"ECOLOGIA, HISTÓRIA EVOLUTIVA E CONSERVAÇÃO DE *THALASSEUS SANDVICENSIS/ ACUFLAVIDUS/ EURYGNATHUS* (AVES: STERNIDAE)".

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Márcio Amorim Efe
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SUMÁRIO

DEDICO

ESTE VÔO ARRISCADO, LONGO E DESAFIADOR

Ao meu pai,

que nos momentos finais, se foi sem me ver aterrizar ...

À **Tina,** que esteve sempre ao meu lado e me manteve em

vôo durante a longa jornada.

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MUITO OBRIGADO

RESUMO

O primeiro capítulo apresenta dados sobre a ecologia reprodutiva do Trinta-réis-de-bico-amarelo, *Thalasseus sandvicensis eurygnathus* do Trinta-réis-de-bico-vermelho, *Sterna hirundinacea* e da Pardela-de-asa-larga, *Puffinus lherminieri* e o importante trabalho de conservação do ambiente insular desenvolvido no Estado do Espírito Santo. Todos os anos milhares de indivíduos de T. *s. eurygnathus* e *S. hirundinacea* usam as ilhas costeiras do sul do Espírito Santo para reproduzir. Os estudos foram desenvolvidos principalmente nas ilhas Itatiaia, Escalvada e Branca. O segundo capítulo apresenta dados sobre a ecologia reprodutiva, estado e ameaças à conservação, estado populacional e recomendações a cerca de temas de pesquisa e estratégias de conservação do Trinta-réis-real (*Thalasseus maximus*) e Trinta-réis-de-bico-amarelo na América do Sul onde nidificam principalmente na Argentina e Brasil. Trinta-réis-real tem reprodução registrada em no mínimo 22 localidades. Trinta-réis-de-bico-amarelo tem reprodução registrada em no mínimo 38 localidades. Em 15 localidades, a maioria na Argentina, as espécies nidificam em associação, frequentemente com seus ninhos entremeados. A população total para o Trinta-réis-real foi estimada em no mínimo 750 pares no Brasil e menos de 5000 na Argentina, enquanto que para o Trinta-réis-de-bico-amarelo foi estimado em no mínimo 8000 pares no Brasil e menos de 10000 na Argentina. As principais ameaças para suas populações em ambos os países são os distúrbios humanos, a pesca, a coleta de ovos e a expansão populacional do Gaivotão (*Larus dominicanus*). Ações prioritárias de pesquisa e conservação são apresentadas. O principal objetivo do terceiro capítulo foi esclarecer o relacionamento entre *T. s. sandvicensis*, *T. s. acuflavidus* e *T. s. eurygnathus* baseado em seqüências moleculares de DNA mitocondrial e seqüências nucleares, uma vez que ainda restam incertezas taxonômicas na tribo Sternini e na classificação do complexo *sandvicensis/ acuflavidus/ eurygnathus*. Material foi coletado para o estudo pelo autor e colaboradores em uma ampla área de distribuição geográfica da espécie. Os relacionamentos filogenéticos estimados pelos diferentes métodos e seqüências (MtDNA, nuclear, and MtDNA+nuclear) foram similares. Árvores construídas com as técnicas de Neighbor-Joining e análise Bayesiana do código-de-barras (*barcodes*) da Citocromo-Oxidase I também foram congruentes. Nossas análises indicaram que as populações dos trinta-réis do Velho Mundo (*T. s. sandvicensis*) e do Novo Mundo (*T. s. acuflavidus/eurygnathus*) são geneticamente tão divergentes como as diferentes espécies do gênero e não formam um grupo monofilético. Nós propomos que o tratamento taxonômico apropriado para o complexo *acuflavidus/eurygnathus* passe a ser como *Thalasseus acuflavidus*. O quarto capítulo apresenta o primeiro estudo genético com a espécie usando seqüências mitocondriais e nucleares, assim como dados de microsatélites. A diversidade do MtDNA é baixa na espécie. Todas as três populações apresentam sinais de efeito gargalo e expansão populacional. Por outro lado, dados de microsatélites sugerem um recente fluxo gênico entre as populações. Os resultados sugerem a ocorrênca de uma zona de hibridização entre o Brasil e a América do Norte. A diferença entre os períodos reprodutivos no Brasil e Argentina pode ser importante no recente isolamento destas aves costeiras. O último capítulo avalia o estado populacional de *T. acuflavidus* no Brasil e discute sua categoria de ameaça. A população brasileira está principalmente confinada na costa do Espírito Santo. Nossa avaliação do estado de conservação da espécie seguiu os critérios e categorias adotadas pela UICN. Nós revisamos vários parâmetros incluindo o nível taxonômico, as principais ameaças, a área e a extensão de ocorrência e o atual tamanho populacional. Nós recomendamos que a espécie seja categorizada como Vulnerável no nível nacional. Ela pode também ser classificada como Em Perigo no nível regional. Finalmente sugerimos que esforços de pesquisa e conservação sejam ampliados na costa do Espírito Santo e que ações semelhantes de conservação sejam implementadas ao longo da costa brasileira.

ABSTRACT

The first chapter presents data about breeding ecology of the Cayenne Tern, also nominated as *Thalasseus sandvicensis eurygnathus*, South American Tern, *Sterna hirundinacea* and Audubon's Shearwater, *Puffinus lherminieri* and the important work of insular environmental conservation developed in the State of Espírito Santo. Every year thousands of individuals of S. *s. eurygnatha* and *S. hirundinacea* use the coastal islands of the southern coast of the state of Espírito Santo to breed. Studies on the bioecology of this species are being developed at the breeding sites and resting and feeding areas since 1985. The studies were developed mainly on the Itatiaia Islands, Escalvada Island and Branca Island. The second chapter presents data about the reproductive ecology, status and threats to conservation, populational status and recommendations about the topics of research and strategies for conservation of the Royal Terns (*Thalasseus maximus maximus*) and Cayenne Terns (*Thalasseus sandvicensis eurygnathus)* in South America, where breeding mostly in Argentina and Brazil. Royal Terns have been recorded in at least 22 locations. Cayenne Terns have been recorded in at least 38 locations. At 15 locations, mostly located in Argentina, Royal and Cayenne terns breed in association, often with their nests intermingled. Total population size for Royal Terns was estimated in at least 750 pairs in Brazil and less than 5000 in Argentina, while that of Cayenne Terns was estimated in at least 8000 pairs in Brazil and less than 10000 in Argentina. Main threats faced by their populations in both countries are human disturbance, fisheries, egging, and expanding Kelp Gull (*Larus dominicanus*) populations. Priority research and conservation actions are presented. The aim of the third chapter is to clarify the relationships among the Sandwich, Cayenne, and Cabot's terns based on nuclear and mtDNA sequences, because one of the remaining taxonomic uncertainties in the Sternini is in the classification of the species complex. Material was collected for this study by the authors and collaborators, from a wide range of geographic locations. Phylogenetic relationships estimated by the different methods and sequence partitions (mtDNA, nuclear, and mtDNA+nuclear) were similar. Trees recovered with Neighbor-Joining and BI analysis of COI barcodes too were congruent. Our analysis indicates that the Old World (*T. s. sandvicensis*) and the New World (*T. s. acuflavidus/eurygnathus*) tern populations are genetically as divergent as different species in the genus, and do not form a monophyletic group. We propose that the appropriate taxonomic treatment of the *acuflavidus/eurygnathus* complex should be as Cabot's Tern, *Thalasseus acuflavidus*. The fourth chapter presents the first genetic study of this species using mitochondrial and nuclear sequences as well as microsatellites data. MtDNA diversity is low in the species. All three populations present signals of bottleneck and population expansion. On the other hand, microsatellites data support a recent gene flow among populations. Results suggest the occurrence of a hybridization zone between Brazil and North America. The different breeding periods in Brazil and Argentina could be important in the recent isolation of these coastal birds. The last chapter evaluates the conservation status of *T. acuflavidus* in Brazil and discusses its threat category. The Brazilian population is mainly confined to the coast of Espírito Santo state. Our evaluation of the conservation status of this species follows the criteria and categories adopted by the IUCN. We review several parameters, including taxonomic level, main threats, area and extent of occurrence, and current population size. We recommend that this species should be defined as Vulnerable at the national level. It may also qualify as Endangered at the regional level. Finally, we suggest that research and conservation efforts should be increased on Espírito Santo coast, and that conservation actions should be implemented across the whole Brazilian coast.

