Invited Review

Endo- and ectoparasites of large whales (Cetartiodactyla: Balaenopteridae, Physeteridae): Overcoming difficulties in obtaining appropriate samples by non- and minimally-invasive methods

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A R T I C L E  I N F O
Article history:
Received 10 September 2015
Received in revised form 15 November 2015
Accepted 18 November 2015

Keywords:
Cetaceans
Whales
Neozoan parasites
Entamoeba
Balamandium
Giardia

A B S T R A C T

Baleen and sperm whales, belonging to the Order Cetartiodactyla, are the largest and heaviest existent mammals in the world, collectively known as large whales. Large whales have been subjected to a variety of conservation means, which could be better monitored and managed if physiological and pathophysiological information, such as pathogen infections, could already be gathered from free-swimming animals instead of carcasses. Parasitic diseases are increasingly recognized for their profound influences on individual, population, and even ecosystem health. Furthermore, a number of parasite species have gained importance as opportunistic neozoan infections in the marine environment. Nonetheless, traditional approaches to study parasitic diseases have been impractical for large whales, since there is no current routine method for the capture and handling of these large animals and there is presently no practical method to obtain blood samples remotely from free-ranging whales. Therefore, we here not only intend to review the endo- and ectoparasite fauna of large whales but also to provide new insights in current available methods for gathering parasitological data by using non- or minimally invasive sampling techniques. We focus on methods, which will allow detailed parasitological studies to gain a broader knowledge on parasitoses affecting wild, free-swimming large whale populations. © 2015 The Authors. Published by Elsevier Ltd on behalf of Australian Society for Parasitology. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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http://dx.doi.org/10.1016/j.ijppaw.2015.11.002
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1. Introduction

Over the last two centuries, most large whale species were subjected to severe overexploitation by commercial whaling to the point of becoming almost extinct (Clapham et al., 1999). Fortunately, since the international moratorium on commercial whaling, that has been in place since 1986, some populations have slowly recovered, although most populations have not yet recuperate their historical numbers, and others are still under threat or seriously endangered (Kraus et al., 2005; Freeman, 2008; Reynolds et al., 2009; IUCN, 2012). Moreover, large whales still experience numerous anthropogenic pressures, which warrant closer investigations from a conservation physiology perspective in order to better understand their resilience and ability to recover from overexploitation. Even with the cessation of commercial whaling in most countries, large whales still face chronic exposure to toxins and pollutants, global climate change, as well as several pathologies including naturally occurring parasite infections but also from water contamination by human activities (Van Bressem et al., 2009; Kleinertz et al., 2014b; Moore, 2014).

Large cetaceans can be affected by a wide range of endo- and ectoparasites, which have been the focus of numerous reports mainly dealing with taxonomy, distribution and ecology. Furthermore, a number of parasite species have gained importance as opportunistic neozoon infections in the marine environment. This review focuses on those organisms which we perceive as pathogenic for baleen and sperm whales (known collectively as large whales) as well as on new emerging and neglected neozoon parasites, mainly protozoan parasites. Nonetheless, traditional approaches to studying parasitic diseases have been impractical for large whales, because there is no current routine method for capturing the largest whale species and there is presently no practical method for obtaining blood samples remotely from free-ranging whales. Thus, here we intend, additionally, to review current available methods for gathering parasitological information by using a variety of non- and minimally-invasive sample collection techniques. The following five types of non- minimally invasive sample collections are discussed: faecal samples, vomitus samples, respiratory samples, skin/blubber samples and photograph monitoring.

2. Endoparasites of large whales (Physeteridae, Balaenopteridae)

Sperm whales (Physeter macrocephalus) are the largest of the toothed whales and it is the only living member of the family Physeteridae. Sperm whales are apex predators and have a catholic diet: females preferentially prey upon mesopelagic cephalopods, in addition to cephalopods, and males consume demersal fish species including gadoids, sharks and rays (Whitehead, 2009). A wide range of helminths including mainly cestodes, nematodes, trematodes and acanthocephalan parasite species can parasitize sperm whales (see Table 1).

