* var. *ceti* is believed to protect the pathogens from the host immune response (Taborda et al. 1999b). Vilani-Moreno and Opromolla (1997) reported that the viability of *L. loboi* yeast-like cells in humans is reduced, and probably only ~40% of the cells are still viable in the infected host tissues. A similar finding was reported in infected dolphins (Lane et al. 2014; Reif et al. 2006). A dramatic decrease in the number of circulating B- and T-helper cells was observed, which might contribute to impairment of adaptive immunity. Antibodies against *P. brasiliensis* var. *ceti* were found to cross-react with the antigens of the culturable *P. brasiliensis* isolates (Landman et al. 1988; Mendes et al. 1986; Puccia and Travassos 1991; Silva et al. 1968; Vidal et al. 1997).

9.5 Clinical Signs and Lesions

The disease in humans and dolphins is usually chronic and takes time to develop into large lesions (Bossart et al. 2015; Reif et al. 2006; Rodriguez-Toro 1993; Tapia et al. 1978; Talhari and Talhari 2012). Typical lesions are monomorphic or multimorphic and painless but may develop mild pruritus. The most affected anatomical sites in humans are ears, shoulders, limbs, back, and abdominal areas. Human lacaziosis clinical features have been subjected to extensive reviews since the first case was reported (Cardoso de Brito and Quaresma 2007; Francesconi et al. 2014; Talhari and Talhari 2012). The first effort to describe the polymorphic clinical characteristics of the disease in humans came from Silva and Brito (1994). These authors described five clinical forms including the typical parakeloidal granuloma and the gummatous, infiltrate, ulcerated, and verrucous form. The same year Machado (1972) described two basic forms. The first one is a hyperergic state that includes the macular, gummatous, and nodular forms described by Silva and Brito (1994). The second form is a hypoergic state including the parakeloidal and verrucous forms (Fig. 9.3). In the polymorphic form, the host immune system may react to the antigens presented during infections in a similar way as in the paucibacillary and multibacillary forms of *M. leprae* infection (Eichelmann et al. 2013). In a revision of 40 cases in Acre, Brazil, Opromolla et al. (2000) also described the above forms and indicated that the most frequent anatomical area was the ear (Fig. 9.3a). Differential diagnosis in humans includes chromoblastomycosis, leishmaniasis, leprosy, neoplasia, and paracoccidioidomycosis (Eichelmann et al. 2013; Lacaz et al. 1986).