APRESENTAÇÃO

O maior enigma taxonômico na sub-família Sterninae é a classificação do grupo *Thalasseus sandvicensis/acuflavidus/eurygnathus* um taxon que foi muito debatido durante o último século. Originalmente, *Thalasseus sandvicensis* e *T. eurygnathus* (antigamente *Sterna eurygnatha*) foram consideradas espécies válidas (Moynihan 1959). Posteriores tratamentos taxonômicos consideraram *T. eurygnathus* uma subespécie, raça ou morfo de *T. sandvicensis*. Atualmente *T. s. eurygnathus* é amplamente reconhecido como a subespécie sul-americana e *T. s. acuflavidus* como a subespécie norte-americana (Sibley and Monroe 1990, Gochfeld and Burger 1996, Shealer 1999).

O relacionamento taxonômico entre *T. s. acuflavidus* e *T. s. eurygnathus* é pouco conhecido o qual parece estar envolvido como parte de uma clina e/ou hibridização ao longo da costa da Venezuela (Hayes 2004). Sibley & Monroe (1990) com base em estudos filogenéticos caracterizam a subespécie como *Thalasseus sandvicensis eurygnathus* e afirmam que ela é freqüentemente tratada como espécie separada, mas ocorre intercruzamento em colônias mistas onde as raças estão em contato. Efe *et al*. (2004) concluiu que estudos genéticos analisando indivíduos das sub-populações do Brasil e futuramente comparando-os com aves das populações nidificantes na Argentina e Caribe seriam de extrema importância para a elucidação do enigma que envolve as subespécies do grupo *sandvicensis/eurygnathus*.

Apesar da controvérsia existente há muito tempo sobre a taxonomia e relações filogenéticas entre *T. s. sandvicensis* , *T. s. acuflavidus* e *T. s. eurygnathus* Recentemente, Bridge *et al*. (2005) publicaram uma análise completa sobre a filogenia da família Sternidae inferida através de seqüências nucleotídicas de DNA mitocondrial, no entanto não chegaram a nenhum

consenso sobre o arranjo filogenético do grupo *sandvicensis/acuflavidus/eurygnathus* e não examinaram aves pertencentes às populações brasileiras e européias.

No Brasil o trinta-réis-de-bico-amarelo, *T. s. eurygnathus* (Figura 1), conhecido também como trinta-réis-de-bando ou andorinha-do-mar-de-bico-amarelo reproduz-se preferencialmente em ilhas rochosas próximas à costa, sendo comumente observada entre os meses de abril e outubro freqüentando bóias sinalizadoras e pedras próximas à costa ou sobrevoando e alimentando-se em águas costeiras.

Figura 1. Fotos de adulto, ovo e filhote característicos do trinta-réis-de-bico-amarelo.

Moure *et al*. (1985) registraram pela primeira vez a colônia de trinta-réis-de-bico-amarelo no litoral do Espírito Santo, até então desconhecida dos pesquisadores brasileiros. Mais tarde *T. s. eurygnathus* foi considerada por Antas (1991) como a espécie costeira mais vulnerável do Brasil,

quando registrou que as colônias do Espírito Santo vinham sofrendo extensivas coletas de ovos por parte dos pescadores, o que podia afetar severamente o sucesso reprodutivo da espécie.

O presente trabalho tem por objetivo contribuir com o conhecimento a respeito da ecologia reprodutiva de *T. s. eurygnathus* no País, principalmente nas ilhas do Espírito Santo, considerado o maior sítio reprodutivo do trinta-réis-de-bico-amarelo no Brasil (Efe *et al*. 2000), além de abordar aspectos do seu congênere *T. maximus* (trinta-réis-real). O trabalho aborda também a caracterização da filogenia do grupo *sandvicensis/acuflavidus/eurygnathus* e propõe um novo arranjo taxonômico para o táxon. Contribui ainda com o conhecimento sobre a história evolutiva e variabilidade genética do trinta-réis-de-bico-amarelo nas Américas e avalia e propõe alterações no estado de conservação da espécie no Brasil. O trabalho segue apresentado através de cinco artigos que tratam dos temas propostos.

O primeiro artigo, de cunho introdutório nesta tese, versa sobre a ecologia reprodutiva do trinta-réis-de-bico-amarelo nas ilhas do Espírito Santo e de outras aves marinhas, tais como o trinta-réis-de-bico-vermelho, *Sterna hirundinacea* e a pardela-de-asa-larga, *Puffinus lherminieri* além do importante trabalho de conservação dos ambientes insulares desenvolvido no Estado, considerado o maior sítio reprodutivo do trinta-réis-de-bico-amarelo no Atlântico sul (Efe *et al*. 2000). Entre outras informações, o trabalho apresenta dados inéditos para o Brasil sobre aspectos comportamentais do trinta-réis-de-bico-amarelo durante a côrte e cuidade com a prole. O artigo foi publicado no livro "Aves marinhas e insulares brasileiras: bioecologia e conservação", editado pela UNIVALI, Itajaí, SC em 2004.

O segundo artigo apresenta dados a respeito da ecologia reprodutiva, estado e ameaças à conservação, estado populacional e recomendações acerca de temas de pesquisa e estratégias de conservação dos trinta-réis-de-penacho (trinta-réis-de-bico-amarelo e trinta-réis-real, *T. maximus*)

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no Brasil e na Argentina. O artigo foi apresentado na IV *North American Ornithological Conference*, Veracruz, México em 2006 e será publicado em breve no periódico *Waterbirds*.

O terceiro artigo estuda o relacionamento filogenético entre uma população européia e duas populações americanas do grupo *sandvicensis/acuflavidus/eurygnathus* com base em seqüências nucleotídicas de DNA mitocondrial e nuclear e propõe um novo arranjo taxonômico para o táxon. O artigo está no formato apropriado para ser publicado como nota no periódico *Molecular Phylogenetics and Evolution*.

Sobre a nova ótica taxonômica o quarto artigo aborda a variabilidade genética do trinta-réis-de-bico-amarelo nas Américas a partir de seqüências nucleotídicas de DNA mitocondrial e nuclear, além de microsatélites, bem como discute a história evolutiva, as diferenças morfológicas e ecológicas e as implicações taxonômicas e conservacionistas para a espécie. O artigo está no formato apropriado para ser publicado no periódico *Molecular Ecology*.

O último artigo faz uma revisão do estado taxonômico, das principais ameaças, da extensão de ocorrência e área de ocupação, do tamanho e condições populacionais da espécie e avalia e propõe alterações no estado de conservação no Brasil. O artigo está no formato apropriado para ser publicado no periódico *Bird Conservation International*.