The cestode fauna of sperm whales consists of species from the Tetrabothriidae, Diphyllobothriidae and Phyllobothriidae: Tetrabothrius curlensis, Tetrabothrium wilsoni, Trigonocotyle sp., Priapocephalus grandis (Rees, 1953), Diplogonoporus sp. (Gubanov, 1951; Rees, 1953; Delyamure, 1955), Hexagonoporus physeters and Phyllobotrium delphini (Baylis, 1932). Most of the tapeworms belonging to the families Tetrabothriidae and Diphyllobothriidae were found in the small intestine of sperm whales, with the exception of the genus Diplogonoporus, which may also reside in the bile ducts. Infections with mature pseudophyllideans, mainly Diphyllolobiotrum spp. are usually innocuous (Arundel, 1978), but may also cause debilitation and even death of heavily infected animals. Adult cestodes have been described to occasionally encyst within the intestine mucosa or to block the gut lumen (Cordes and O’Hara, 1979; Geraci and St Aubin, 1987). Moreover, plerocercoids cysts of Phyllobotrium spp. are the most commonly encountered parasites in cetaceans worldwide (Ruhnke and Workman, 2013). The definitive hosts of adult Phyllobotrium cestodes are several sharks as described elsewhere (Ruhnke and Workman, 2013; Kuris et al., 2015).

Table 1

Groups of endo- and ectoparasite species and their site of infection in large whale species (Balaenopteridae, Physeteridae).

| Parasite | Whale species | Site of infection |
|----------|--------------|------------------|
| Protozoa | | |
| Giardia | | |
| Entamoeba | | |
| Balantidium | | |
| Trematoda | | |
| Zalophotrema | | |
| Ogmogaster | | |
| Icthyocotyle | | |
| Nasitrema | | |
| Cestoda | | |
| Tetrabothrium | | |
| Trigonocotyle | | |
| Priapocephalus | | |
| Diplogonoporus | | |
| Hexagonoporus | | |
| Phyllobotrium | | |
| Acanthocephala | | |
| Bolbosoma | | |
| Corynosoma | | |
| Nematoda | | |
| Anisakis | | |
| Contracaecum | | |
| Porrocaecum | | |
| Macracentema | | |
| Crassinema | | |
| Sertorius | | |
| Arthropoda | | |
| Coronula | | |
| Xenobalanus | | |
| Penella | | |
| Cyamus | | |
| Neocamus | | |
| Agnatha (Fish) | | |
| Entosphenus | | |

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The most prevalent helminths found in sperm whales are gastric species belonging to the family Anisakidae with the species *Anisakis* *iivanizkii* (Delyamure, 1955), *A. physeteris*, *A. simplex* (Baylis, 1932) and *A. skrjabini* (Delyamure, 1955) and the large-sized spirurid nematode *Placentonema gigantissima* (up to 9 m long) which parasitizes the placenta, uterus, mammary gland and subdermis of these marine mammals (Delyamure, 1955) (see Table 1).

Also, intestinal acanthocephalan parasites, belonging to the genera *Bolbosoma* and *Corynosoma*, have been reported to occur in the stomach and intestine of sperm whales, including the species *Balaenoptera brevicolle*, *Balaenoptera capitatus*, *Balaenoptera physeteris* and *Corynosoma curilensis* (Baylis, 1932; Cubanov, 1951; Delyamure, 1955). Nevertheless, the genus *Bolbosoma* is primarily found in cetaceans, while the genus *Corynosoma* occurs mainly in pinnipeds (Golván, 1959). The life cycle of marine acanthocephalans is heteroxenous, involving one or two intermediate hosts, being represented by crustaceans and fishes. Cystacanthis of *Bolbosoma balaenae* were recently found encapsulated in the cephalothorax of the krill species *Nycitphanes couchii* from temperate waters in the Northeast Atlantic Ocean (Gregori et al., 2012). It is therefore suggested that large whales become infected either by the oral uptake of *Bolbosoma*-infected krill species, or by feeding on a second intermediate host (fishes).

The only trematode parasite known so far of sperm whales is the species *Zalophotrema curilensis* (Delyamure, 1955), which resides in the bile duct and pancreas. *Zalophotrema* is associated with parenchymal necrosis, chronic fibrosis and hyperplasia of bile ducts in other cetacean species (Woodard et al., 1969; Geraci and St Aubin, 1987), but very little is known on the pathogenesis of *Z. curilensis* in sperm whales.