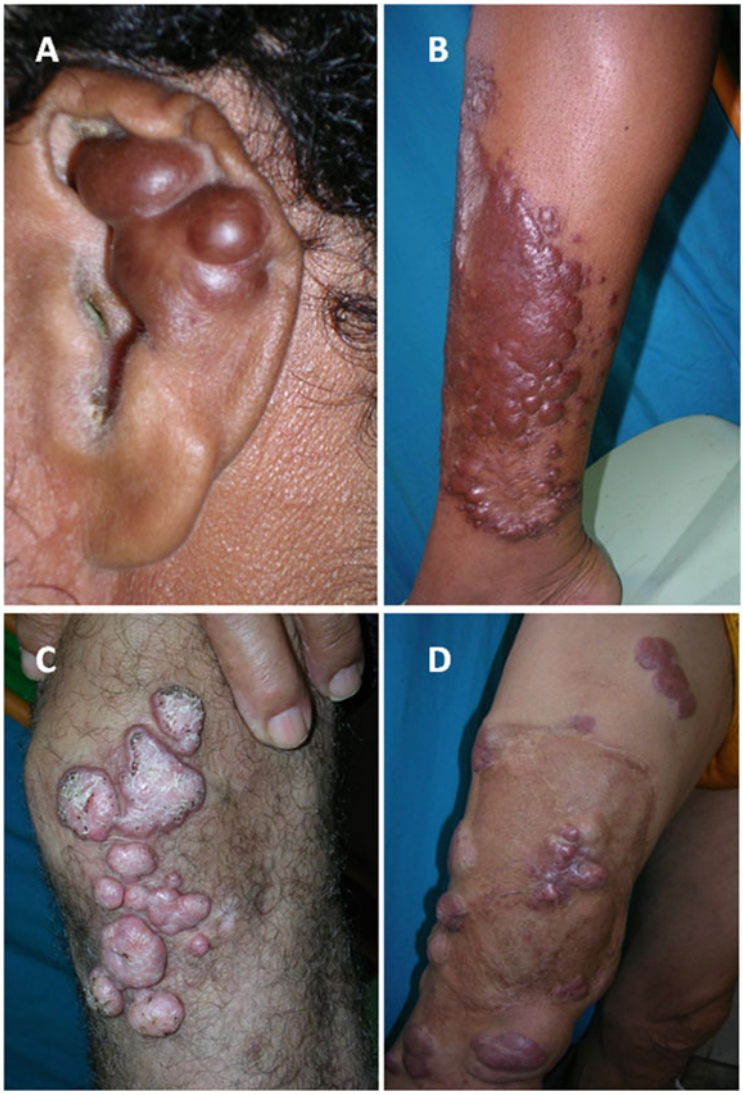

Fig. 9.3 Cases of human lacaziosis from Acre, Brazil. (**a** and **d**) Some of the clinical manifestation of the infection described by Silva and Brito (1994). (**a**) The most common anatomical site followed by different type of granulomatous lesions on the limbs. (**d**) An example of recurrence after surgical removal of old lesions (Courtesy of Dr. P. Rosa)

The infection has been diagnosed in several dolphin species including *Tursiops truncatus*, *T. aduncus*, *Sotalia guianensis*, and *Lagenorhynchus obliquidens* (Bossart et al. 2015; De Vries and Laarman 1973; Kiszka et al. 2009; Minakawa et al. 2016; Van Bressem et al. 2009). The terms "lacaziosis-like" and "lobomycosis-like" are currently used to identify the clinical features of putative cases of the

disease based only on gross anatomical observations, but lacking histopathological confirmation (Daura-Jorge and Simões-Lopes 2011; Kiszka et al. 2009; Reif et al. 2006; Sacristan et al. 2016; Tajima et al. 2015). However, due to the new proposed names for the disease, the use of paracoccidioidomycosis ceti-like is recommended. The clinical features of the disease in dolphins are characterized by the formation of white, gray to reddish nodular or verrucous lesions (cauliflower-like), sometimes with prominent elevation over non-infected skin areas (Fig. 9.4) (Bossart et al. 2015; Minakawa et al. 2016; Rotstein et al. 2009; Reif et al. 2006; Ueda et al. 2013). These areas could ulcerate and form papillary nodules becoming large plaques. The lesions bleed easily after small traumas (Fig. 9.4a). The most frequently affected anatomical areas are anterior dorsum, dorsal and pectoral fins, flukes, rostrum, dorsal cranial surface, and the mid body (Bossart et al. 2015; Reif et al. 2006). Photographic

Fig. 9.4 (**a**) A common bottlenose dolphin (*Tursiops truncates*) with extensive plaques over the frontal anterior section of the dorsal fin caused by *Paracoccidioides brasiliensis* var. *ceti*. Note numerous elevated verrucous gray and white plaques some of them bleeding. New nodular small satellite lesions adjacent to the large plaque are observed (Courtesy of Dr. J. St. Leger). (**b** and **c**) Two different dolphins with large nodular white plaques typical of paracoccidioidomycosis ceti (Courtesy of Drs. G.D. Bossart, P.A. Fair and J.S. Reif)

observations for long periods revealed that small lesions in dolphins slowly progressed to form extensive granulomatous plaques. Most veterinarians agreed that environmental factors might play a key role in some epizootic reports of the disease in the coastal areas of Florida and North Carolina, USA (de Moura et al. 2014; Lane et al. 2014; Reif et al. 2006, 2009; Tajima et al. 2015).