ÁREAS DE ESTUDO

O primeiro artigo estudou a espécie no litoral do Espírito Santo (Figura 2), o qual possui várias ilhas costeiras onde ocorre a reprodução do trinta-réis-de-bico-amarelo e de outras aves marinhas. Entre as ilhas mais representativas estão as Ilhas Itatiaia, em Vila Velha (20º 21' 30" S; 40º 16' 45" W), a Ilha Escalvada em Guarapari (20º 42' S; 40º 24' 24" W) e a Ilha Branca em Marataízes (21º 00' S; 40º 47' W).
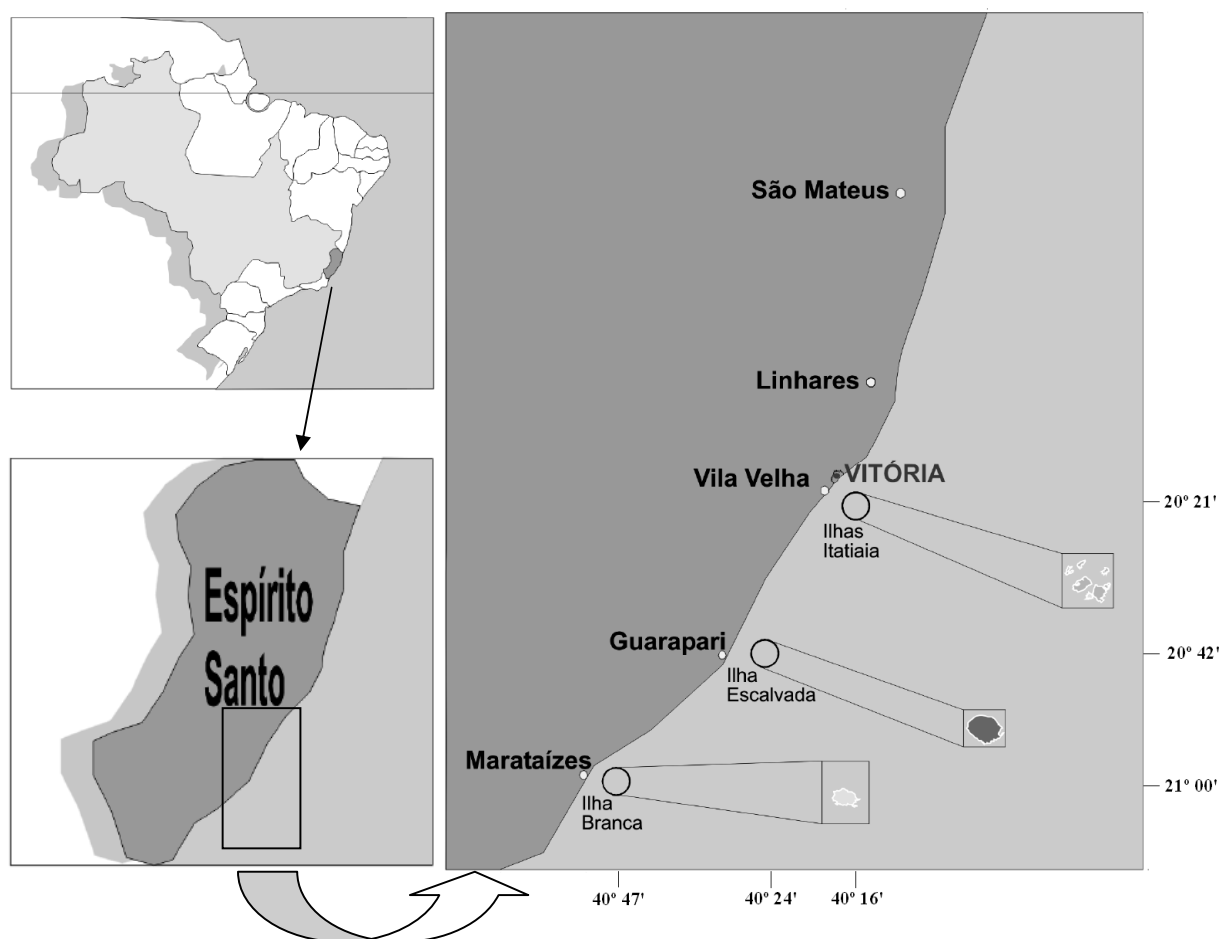
Figura 2. Mapa com as ilhas do litoral do Espírito Santo utilizadas para reprodução do trinta-réis-de-bico-amarelo.

O arquipélago das Ilhas Itatiaia (Figura 3) é formado por sete ilhas e situa-se a 1000 m da Praia de Itapoã, em Vila Velha. Algumas delas são pequenas ilhas rochosas sem vegetação,

enquanto outras são maiores e possuem vegetação rasteira, como gramíneas e cactos e arbustiva e arbórea, como mangue e castanheiras. A Ilha Escalvada (Figura 3) localizada à aproximadamente oito quilômetros da praia de Setiba no município de Guarapari. Tem aspecto circular, altura de aproximadamente 15 metros e apresenta em sua região central, vegetação rasteira composta principalmente por gramíneas. Sua região periférica é rochosa e desprovida de vegetação. No topo da ilha existe um farol de sinalização com 20 metros de altura e um antigo reservatório d'água, atualmente desativado, que funciona como base de apoio aos trabalhos desenvolvidos na ilha. A ilha Branca (Figura 3) localiza-se em frente a barra do rio Itapemirim, a 1.400 m da costa, no município de Itapemirim é também conhecida como ilhas dos Ovos e apresenta vegetação rasteira formada principalmente por cactos.

Figura 3. Fotos do arquipélago das Ilhas Itatiaia (alto à esquerda), da Ilha Escalvada (alto à direita) e da Ilha Branca ou dos Ovos (embaixo).

O segundo artigo apresenta informações sobre sítios de reprodução do trinta-réis-real e do trinta-réis-de-bico-amarelo no Brasil e na Argentina abrangendo áreas na costa sul e sudeste do Brasil e Patagônia Argentina (Figura 4).
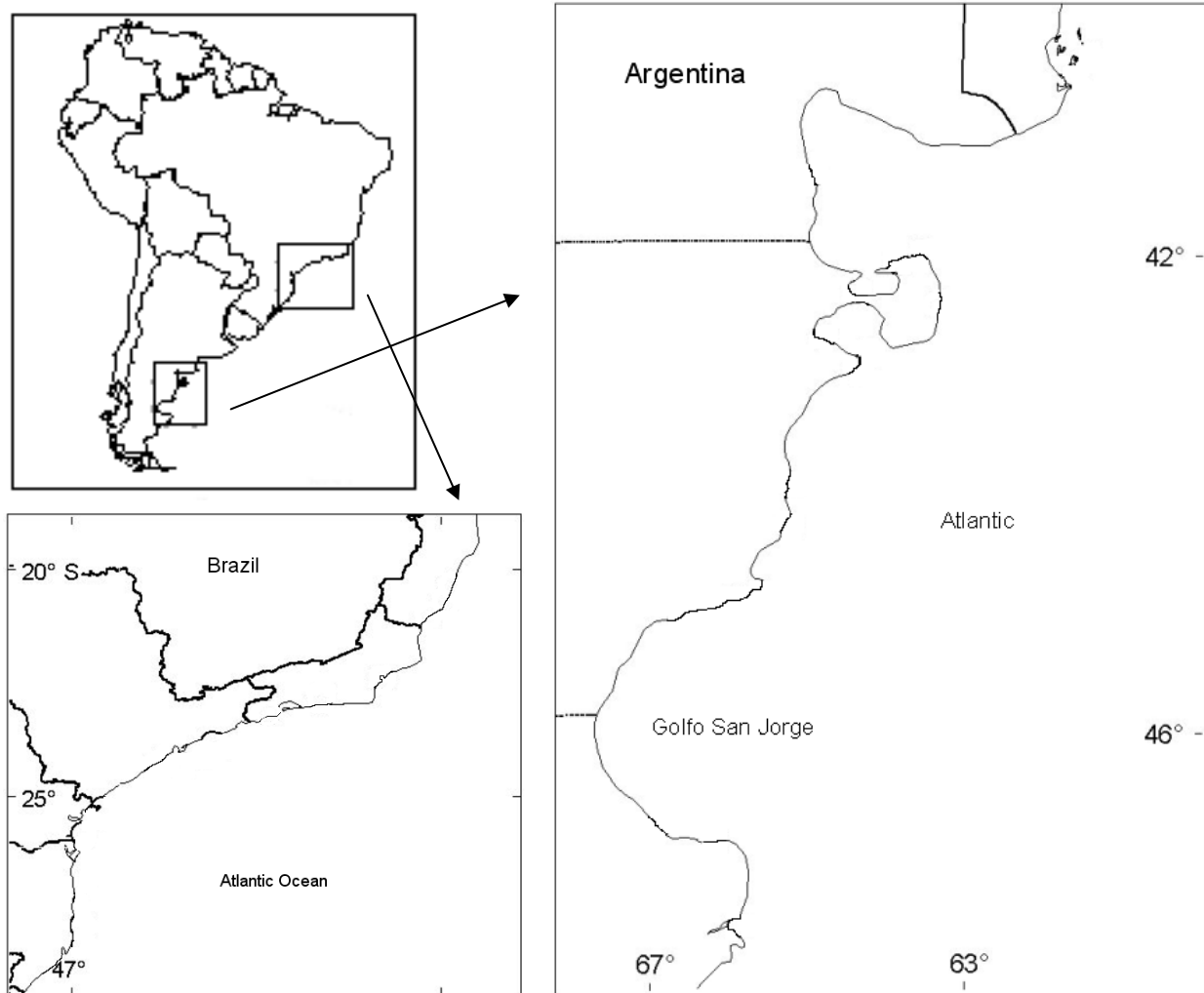
Figura 4. Mapa da costa sul e sudeste do Brasil e Patagônia Argentina onde se encontram as colônias reprodutivas do trinta-réis-real e do trinta-réis-de-bico-amarelo no Brasil e na Argentina.