In contrast to sperm whales, the mysticeti [baleen whales; *Balaenopteridae*] are the edentulous whales characterized by having baleen plates for filtering food from water. As a group they feed on a diverse assemblage of schooling planktonic and micronektonic crustaceans and small pelagic schooling fish, but diet composition varies much across species and geographic areas (Bannister, 2009; Groves and Grubb, 2011).

The endoparasite fauna of blue whales (*Balaenoptera musculus*), sei whales (*Balaenoptera borealis*) and fin whales (*Balaenoptera physalus*) resembles in principle the fauna of sperm whales, although they rely on entirely different diets. The gastrointestinal trematode fauna of large baleen whales is richer in species including *Ogmoagaster antarcticus*, *O. plicatus*, *Lecithodema goliath* and *L. spinosus*, which have been reported to parasitize the stomach as well as the small intestine of large whales (Baylis, 1932). Unfortunately, little is known on the pathogenesis of the genera *Ogmoagaster* and *Lecithodema* in these marine mammals. No helminths have been described so far in any large baleen whale species.

The spectrum of cestode species found in the small intestine of large baleen whales (blue, fin and sei whales) is rather broad and includes the species *Priapocephalus grandis*, *P. minor*, *Phyllobothrium delphini*, *Tetrabothrius affinis*, *T. arsenevi*, *T. wilsoni*, *T. ruudi*, *Diphyllodotum sp.*, and *Diplagonoporus balaenoptera* (Baylis, 1932; Delyamure, 1955). Furthermore, several nematode species parasitize other mysticeti, including ascariids such as *Anisakis simplex*, *Contracaecum sp.*, *Porrocaecum decipiens*, and giant spirurid nematodes such as *Crassicauda crassicauda*, *C. boops* and *C. pacifica*. Large baleen whales are known as definitive host of the anthropozoonotic nematode *Anisakis* sp. with a worldwide distribution (Delyamure, 1955; Klempf et al., 2004; Nadler et al., 2005; Kleinertz et al., 2014a, 2014b). *Anisakis* nematodes can be found in the lumen of the stomach or firmly attached, often as clusters, to the gastric mucosa (Geraci and St Aubin, 1987). Mucosal penetration by pre-adult larvae (Young and Lowe, 1969) and adults (McClelland, 1980) can lead to severe ulcers, gastritis and even perforation leading to severe peritonitis (Geraci and St Aubin, 1987). Giant nematodes species (up to 9 m in length) of the subfamily *Crassicaudinae* (Anderson, 1992), such as *C. crassicauda* and *Crassicauda delmareana*, are generally found in the kidneys, renal veins and intrarenal ureters of fin, blue and sei whales in the North Atlantic (Baylis, 1932; Skriban, 1966; Lambertsen, 1985). The transmission routes of these parasites have not been adequately studied yet, but as typical members of the heteroxenous spirurids, it appears likely that they use arthropods as intermediate hosts promoting infective stage larvae development (Anderson, 1992).

In addition to these metazoans, protozoan parasites have been recently reported to occur in large baleen whales inhabiting the North Atlantic Ocean. Thus, the enteropathogens *Entamoeba* *Giardia* and *Balantidium* have recently been described for the first time in blue, sei and fin whales (Hermosilla et al., 2015). In the past, particularly protozoan enteropathogens have not obtained sufficient attention in whale investigations, although they endemically occur in the marine environment (Raga et al., 1997; Hughes-Hanks et al., 2005; Raga et al., 2008; Van Bressem et al., 2009; Oliveira et al., 2011; Kleinertz et al., 2014b; Reboredo-Fernandez et al., 2014).

3. Ectoparasites of large whales (*Physeteridae, Balaenopteridae*)

Referring to marine mammal studies, the term ‘ectoparasite’ has rather loosely been used for any type of organisms (ranging from algae to fish) that somehow clings or attaches to the surface of a marine mammal, and whose mode of attachment, feeding behaviour, or relationship with the definitive host or transport animal (phoresis) are somehow obscure so that a parasitic origin cannot be excluded (Geraci and St Aubin, 1987). What seems doubted is the fact that many of these organisms have evolved as real ectoparasites of large whales and thus bear the potential to damage their sensitive epidermis. As such, blue whales entering the cold waters of the Antarctic often acquire a yellowish film on their bodies (commonly known as ‘sulfur bottoms’) resulting from diatom infestations, such as *Cocconeis ceticola* and *Navicula* spp. (Hart, 1935). These organisms attach firmly to the whale skin surface by sucker-like valves. Occasionally they may penetrate the epidermis thereby becoming saprophytic ectoparasites (Hart, 1935). However, most large whales being infested with diatoms shed these when returning to warmer or subtropical waters (Hart, 1935).