9.6 Pathological Findings

The diagnosis of the lacaziosis in humans is confirmed after biopsy collection from the infected areas. Hematoxylin-eosin (H&E)-stained tissue samples show the presence of atrophy of the epidermis with extensive fibrosis interspersed with microabscesses containing numerous lymphocytes, plasma cells, foamy histiocytes, and multinucleated giant cells, some enclosing the pathogens (Fig. 9.5) (Baruzzi et al. 1981; Carneiro et al. 2009; Francesconi et al. 2014; Lacaz et al. 1986; Talhari and Talhari 2012). Acanthosis with hyperkeratosis is commonly observed. The Splendore/Hoeppli phenomenon enclosing one or more *L. loboi* yeast-like cells has been occasionally reported (Opromolla et al. 2000). In H&E *L. loboi* yeast-like cells appear as unstained hyaline structures resembling ghost-like bodies (Fig. 9.5a). In Gomori methenamine silver (GMS) stain, *L. loboi* cells appear uniform in shape (4–12 μm in diameter) developing one or more chains of three or more yeast-like cells connected by slender tubes (Fig. 9.5b). In some instances, few chains of yeast-like cells are present forming individual or budding spherical structures. The yeast-like cells shape varied between spherical to oval (lemon shape).

Dolphins are protected mammalian species; thus the collection of material for histopathology is strictly regulated. Biopsies are collected after the diseased dolphin is restrained and precautions are taken to avoid traumas that may have future repercussions. In H&E, the inflammatory process and the pathogen seem very similar to that in cases of human lacaziosis (Esperon et al. 2012; Minakawa et al. 2016; Rotstein et al. 2009; Ueda et al. 2013). Minor differences in the pathogen cell size in humans and dolphins have been mentioned (Haubold et al. 2000; Minakawa et al. 2016). Acanthosis, hyperkeratosis, hyperpigmentation, and extensive progressive fibrosis are the main pathological features in dolphins (Bossart et al. 2015; Tajima et al. 2015; Ueda et al. 2013). Microabscesses containing numerous unstained thick-walled hyaline yeast-like cells are often present (Fig. 9.5c, d). Histiocytes, giant cells, few lymphocytes, and plasma cells can also be observed. In GMS, the presence of numerous dark, 2–10 μm wide, oval yeast-like cells connected by short isthmuses is diagnostic of dolphin cases (Fig. 9.5e, f).