Para o terceiro e quarto artigos o material foi especialmente coletado pelo autor e colaboradores em uma ampla área geográfica tanto na Europa, em Ebro Delta, Espanha (40º 37'

N; 00º 35' E), como nas Américas, na Carolina do Norte, EUA (35º 32' N; 75º 59' W), na ilha

Escalvada, Brasil e em Punta León, Argentina (43º 03' S; 64º 27' W) (Figura 5).
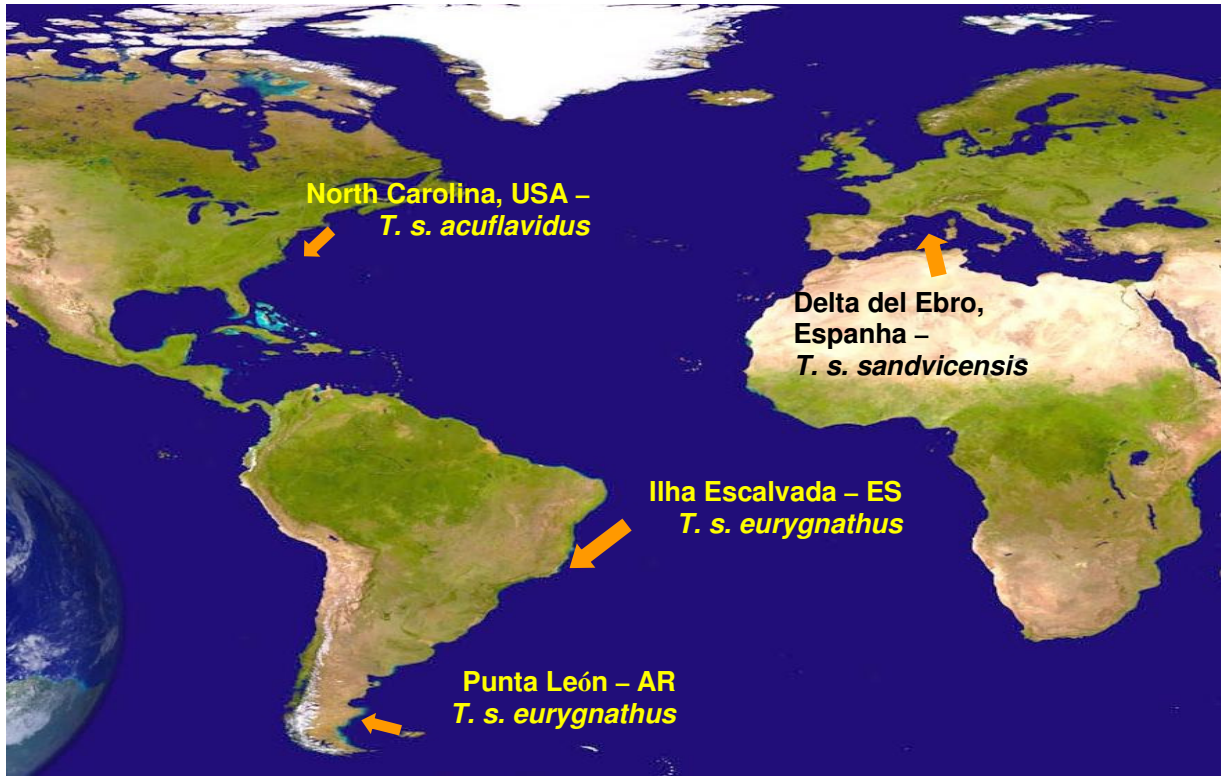
Figura 5. Mapa das áreas onde foram coletadas amostras de sangue para as análises filogenéticas e filogeográficas.

CAPÍTULO 1

Aves Marinhas das Ilhas do Espírito Santo

Publicado no livro **Aves marinhas e insulares brasileiras: bioecologia e conservação** (Organizado por Joaquim Olinto Branco). Editora da UNIVALI, Itajaí, SC.

CAPÍTULO 5

AVES MARINHAS DAS ILHAS DO ESPÍRITO SANTO

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ABSTRACT

Sea birds of Espírito Santo Islands. This chapter presents data about breeding ecology of the Cayenne Tern, *Sterna sandvicensis eurygnatha*, South American Tern, *Sterna hirundinacea* and Audubon's Shearwater, *Puffinus lherminieri* and the important work of insular environmental conservation developed in the State of Espírito Santo. Every year thousands of individuals of *S. s. eurygnatha* and *S. hirundinacea* use the coastal islands of the southern coast of the state of Espírito Santo to breed, between the months of May and September. Studies on the bioecology of this species are being developed at the breeding sites and resting and feeding areas in Brazil since 1985, and part of these data is discussed and presented in this chapter. The studies were developed mainly on the Itatiaia Islands, Escalvada Island and Branca Island. The terns began to arrive in mid-April. The settlement at the colony site occurs from May onwards. The first chicks began to be born in the first weeks of June. In mid-September the birds begin to leave the colonies and after the end of October are rarely found on the coast of the State of Espírito Santo. In August 1993, a single Audubon´s Shearwater, *Puffinus lherminieri* was found nesting in a cavity under a rock on one of the islands of the Itatiaia Archipelago, State of Espírito Santo. In later nocturnal visits to the islands, four other nests were found, with fledglings, in natural cavities under stones. The young left the nests in December. This new record for the State of Espírito Santo may indicate that this species is dispersing and colonizing new areas in the tropics, or simply that other breeding areas for the species are as yet unknown. More recent initiatives are investing in the transformation of the coastal islands of the state of Espírito Santo and surroundings into environmental protection areas, ensuring the conservation of breeding sites by legal instruments to protect them.

INTRODUÇÃO

O litoral brasileiro é um dos mais extensos do mundo e abriga uma importante diversidade de espécies de aves marinhas costeiras e oceânicas. No entanto, experimentou, especialmente, nas últimas décadas, um processo de degradação ambiental que começou pelo desmatamento para a implantação dos primeiros grandes aglomerados urbanos e culmina hoje com a má condução dos despejos de esgotos domésticos e industriais, aterros e ocupação da orla, os quais atinge os manguezais, lagoas, cursos d'água, praias e áreas marinhas com conseqüente comprometimento da qualidade de vida das populações envolvidas. Aliou-se a esses fatores uma

exploração inadequada do turismo e dos recursos naturais, que não valorizou a conservação do patrimônio natural e cultural. Essa exploração inadequada dos recursos naturais e a crescente explosão demográfica ao longo dos últimos anos alteraram de forma significante a paisagem dos ambientes litorâneos brasileiros.

Da mesma forma, as ilhas costeiras, ao longo dos anos vêm sofrendo enorme degradação de seus ecossistemas, principalmente por estarem próximas ao continente urbanizado e receberem visitas periódicas de pescadores e turistas. Essas agressões constantes vêm agravando a recomposição natural da vegetação e transformando essas ilhas em ambientes inadequados para a fauna existente nas ilhas.

Ao longo da costa brasileira as aves marinhas enfrentam vários problemas, sendo os mais freqüentes a coleta de ovos por parte de pescadores e visitantes, a perturbação das colônias e a poluição dos mares, especialmente por petróleo e derivados, que a médio e longo prazo causa problemas tanto no nível de sobrevivência do indivíduo como interfere no seu sucesso reprodutivo (Vooren & Fernandes, 1989). Outro grave problema é a introdução de animais exóticos nos ambientes insulares, que afetam as aves marinhas através da predação direta por gatos e ratos e da destruição do habitat de reprodução por caprinos, eqüinos e bovinos.

Os principais fatores que dificultam a reprodução das espécies marinhas no Espírito Santo são a alteração do habitat e a interferência antrópica direta através da presença humana na área e coleta de ovos, impostas pelo crescente desenvolvimento populacional das grandes cidades e, a carência de programas conservacionistas voltados para essas áreas, como já evidenciado por Antas (1990).

As ilhas existentes ao longo do litoral sul do Espírito Santo têm papel importante como refúgio para várias espécies de aves, tanto residentes quanto migratórias. Das aves migratórias, as que mais dependem desses ambientes são, sem dúvida, os trinta-réis do gênero *Sterna*.

Nos meses de maio a setembro, essas ilhas funcionam como sítio reprodutivo de duas espécies de andorinhas-do-mar, o trinta-réis-de-bico-amarelo, *Sterna sandvicensis eurygnatha* e o trinta-réis-de-bico-vermelho, *Sterna hirundinacea*.

Para o monitoramento dessas aves foi criado em 1998 o Projeto Andorinhas do Mar, desenvolvido pela Associação Vila-Velhense de Proteção Ambiental – AVIDEPA em conjunto com o Centro Nacional de Pesquisas para Conservação das Aves Silvestres - CEMAVE/IBAMA. O Projeto vem desenvolvendo atividades de conservação, educação ambiental e pesquisa, tanto no sítio reprodutivo do Espírito Santo como em outras áreas onde as andorinhas-do-mar reproduzem-se e passam durante sua migração, integrando instituições e esforços na conservação dessas espécies.