Most ectoparasite infestations are caused by arthropods, which have adapted to the oceanic environment. Such are the epizoic sessile barnacles *Coronula diadema*, *C. reginae* and *Crytophasa rhiachia* which attach so deeply onto their host skins that a pit remains when being removed or shed (Felix et al., 2006) which eventually results in a scar (Scheffer, 1939). In addition, the closely related aberrant sessile barnacle, *Xenobalanus globicipitis*, may also cause characteristic star-shaped scars on the epidermis of whales (Bane and Zullo, 1980).

A more serious skin-invader of large whales is *Penella balaenoptera* (Penellidae), one of the largest parasitic copepods in the ocean. It actively penetrates the skin of mysticetes and anchors deeply in the blister, feeding on whale tissue (Clarke, 1966). Sadly, nothing is known on the vector-borne capacity of *P. balaenoptera* so far, but it can be assumed that it might play a pivotal role in the
transmission of invasive pathogens as demonstrated for other marine ectoparasites, i.e. the seal lice *Echinophthirius horridus* transmitting the heartworm *Acanthochelonea spirucauda* (Leidenberger et al., 2007).

Although whale lice (Amphipoda) are grouped into a single compact family (Cyamidae), they are heterogeneous and include at least 11 different species (Leung, 1970). Whale lice species, such as *Cymus catodontis*, *C. bahamondi*, *C. ovalis* and *Neocymus physeteris* parasitizing the skin of sperm whales, are rather considered as euryxenous parasites, whilst the whale lice *C. balaenoptera* tend to be more host-specific (for review see Leung, 1970). In general, cyamid ectoparasites reside in fissures and crevices of the skin and are often found close to sessile barnacles and on the callosities of the head (Scheffer, 1939; Leung, 1970). The detrimental impact of these organisms is low, nonetheless, in heavily infested animals dermatitis may occur (Leung, 1970).

Primitive fishes (Agnatha), belonging to the lamprey species such as *Entosphenus tridentatus*, are by far the largest ectoparasites firmly attaching to the skin of large whales. These are polyxenous ectoparasites feeding on cetacean skin as a blood/nutrient source amongst others. Nonetheless, while being firmly attached by the teeth of their sucking disc, the lampreys can cause severe and deep haemorrhagic skin lesions (Pike, 1951). As such, they represent one of the largest and most voracious marine mammal parasites on earth (Pike, 1951; Geraci and St Aubin, 1987).

4. New emerging neozoan parasites within the marine environment

The introduction of opportunistic emerging neozoan infections into the marine ecosystem is of major importance for animal and human welfare (Van Bressem et al., 2009; Kleinertz et al., 2014b; Moore, 2014). In this context, protozoan parasites, such as *Toxoplasma gondii*, *Neospora caninum*, *Sarcocystis canis*, *Giardia* sp., *Cryptosporidium* sp., *Balantidium* sp. and *Entamoeba* sp., behaved in the past decades as classical neozoan parasites within the marine habitat (Resendes et al., 2002b; Hughes-Hanks et al., 2005; Fujii et al., 2007; Raga et al., 2008; Van Bressem et al., 2009; Kleinertz et al., 2014b; Reichel et al., 2015) and have emerged as devastating pathogens for several pinnipeds (Cabezón et al., 2011), dolphins (Resendes et al., 2002b; Dubey et al., 2008), sea otters (Conrad et al., 2005; Miller et al., 2008) and whales (Inskeep et al., 1990; Omata et al., 2006; Van Bressem et al., 2009; Mazzariol et al., 2012). Apicomplexan-derived diseases such as toxoplasmosis in marine mammals is intriguing and at present indicative of contamination of the oceans and coastal waters with sporulated oocysts (Conrad et al., 2005; Dubey et al., 2008; Dubey, 2009; Reichel et al., 2015). As in the case of terrestrial mammals primary *T. gondii*-infections in facultative intermediate hosts, congenital toxoplasmosis have also been reported to occur in small-sized cetacean species such as the Indo-Pacific bottlenose dolphin (*Tursiops aduncus*) (Jardine and Dubey, 2002). Nonetheless, so far nothing is known for large whales with regards to apicomplexan parasite infections. Thus, protozoan parasite infections of humans as well as animals most probably occur by contaminated terrestrial and aquatic sources, emphasizing the need for joint examination of humans, domestic animals and wildlife populations (Resendes et al., 2002a; Kleinertz et al., 2014b) as proposed by the new ‘One Health’ concept (Kahn, 2015).