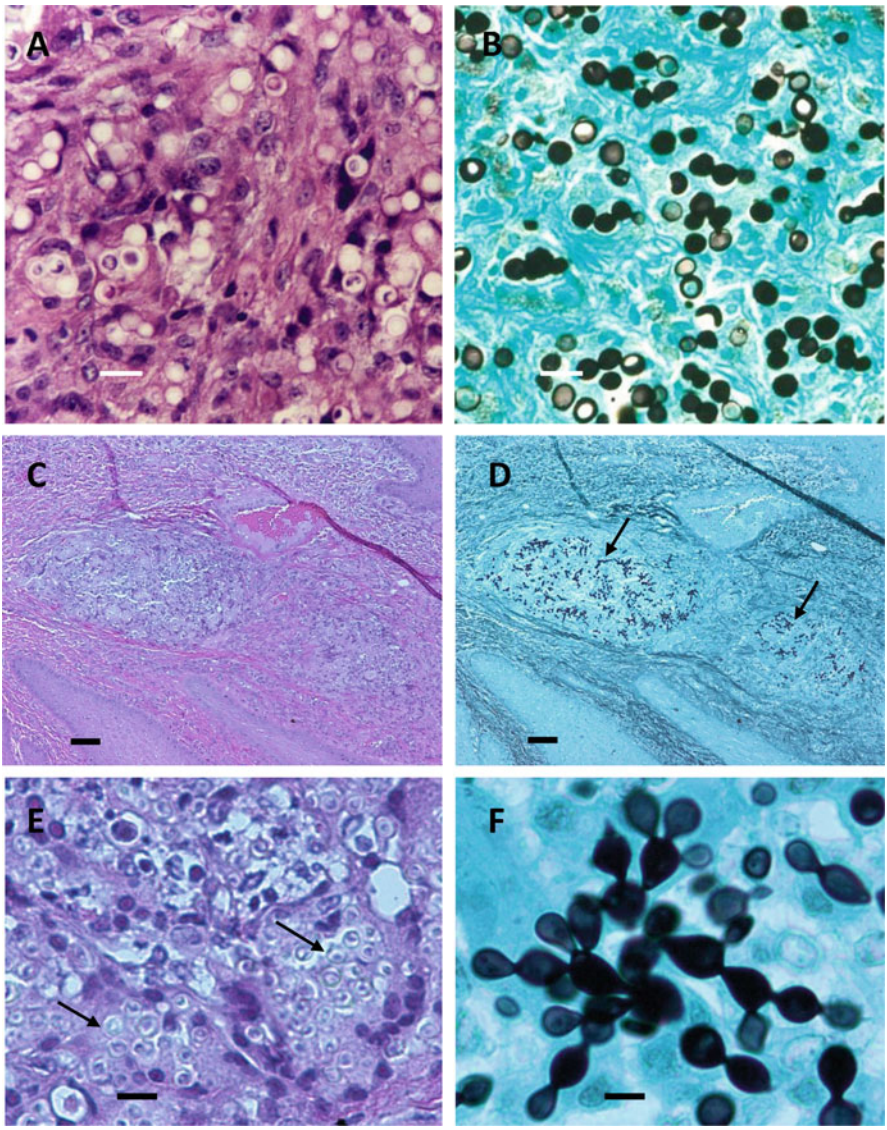

Fig. 9.5 (**a**) Unstained *Lacazia loboi* yeast-like cells (H&E) (arrows) from a case of human lacaziosis Acre, Brazil (bar = 18 μm). (**b**) A Gomori methenamine silver stain sample of panel A (bar = 18 μm). Small yeast-like cells chain of more than two cells connected by slender isthmuses are visible (Courtesy of Dr. Patricia Rosa). (**c** and **e**) H&E stained histological sections of a dolphin with paracoccidioidomycosis ceti. Presence of microabscesses containing inflammatory infiltrate, extensive fibrosis, acanthosis (Panel C; bar = 65 μm), and numerous unstained *Paracoccidioides brasiliensis* var. *ceti* yeast-like cells (Panel E; bar = 20 μm) (arrows). (**d**) A low magnification of panel C stained with Gomori methenamine silver. Presence of numerous black chains of yeast-like cells (arrows) (bar = 65 μm). (**f**) A close-up of Panel E. Presence of the yeast-like cells uniform in size and formation of branching chain connected by small bridges (bar = 10 μm)

9.7 Laboratory Diagnosis

The ideal clinical specimen to be sent to the laboratory is a biopsy in both humans and dolphins (Vilela and Mendoza 2015). However, skin smears of the granulomas collected with a scalp blade (Talhari et al. 2009) or the Scotch tape technique (Miranda and Silva 2005) are also valuable tools for the microscopic visualization of the pathogens. Both *L. loboi* and *P. brasiliensis* var. *ceti* resist culture; thus the use of traditional laboratory media has to be performed with the purpose only of ruling out other pathogens.

To perform the wet mount technique, the biopsy is divided into 2–3 mm cubes and placed onto a slide containing two drops of 10 or 20% KOH (Vilela and Mendoza 2015). The addition of calcofluor enhances sensitivity when observed under fluorescence. The samples are heated for 5 min and then left on the counter. In wet mount preparations, *L. loboi* from humans and *P. brasiliensis* var. *ceti* from dolphins appear as single spherical or oval yeast-cells developing branching chains of cells (Fig. 9.6). The yeast-like cells in dolphins measure ~2–10 μm, whereas in humans ~4–14 μm, according to Haubold et al. (2000). In 10% KOH, the cells appear hyaline with a thick cell wall (Fig. 9.6).

Experimental inoculation of *L. loboi* has been successfully obtained in several mammalian species including humans (Belone et al. 2001; Borelli 1962; Madeira et al. 2000). The mouse model proposed by Madeira et al. (2000) showed better results using propagules from human lacaziosis. Inoculated mice on the injected pads developed small granulomas that slowly enlarged. In histopathology, diffuse infiltrate-containing macrophages, lymphocytes, plasma cells, fibrosis, and numerous yeast-like cells can be observed. There are no official reports of *P. brasiliensis* var. *ceti* experimental infection in mice. Incidentally, few years ago, one of the authors (LM) was informed of a successful experimental inoculation in a mouse injected with yeast-like cells from an infected dolphin (Dr. Libero Ajello, personal communication).