As atividades visando à proteção dos sítios de reprodução são importantes como iniciativa de conservação dessas espécies e funcionam

como catalisador de uma postura conservacionista, que vem contribuindo para a melhoria da qualidade de vida das populações humanas. A presença de colônias de reprodução de aves migratórias em ilhas próximas a costa, é um evidente indicador biológico das condições de conservação dos ecossistemas costeiros.

ÁREA DE ESTUDO

O litoral do Espírito Santo possui várias ilhas costeiras nas quais ocorre reprodução de aves marinhas (Fig. 1). Entre elas as mais representativas são as Ilhas Itatiaia em Vila Velha (20º 21' 30" S 40º 16' 45" W), Ilha Escalvada, em Guarapari (20º 42' S 40º 24' 24" W) e a Ilha Branca, em Marataízes (21º 00' S 40º 47' W).

As Ilhas Itatiaia formam um arquipélago composto por sete ilhas rochosas situadas a 1.000 metros da praia de Itapoá, no município de Vila Velha. Apenas as duas maiores ilhas apresentam vegetações compostas principalmente por cactáceas e gramíneas.

A Ilha Escalvada está localizada a oito quilômetros da praia de Setiba no município de Guarapari. Tem aspecto circular, altura de aproximadamente 15 metros e apresenta em sua região central, vegetação rasteira composta principalmente por gramíneas. Sua região periférica é rochosa e desprovida de vegetação. No topo da ilha existe um farol de sinalização com 20 metros de altura e um antigo reservatório d'água, atualmente desativado, que funciona como base de apoio aos trabalhos desenvolvidos na ilha.

A Ilha Branca ou dos Ovos, como também é conhecida, situa-se a 1.400 metros da foz do rio Itapemirim no município de Marataízes. Ilha rochosa, coberta por vegetação rasteira composta por gramíneas e cactos na porção central, também possui um farol para sinalização marítima.

MATERIAL E MÉTODOS

Os dados e informações foram obtidos entre os anos de 1993 e 1996 nas Ilhas costeiras do litoral sul do Espírito Santo.

Dentre as atividades desenvolvidas pelo Projeto Andorinhas do Mar, o anilhamento tem sido a mais intensificada. A marcação se deu com anilhas do CEMAVE - Centro de Pesquisas para Conservação das Aves Silvestres, órgão do IBAMA que coordena o Sistema Nacional de Anilhamento de Aves Silvestres.

Nas ilhas do Espírito Santo, durante a temporada reprodutiva das andorinhas-do-mar, os filhotes recém-nascidos foram marcados ainda no ninho. A captura dos filhotes com mais de uma semana de vida foi realizada utilizando-se um cercado, para onde foram encaminhados os filhotes que se
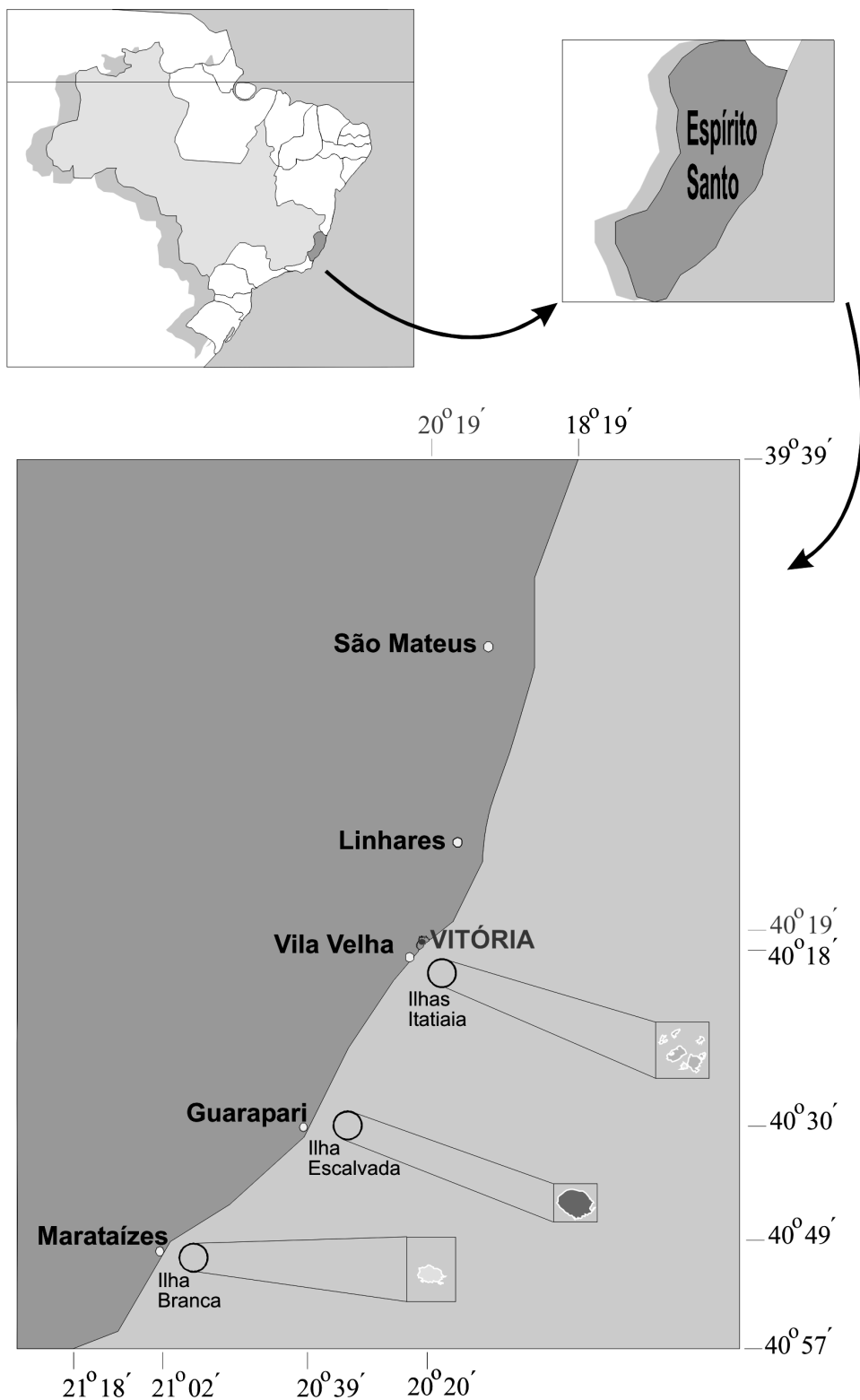
Figura 1 - Mapa com a localização das ilhas costeiras no litoral sul do Espírito Santo.

encontravam na região periférica da ilha, conforme descrito em Efe *et al.* (2000). Os adultos foram capturados utilizando-se redes ornitológicas (*mist-nets*). As observações do comportamento reprodutivo da andorinha-do-mar-de-bico-amarelo, *Sterna sandvicensis eurygnatha* foram realizadas nas ilhas Itatiaia e Escalvada. Para isso, utilizou-se o método de varredura, no qual se percorre com os olhos toda a área de estudo registrando os eventos comportamentais realizados. Durante a observação os eventos foram descritos oralmente e gravados em micro-cassetes. Nas Ilhas Itatiaia foram coletadas informações a respeito do comportamento de "corte" utilizando-se uma luneta instalada em uma ilha, próxima à ilha em que os indivíduos se encontravam pousados. Na Ilha Escalvada o ninhal estudado estava instalado junto à base de apoio, o que proporcionou a coleta visual de informações a respeito do comportamento de "cuidados com a prole". Outro repertório comportamental estudado nessa ilha foi denominado de "creche", para o qual utilizou-se o farol como ponto de observação e um binóculo como equipamento visual.

Os registros comportamentais de "corte" foram realizados em quatro sessões diárias de 20 minutos, com intervalo de duas horas entre elas, durante um dia e os registros a respeito dos "cuidados com a prole" e "creche" utilizaram o mesmo intervalo de tempo, sendo realizados durante dois dias.

A realização destes trabalhos com as andorinhas-do-mar intensificou as visitas às ilhas e possibilitou estudos com outras espécies que ocorrem e nidificam no litoral do Espírito Santo.

O trabalho com a pardela-de-asa-larga se deu exclusivamente nas Ilhas Itatiaia. Os filhotes e adultos foram capturados manualmente durante a noite e marcados com anilhas metálicas adaptadas à forma triangular da pata da ave.