5. Alternative methods to overcome the challenges in studying large whale ecto- and endoparasites

Given that the direct study of physiological parameters and pathogen infections in free-ranging large cetaceans is necessary to monitor the population health, several methods were established for non-lethal sample assessment (Hunt et al., 2013). We here intend to review non- and minimally-invasive techniques, which can be applied to obtain faecal samples, skin and blubber biopsies, vomitus samples, respiratory vapour samples and photographs.

6. Faecal samples

Large whale faecal consistency varies with the species, season and diet, ranging from well-formed floating semi-solid clumps (see Fig. 1) to a more fluid and dispersed plume. Floating semi-solid scat samples can be collected from the water surface (see Fig. 1B) using a fine-mesh nylon net fixed on a telescope (see Fig. 1C). More fluid faeces may be collected in plastic containers. Even heavier and rapidly sinking cetacean faecal material can be collected successfully by divers using plastic tubes as described elsewhere (Kleinertz et al., 2014b).

Several large whale species defecate on the ocean’s surface prior to diving (Fig. 1). Consequently, faecal samples can be collected opportunistically during any large whale monitoring surveys. Faecal samples have been collected by researchers diving in close proximity to cetaceans (Kleinertz et al., 2014b). Moreover, large whale faeces can be identified on the water surface area by trained dogs, as recently demonstrated for North Atlantic right whales and killer whales (Rolland et al., 2007; Hunt et al., 2013).

7. Skin biopsies and blubber samples

Since the early 1990s, the ‘minimally invasive’ dart-based biopsy method has become one of the most common skin sampling techniques for free-swimming large whale species (Barrett-Lennard et al., 1996). Therefore, either a crossbow (see Fig. 2) or a pneumatic rifle with modified dart tips are used (Lambertsen, 1987; Mathews et al., 1988). Additionally, it might be feasible to collect sloughed skin samples at the water surface after vigorous whale activities, such as mating, diving (e.g. sperm whales) or parturition. Such samples have been successfully used before (Whitehead et al., 1990; Valsecchi et al., 1998; Piersalowski et al., 2013), but their potential for parasitological analyses has not been clarified so far.

Especially polyxenous apicomplexan parasites such as *T. gondii* and *N. caninum*, being capable of parasitizing all type of nucleated host cells may be detected in host epithelial cells, fibroblasts, keratinocytes or even adipocytes. In summary, skin/blubber samples bear a wide spectrum of invaluable physiological information, ranging from epidermal lipidomics, proteomics, transcriptomics and hormones (Hunt et al., 2013), but in addition are also useful for the detection of ectoparasitic and dermal microbial diseases.

8. Vomitus samples

Although vomitus has seldom been reported to occur in large cetaceans and it is most probably happening particularly in marine mammals suffering acute oesophagitis or gastritis, it can be investigated. The option of sampling vomitus content is not a new one, since in recent years it has been realized that vomitus might represent a valuable, entirely non-invasive, physiopathological sample that could be collected with relative ease. In this context, a recent study on the gastrointestinal parasite fauna of free-ranging Indo-Pacific bottlenose dolphins (*T. aduncus*), demonstrated the usefulness of vomitus contents in addition to faeces (Kleinertz et al., 2014b). Consequently, large whale vomitus sampling should be included in the near future to efficiently monitor the animal infection status.
9. Respiratory samples (‘blow cloud’)