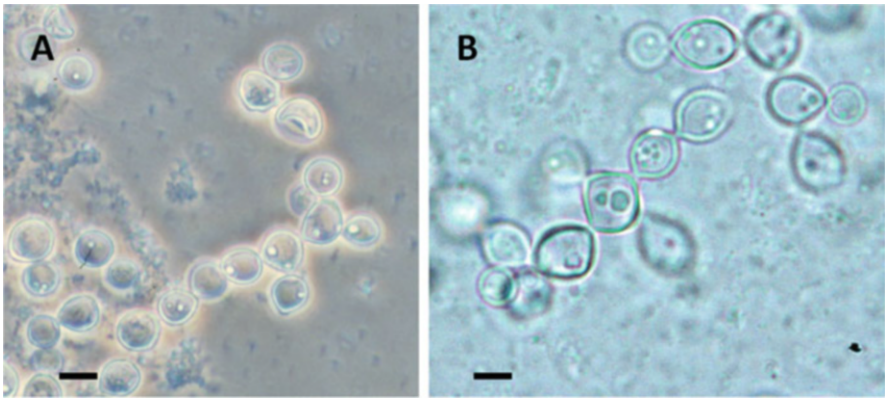

Fig. 9.6 (**a**) *Paracoccidioides brasiliensis* var. *ceti* yeast-like cells in 10% KOH wet mount preparation from a case of dolphin with cutaneous granulomas (bar = 10 μm). Presence of uniform single cells and branching chains of yeast-like cells (Nomarski microscopy). (**b**) A wet mount (10% KOH) preparation from a human lacaziosis case (bar = 10 μm). Presence of yeast-like cells in chain

9.8 Treatment

Currently, there is no effective drug or therapeutic protocol for the management of the infections caused by *L. loboi* in humans or *P. brasiliensis* var. *ceti* in dolphins. Surgery in the early stages of the disease remains the treatment of choice in both conditions (Baruzzi et al. 1981; Bossart et al. 2015; Lacaz et al. 1986). In advanced cases, surgery can be also performed, but recurrence rate is high (Lacaz et al. 1986; Opromolla et al. 2000; Rodriguez-Toro 1993; Talhari and Talhari 2012). These two atypical microorganisms were always classified within the fungi, thus treated with antifungal drugs (Bustamante et al. 2013; Cucé et al. 1980; Dudok van Heel 1977; Lawrence and Ajello 1986). The response of infected hosts to antifungal therapy has been unsatisfactory (Cucé et al. 1980; Lacaz et al. 1986; Woods et al. 2010). For instance, amphotericin B, itraconazole, 5-fluorocytosine, ketoconazole, posaconazole, terbinafine, and others resulted in the reduction of the granuloma size, but cure was achieved only in few instances (Francesconi et al. 2014). On the other hand, antibacterial drugs such as sulfadimethoxine and sulfamethoxypyridazine were found to improve the nodular lesions, but the presence of yeast-like cells persisted in the infected areas (Woods et al. 2010). Because leprosy is the differential diagnosis of lacaziosis, drugs such as clofazimine have been used in patients with dual infection. According to studies conducted in Acre, Brazil (Opromolla et al. 2000; Silva 1972; Talhari and Talhari 2012), the use of dapsone and clofazimine dramatically improved lesion size and itching, usually associate to active lacaziosis, and in some cases the lesions decreased or disappeared (Woods et al. 2010). Moreover, when surgery and these two drugs were combined, no recurrence was reported (Woods et al. 2010).

Only a handful of wild infected dolphins have been kept in captivity for treatment; thus there is limited information on the management strategies in animals. Dolphins treated with the strategies used in humans showed similar responses (Minakawa et al. 2016). Surgical removal of small lesions is the best option. However, surgery in large or multicentric lesions is not recommended. In 1977, a dolphin captured in Florida, USA, was successfully treated with miconazole (Dudok van Heel 1977). But, this antifungal drug has not been used ever since. More recently, a dolphin treated with topical itraconazole and ketoconazole did not improve (Esperon et al. 2012). The same animal was then treated with oral 2.5 mg/kg itraconazole and 2.0 mg/kg terbinafine. With this treatment protocol, cutaneous lesions reduced to small nodules that later disappeared, and no relapse was reported. Currently, there is little information on the best antifungal approach to treat dolphins. This is probably in part due to the price of antifungals and the difficulties to keep wild animals for long periods outside their natural ecological habitat.

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