As Espécies

Trinta-réis-bico-amarelo, *Sterna sandvicensis eurygnatha*

O trinta-réis-de-bico-amarelo ou andorinha-do-mar-de-bico-amarelo, *Sterna sandvicensis eurygnatha* é amplamente distribuído na costa atlântica da América do Sul, desde as ilhas do Caribe (12º N) até a região de Porto Deseado (46º S) (Escalante, 1970). Segundo Sibley & Monroe (1990), eles invernam ao longo da costa da América do Sul até a Argentina e vagam até a Ilha Cozumel na Península Yucatán. Spaans (1978) considera a costa do Suriname um importante ponto de descanso para as aves oriundas do norte, entretanto não descarta a possibilidade de aparecerem indivíduos provenientes das populações que se reproduzem ao sul. Yorio *et al.* (1994) afirmam que *Sterna spp*. são escassas na Patagônia e existem muito poucas colônias de *S. sandvicensis eurygnatha* na Argentina.

Historicamente, existem registros de colônias de reprodução nas Ilhas da Baía de Guanabara e Ilha do Papagaio, no Rio de Janeiro (Sick, 1997), na Ilha da Figueira em São Paulo (Scherer-Neto, 1985 *in* Efe, 2000) e na Ilha Deserta em Santa Catarina (Escalante *et al*., 1988), no entanto atualmente estes locais não têm sido utilizados com freqüência e as contagens da espécie nestas localidades, nunca ultrapassam poucas dezenas.

No Brasil a espécie reproduz-se preferencialmente em ilhas rochosas próximas à costa, sendo comumente observada entre os meses de abril e outubro freqüentando bóias sinalizadoras e pedras próximas à costa ou sobrevoando e alimentando-se em águas costeiras. De acordo com Efe *et al*. (2000) das 25.733 aves anilhadas no Espírito Santo entre 1988 e 1997, 169 (0,66 %) foram recuperadas, até 1999, ao longo da costa da América do Sul, desde o Maranhão até o norte da Argentina.

Moure *et al*. (1985) *in* Efe *et al*. (2000) registraram pela primeira vez a colônia de trinta-réis-de-bico-amarelo no litoral do Espírito Santo, até então desconhecida dos pesquisadores brasileiros. Mais tarde *Sterna sandvicensis eurygnatha* foi considerada por Antas (1990) como a espécie costeira mais vulnerável do Brasil, quando registrou que as colônias do Espírito Santo vinham sofrendo extensivas coletas de ovos por parte dos pescadores, o que podia afetar severamente o sucesso reprodutivo da espécie. Atualmente, Efe *et al*. (2000) estimam a população total do Espírito Santo entre 10.000 e 13.000 indivíduos, e considera a região como o maior sítio reprodutivo da espécie em todo o Atlântico Sul.

Na costa do Espírito Santo, *S. sandvicensis eurygnatha* surge em meados de abril e no início de setembro as aves começam a deixar as colônias. Após o final de outubro, raramente são encontradas na costa do Espírito Santo. De acordo com Efe *et al.* (2000), o estabelecimento da colônia reprodutiva ocorre a partir de maio e os primeiros filhotes começam a nascer nas primeiras semanas de junho.

Os casais de *S. sandvicensis eurygnatha* formam densas colônias. Os ovos e filhotes do trinta-réis-de-bico-amarelo, em geral são brancos e cobertos por manchas, necessitando de maior proteção contra predadores. Nas colônias do Espírito Santo, Efe (2001) registrou um ovo por ninho depositado em pequenas depressões no solo ou em ninhos confeccionados com gravetos na vegetação rasteira. Dados de 150 ovos medidos apresentaram o comprimento médio de 51,83 mm ± 2,0 (48,7 mm - 57,1 mm) e largura média de 35,91 mm ± 1,36 (31,3 mm - 39,6 mm). A massa média dos 150 ovos foi de 35,49 g ± 3,15 (25g - 45g).

Os filhotes, após a primeira semana, se agrupam em creches na região periférica dos ninhais onde são protegidos por adultos.

Outra estratégia de proteção é o fato de que a população costuma variar na escolha do local de reprodução a cada temporada reprodutiva, utilizando assim, de forma diversa, as ilhas disponíveis na costa do Espírito Santo. Segundo Efe (2001), a freqüência de ocorrência de reprodução foi

maior nas Ilhas Escalvada e Itatiaia, cujos valores corresponderam a 90% e 70%, respectivamente.

Quando se sentem ameaçados os indivíduos adultos de Trinta-réis-de-bico-amarelo levantam vôo e permanecem vocalizando e sobrevoando o ninhal em uma constante algazarra. Passada a ameaça, retornam todos, sincronizadamente, a seus ninhos.

A partir do estudo comportamental foi possível identificar e descrever os principais eventos realizados pelas aves.

As observações relacionadas à "corte" totalizaram 80 minutos de observação. Verificou-se que os eventos foram mais intensos nas primeiras horas do dia, quando a temperatura era mais amena. Pelo fato da espécie não apresentar dimorfismo sexual aparente, durante todo o trabalho os indivíduos serão tratados como "provável macho" e "provável fêmea". Durante o estudo, alguns eventos foram identificados e são descritos a seguir:

Caminhada: Neste evento o "provável macho" empurra a "provável fêmea" com o bico, obrigando-a a caminhar por entre o bando de indivíduos pousados. Foi comum observar durante a caminhada a "provável fêmea" com as asas entreabertas.

Dança: O "provável macho" se exibe para a "provável fêmea", fazendo movimentos de abrir e fechar as asas e levantar e abaixar o bico enquanto a rodeia.

Oferecendo alimento: Durante a corte foi comum observar a "provável fêmea" pousada no solo, se abaixar e vocalizar ao perceber a chegada de seu parceiro em vôo com alimento preso ao bico. Após o pouso do "provável macho", a "provável fêmea" bicava por várias vezes o peito do "provável macho" e, agachada, esticava o pescoço tentando alcançar o alimento preso ao bico do parceiro. Este por sua vez, fazia movimentos de abaixar e levantar a cabeça. Após algum período nesse ritual, o "provável macho" liberava o alimento para a "provável fêmea".

Cópula: A cópula, geralmente é precedida por um ou mais dos eventos descritos anteriormente. No ato da cópula, como acontece nas outras espécies de aves, o macho sobe no dorso da fêmea e batendo as asas mantém o equilíbrio, até que, com sua cauda, consegue afastar lateralmente a cauda da fêmea e encostar sua cloaca à dela. Esse movimento pode se repetir por várias vezes em um curto período de tempo.

As observações relacionadas aos "cuidados com a prole" foram realizadas na Ilha Escalvada e totalizaram 160 minutos de observação. Nesta fase do ciclo reprodutivo foram identificados os seguintes eventos:

Incubação: Evento no qual o indivíduo permanece chocando o ovo. Freqüentemente o indivíduo que choca, levanta-se e arruma o ovo com o bico.

Troca de turno: Este evento caracteriza o revezamento realizado por ambos os pais, na incubação dos ovos e cuidados com o filhote. Durante o dia são feitas várias trocas de turno. Na maioria das vezes, a mesma acontece de forma bastante rápida, devido à proximidade dos ninhos e enorme agressividade por parte dos vizinhos. Neste evento, o indivíduo que chega, pousa ao lado do indivíduo que está chocando e começa a receber bicadas dos seus vizinhos. Imediatamente o indivíduo que chocava levanta-se e deixa o ovo ou o filhote expostos. O indivíduo que chegou assume a proteção e o que chocava, passa também a receber bicadas até levantar vôo. Quando o casal apresenta filhote, geralmente o indivíduo que chega, traz alimento preso ao bico, que é repassado ao filhote por ele ou por intermédio do adulto que choca.

Defesa do território: A espécie, por reproduzir em colônias densas e numerosas se mostrou fortemente territorialista, exibindo constantemente eventos agressivos em defesa de seu ninho. Foi comum observar a ave pousada em seu ninho, com ovo ou filhote, vocalizar e trocar bicadas fortes com os vizinhos ao seu redor. O território de cada par apresenta seus limites relacionados com o círculo coberto pelo alcance dos seus bicos.

Manutenção do par: Durante a incubação e cuidados com o filhote, foi comum observar a identificação dos parceiros quando se encontravam, principalmente, durante a troca de turno. Quando um indivíduo pousava junto ao outro que chocava no ninho, imediatamente ele recebia bicadas leves de seu parceiro e ambos vocalizavam até que se reconheciam. Eventualmente foram observados movimentos de abaixar e levantar a cabeça e abrir as asas, que foram realizadas pelos dois parceiros. Em algumas ocasiões o indivíduo que chocava, foi alimentado pelo parceiro que chegou.