Large whales produce clouds of ‘blow’, which consist of exhaled droplets of condensed respiratory vapour when they exhale at the water surface. Whales usually blow several times during a single surfacing interval, and they can often be approached closely by floating boats during this event. In recent years, it has been realized that ‘blow clouds’ may represent valuable, entirely non-invasive samples, since studies on humans and other terrestrial animals showed that breath contains a varying mixture of volatile compounds in the gaseous phase (see Hunt et al., 2013). Furthermore, breath samples may also be used to detect the presence of bacterial, fungal, viral and parasitic infections. There is no doubt that particularly pseudalid nematodes (Metastrongylidae) can cause severe pneumonia in whales. Cetaceans may act as definitive hosts for the lungworms Pseudalis inflexus, Stenurus minor, Torynurus convolutus and Halocercus invaginatus, all of them residing in the main-stem bronchi (Arnold and Gaskin, 1975; Geraci and St Aubin, 1987). Thus the ‘blow cloud’ might be used for the detection of either lungworm larvae or eggs as described by Woodard et al. (1969). ‘Blow cloud’ droplets have been collected from large whales using a variety of sampling devices being attached to elongated poles and being positioned over the blowholes (Hogg et al., 2009; Schroeder et al., 2009; Hunt et al., 2012).

10. Imaging techniques for large whales

The visual assessment of the external appearance of an individual animal is essential for the determination of its health and nutritional status. Furthermore, visible aspects, such as skin condition, fresh wounds and ectoparasite loads and distribution (e.g. diatoms, sessile barnacles, whale lice and lampreys) provide additional information on individual and population health. In contrast to small cetaceans, where skin health is assessed by capture and release of the animals as shown elsewhere (Wells et al., 2004), this procedure is not applicable to large whales. Thus, a series of
photography-based studies were performed in the last decades using remote observational methods. Most imaging techniques to assess whole body condition and appearance of large whales have been based on photographs taken by airplanes, drones or large-sized ships. In contrast, boat-based photography permits close-up views of the skin. The presence, severity and extent of macroscopic ectoparasite infestations around the head, eyes and the blowholes, and the presence of scars due to past parasite infestations can be easily evaluated by this non-invasive method.

11. Conclusions

In contrast to past parasitological investigations carried out exclusively on killed or stranded large whales, we here review several newly minimal invasive sample collection methods which can be used for future studies on cetacean ecto- and endoparasite fauna. More importantly, these methods will allow us to gain a broader knowledge on the current status of originally terrestrial parasitoses (e.g. neosporosis, toxoplasmosis, giardiasis, cryptosporidiosis, and sarcocystosis) and also on new emerging anthropozoonotic parasites (e.g. entamoebiasis, balantidiasis) affecting endangered marine species such as large whales. Large whales will continue to be subjected to a variety of natural- and human-related pressures. The constant surveillance of whale health status within their natural habitats via these techniques will be of benefit for monitoring purposes. As a further step, conservation studies on large whales will greatly benefit from cross-disciplinary approaches which may include not only researchers on behavioural ecology but also experts from other fields, i.e. parasitologists, microbiologists, nutritionists, toxicologists, endocrinologist and clinicians.

Conflict of interest

The authors of this review declare that they do not have any conflicts of interest. Furthermore, the authors declare that they have no competing interests.

Acknowledgements

We are deeply thankful to those who contributed to the pictures included in this review: Marta Tabena (Fig. 1B, C) and N. Liebsch (Fig. 2). We further acknowledge funds and support from the Portuguese Fundação para a Ciência e a Tecnologia (FCT), Fundo Regional da Ciência, Tecnologia (RFCT), through research projects TRACE-PTDC/MAR/74071/2006 and MAPCTE-M2.1.2/F/012/2011 [FEDER, the Competitiveness Factors Operational (COMPETE), QREN European Social Fund, and Proconvergencia Açores/EU Program]. We acknowledge funds provided by FCT to MARE and by the FCT – Government of the Azores pluriannual funding. RP is supported by a research grant from the Azores Regional Fund for Science and Technology (M3.1.5/F/115/2012). MAS is supported by a research grant from the Azores Regional Fund for Science and Technology (M3.1.5/F/115/2012). MAS is supported by a research grant from the Azores Regional Fund for Science and Technology (M3.1.5/F/115/2012).

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