Cuidados com a plumagem: Durante a fase de incubação e desenvolvimento do filhote, o indivíduo que chocava, constantemente, arrumava sua plumagem com o bico.

As observações relacionadas ao comportamento de "creche" foram realizadas na Ilha Escalvada em uma creche de aproximadamente 170 filhotes com idades entre três e quatro semanas, totalizando 160 minutos de observação.

Após os filhotes completarem uma semana de vida, a maioria abandonava a região do ninhal e concentravam-se em grupos na região periférica da ilha. Geralmente esses grupos são protegidos e acompanhados de perto por alguns adultos que caminham na borda dos grupos. A formação dessas creches é uma das formas de proteção da espécie contra seus predadores, assim como a reprodução em colônias e ninhais densos. Abaixo seguem descritos alguns eventos identificados:

Alimentação do filhote: Durante todo o tempo foi comum observar adultos com peixe no bico sobrevoando o local e vocalizando sobre a creche. Nesse

momento os filhotes reagiram de duas maneiras, (1) os filhotes que identificavam os adultos como sendo um de seus pais saíam do centro da creche e iam receber o alimento; (2) os outros filhotes, que não tinham relação parental com os adultos em vôo, agachavam-se, esticavam o pescoço e vocalizavam para o alto como forma de pedir alimento. Quando o adulto, eventualmente, pousava próximo do seu provável filhote com o peixe no bico, imediatamente ele era atacado por outros filhotes famintos, que muitas vezes roubavam o alimento do bico do adulto. Durante esses eventos os filhotes eram acompanhados de perto pelos adultos que acompanham e protegem a creche. Esses, por vezes, podem espantar inclusive outros adultos que pousem próximo à creche. Esses adultos são responsáveis também pela condução e acompanhamento do filhote que se "desgarra" da creche, trazendo-o de volta ao grupo. Efe *et al.* (2000) estimaram a população na Ilha Branca em 1990 em 10.000 indivíduos, na Ilha Escalvada em 1994 em 10.000 indivíduos e em 13.000 indivíduos na Ilha Escalvada em 1996. Nos anos de contagem estas ilhas concentraram os maiores números de indivíduos entre as colônias utilizadas para reprodução no Espírito Santo.

Gochfeld & Burger *in* Hoyo *et al.* (1996) com base na análise fenética de esqueleto e morfologia externa realizada por Schnell (1970) indicam seis espécies para o gênero *Sterna* e alocam o trinta-réis-de-bico-amarelo, assim como outros trinta-réis grandes com crista no gênero *Thalasseus*. Segundo Efe *et al.* (2000) no grupo dos Trinta-réis, um dos grandes enigmas é a caracterização taxonômica aplicada a *Sterna sandvicensis eurygnatha*, que ultimamente vem sofrendo algumas variações. Sick (1997) trata *Sterna sandvicensis eurygnatha* e *Sterna sandvicensis sandvicensis* de forma independente.

Os adultos da sub-população brasileira apresentam grande variação na quantidade de preto existente, em forma de manchas, nos cúlmens e tarsos. Quanto ao cúlmem, observou-se que a coloração variava do amarelo ao negro, com diversos graus intermediários. Efe (2001) registrou três padrões de cores, na colônia reprodutiva da Ilha Escalvada, onde verificou uma freqüência de 55,22% de aves adultas com cúlmem amarelo e 44,77% de aves com cúlmem mesclado entre amarelo e preto.

Norton (1984) afirma que a área de invernada de *Sterna sandvicensis acuflavida*, que tem cúlmem negro com ponta amarela, se sobrepõe à área de reprodução de *S. sandvicensis eurygnatha* na costa da América do Sul onde vínculos sociais podem ser formados para a próxima primavera, influenciando movimentos extra-limites e recrutamento de jovens em idade reprodutiva procurando habitats de reprodução. Ansingh *et al.* (1960), também defendem que a variação na coloração do cúlmem tem sido atribuída ao resultado das intergradações secundárias da forma do sul de cúlmem amarelo, *eurygnatha* e da forma de cúlmem preto do norte, *acuflavida* (Junge & Voous, 1955). Por outro lado, Buckley & Buckley (1984) afirmam que mesmo com o intercruzamento de *eurygnatha* e *sandvicensis* nas Antilhas Holandesas aparentemente não há evidências para considerar um recente contato ou

que a zona de hibridização esteja em expansão, e isso não pode ser considerado uma indicação de possível troca de alelos entre os grupos de *eurygnatha* e *sandvicensis* até que esta questão seja cuidadosamente examinada. Segundo Harrison (1983) a espécie é considerada por muitos autores como uma raça de *Sterna sandvicensis*, com o qual parece estar envolvido como parte de uma clima e/ou hibridização ao longo da costa da Venezuela. Sibley & Monroe (1990) com base em estudos filogenéticos caracterizam a espécie como *Sterna sandvicensis eurygnatha* e afirmam que ela é freqüentemente tratada como espécie separada, mas ocorre inter-cruzamento em colônias mistas onde as raças estão em contato.

As variações morfológicas destas raças, há muito vêm sendo discutido. Ansingh *et al.* (1960) descreveram e quantificaram a variedade de cores nos ovos, pernas e cúlmens de adultos de Trinta-réis-de-bico-amarelo, *Sterna sandvicensis eurygnatha* em Curaçao, ilha ao sul do Caribe. Sick (1997) trata *Sterna sandvicensis eurygnatha* e *Sterna sandvicensis sandvicensis* de forma independente, indicando o tamanho de 41 cm para a primeira e, de 32 a 35 cm para a última. Efe (2001) estudou a variabilidade morfométrica de ovos, filhotes e adultos no sítio reprodutivo do Estado do Espírito Santo, bem como comparou medidas lineares de adultos da espécie no sítio reprodutivo do Espírito Santo e em áreas de alimentação como o Parque Nacional da Lagoa do Peixe, no Rio Grande do Sul e na Ilha Coroa Vermelha, na Bahia. Os resultados da análise das medidas dos adultos nas três áreas de estudo mostrou diferenças significativas para todos os parâmetros analisados, podendo portanto, aceitar-se a hipótese de que elas pertençam à populações diferentes. Efe (2001) conclui que estudos genéticos analisando indivíduos das sub-populações do Brasil e futuramente comparando-os com aves das populações nidificantes na Argentina e Caribe, serão de extrema importância para a elucidação do enigma que envolve as subespécies do grupo *sandvicensis / eurygnatha*.

Recentemente, Efe (2001) também avaliou a produtividade, mortalidade, sobrevivência e expectativa de vida na idade específica da coorte acompanhada na estação reprodutiva de 1993 na Ilha Escalvada, bem como apresentou dados de sucesso reprodutivo da população nidificante na mesma ilha entre os anos de 1993 e 1997. No estudo o autor verificou uma alta taxa de mortalidade até os sete primeiros dias de vida dos filhotes, quando são mais vulneráveis aos ataques dos predadores e intempéries climáticas. Os dados concordam com vários autores que afirmam que o período mais crítico na sobrevivência dos filhotes está entre os primeiros 10 dias após a eclosão (Klett & Johnson, 1982; Nisbet *et al*., 1990, 1998 e 1999). Efe *et al.* (2000) demonstram que dos 379 filhotes marcados logo no primeiro dia de vida na Ilha Escalvada em 1993, no mínimo 100 deles atingiram a idade de vôo (quinta semana), o que resulta em um sucesso reprodutivo de 0,26 filhotes recrutados por par. Dados apresentados em Efe (2001) resultam em um sucesso ainda maior ao longo dos anos (1993 = 0,65 filhotes/par; 1994 = 0,45 filhotes/par; 1995 = 0,90 filhotes/par; 1996 = 0,63 filhotes/par e 1997 = 0,64 filhotes/par),

levando em conta o total de recrutas em relação ao número total de ovos e concordam com resultados obtidos em outras áreas apresentadas em Shealer (1999), onde as melhores produtividades variaram entre 0,44 e 0,96 filhotes/par. Segundo Efe (2001), o alto sucesso de nascimento nos anos de estudo, com média em torno de 80%, foi superior ao encontrado por Quintana & Yorio (1997) em seus estudos com *Sterna sandvicensis eurygnatha* na Patagônia e ficou próximo ao encontrado nas colônias de *Sterna sandvicensis sandvicensis* na Inglaterra (Shealer, 1999).

Efe (2001) demonstra que o crescimento anual do número de fêmeas férteis para o período estudado, se revelou positivo, com uma taxa de crescimento vegetativo de 1,051 % ao ano, considerando os dados globais em 1997 em relação a 1993. Com base nestes dados projeta-se uma taxa intrínseca de crescimento populacional, r = 0,199. Os resultados revelam que a população do Espírito Santo vem se recuperando. No entanto, mesmo com a tendência aparentemente crescente do tamanho populacional da espécie no Espírito Santo, o recente passado de desaparecimento da espécie em outras áreas da costa brasileira é motivo de alarme e chama a atenção para a continuidade do monitoramento das colônias reprodutivas do Espírito Santo.

Trinta-réis-de-bico-vermelho, *Sterna hirundinacea*

Confinado à América do Sul o trinta-réis-de-bico-vermelho, *Sterna hirundinacea* distribui-se entre o paralelo 25º S (Brasil) na costa atlântica até o paralelo 15º S (Peru) na costa pacífica, com limite sul na Terra do Fogo (Harrison, 1983).

Na costa brasileira, *S. hirundinacea* geralmente nidifica em simpatria com o trinta-réis-de-bico-amarelo, *Sterna sandvicensis eurygnatha*. No litoral do Estado do Espírito Santo durante o acompanhamento da temporada reprodutiva verificou-se que ambas as espécies utilizam estratégias reprodutivas diferentes. Enquanto *S. sandvicensis eurygnatha* forma densas colônias, *S. hirundinacea* faz seus ninhos na região periférica destes ninhais. O trinta-réis-de-bico-vermelho, nidifica isoladamente em ninhos esparsos, seus ovos e filhotes são escuros e bem mimetizados com o ambiente. Os filhotes, após a primeira semana de vida, abandonam os ninhos e buscam abrigo em baixo da vegetação vizinha, semelhante ao comportamento descrito por Palmer (1941), para o trinta-réis-boreal, *S. hirundo*.

O trinta-réis-de-bico-vermelho, *Sterna hirundinacea* é naturalmente mais agressiva e, portanto é ela a responsável pelas reações mais consistentes contra os predadores, quando em colônia mista com *S. sandvicensis eurygnatha*. Durante a reprodução, quando se sentem ameaçados, os trinta-réis-de-bico-vermelho levantam vôo e vão em direção dos invasores, gritando e mergulhando em direção ao seu corpo, por vezes chegando à atingir o intruso com bicadas.

Nas Ilhas Itatiaia, geralmente as colônias das duas espécies ocorrem em ilhas separadas. A maior parte da população de trinta-réis-de-bico-vermelho faz seus ninhos na ilha onde existe a Base de Apoio do Projeto Andorinhas do Mar. Seus ovos são colocados diretamente no solo por entre os cactos e/ou em meio às rochas existentes na ilha. Coloca de um a três ovos de fundo marrom e coberto por manchas pretas. Seus filhotes nidífugos acompanham o mesmo padrão de cor do ovo, ao nascer.

Na temporada reprodutiva de 1994, a espécie com maior número de pares reprodutivos nas Ilhas Itatiaia foi a *S. hirundinacea*, com 490 ninhos. Na ilha onde existe a Base de Apoio do Projeto Andorinhas do Mar 58 ninhos foram marcados e acompanhados diariamente, sendo que 56, desde a postura do primeiro ovo. Nos 58 ninhos foram postos 93 ovos, dos quais 40 chegaram a eclodir, o que representou um sucesso de 43%.

Foram medidos 190 ovos, os quais apresentaram como médias, as seguintes medidas: comprimento – 45,81 mm (39,6 - 52,4 mm), largura – 32,77 mm (29,5 - 35,5 mm) e massa – 24,1g (17 – 31g). Dentre os filhotes nascidos na temporada de 1994 nas Ilhas Itatiaia, 95 foram medidos no primeiro dia de vida. As medidas destes filhotes apresentaram os seguintes valores médios; cúlmen exposto - 10,48 mm (8,9 -12,2 mm), tarso - 15,76 mm (11,2 - 20,7 mm), corda da asa - 17,97 mm (14,3 - 39,2 mm) e massa - 22,1 g (14 -28 g). O acompanhamento dos 40 ovos desde a postura até o nascimento, mostrou um tempo médio de 23 (19-26) dias.

Nas colônias reprodutivas dos trinta-réis no Espírito Santo, Efe *et al.* (2000) verificou a predação por parte do Urubu-comum, *Coragyps atratus*, que geralmente pousa no ninhal furando os ovos e, por vezes, ataca os filhotes menores. O Gavião Caracará, *Polyborus plancus*, foi também freqüente e predou filhotes e adultos. Outro predador verificado com menos freqüência no ataque à adultos e filhotes foi a Gaivota-rapineira, *Stercorarius parasiticus*. No entanto, observações de campo e análise dos dados de ovos inviáveis e filhotes mortos, sugerem que a maior causa de mortalidade nas colônias do Espírito Santo, foram aparentemente, as freqüentes tempestades (Efe, 2001).

Pardela-de-asa-larga, *Puffinus Iherminieri*

No Brasil, a Pardela-da-trindade, *Pterodroma arminjoniana*, com colônia reprodutiva conhecida na Ilha da Trindade (Sick, 1997), tem sido tradicionalmente a única representante da família Procellariidae com registro de reprodução em território brasileiro. Em 1990, *Puffinus Iherminieri* foi registrado pela primeira vez em colônia reprodutiva estabelecida no arquipélago de Fernando de Noronha, mas os autores apresentaram sua descoberta no Congresso Brasileiro de Ornitologia em 1990 como sendo o primeiro registro de *Puffinus assimilis*, cabendo a Efe & Musso (2001) a publicação do primeiro registro de *P. Iherminieri* para o Brasil. Posteriormente a identificação da ave encontrada em Fernando de Noronha

foi questionada por Soto & Filippini (2000). Recentemente, Soto & Filippini (2003) confirmaram a ocorrência e reprodução de *P. lherminieri* em Fernando de Noronha e revisaram os registros de *P. lherminieri* no Brasil.

No Espírito Santo a colônia conhecida está estabelecida em uma das ilhas do arquipélago das Itatiaia. Os adultos começam a freqüentar a ilha a partir de julho, quando começam a pernoitar em seus ninhos. Os ninhos não apresentam material de construção, o único ovo é depositado, em agosto, diretamente no solo em cavidades naturais ou buracos cavados sob rochas. Ao todo são conhecidos cinco ninhos. O filhote é coberto por uma penugem cinza e permanece sozinho no ninho, durante o dia, sendo alimentado apenas durante a noite, período de maior atividade na colônia, quando os adultos chegam, perambulam perto dos ninhos e vocalizam bastante, comportamentos também verificados por Brooke (1990) para o bobo-pequeno, *Puffinus puffinus*. Os filhotes deixam os ninhos em dezembro.

Através da marcação e recaptura dos adultos e filhotes, realizada desde 1993, sabe-se que a espécie apresenta fidelidade ao ninho e ao parceiro, retornando a cada ano para reproduzir-se na mesma cavidade e com o mesmo parceiro.

No período de agosto 1993 a outubro de 1996 foram anilhados os 10 adultos, 11 ninhegos e um indivíduo jovem encontrado, posteriormente, na Praia de Itapoã, em Vila Velha. Foram medidos ao todo oito adultos, os quais apresentaram as seguintes médias em milímetros: cúlmen exposto: 29,7 (29,0 - 31,4); corda da asa: 210,6 (203,0 - 218,0); cauda: 83,8 (80,0 - 87,0); tarso: 41,0 (44,6 - 38,3) e peso (7 indivíduos): 224,1 g (189,0 - 259,0). As médias biométricas em milímetros dos seis ovos medidos foram 52,6 mm (48,5 - 54,8) de comprimento, 36,8 mm (36,2 - 37,4) de largura e 36,3 g (33,0 - 38,0) de peso.

Ao longo dos últimos anos têm surgido diversos trabalhos, onde são descritas novas formas para a espécie e descobertos novos sítios reprodutivos. Os relatos de reprodução da espécie para Trinidad e Tobago, eram considerados por Murphy (1936) como os registros mais ao sul do continente americano e segundo Bourne & Loveridge (1978) não se havia localizado ainda colônias dessa espécie no Atlântico Sul, até que foi relatada a descoberta de fósseis em Santa Helena e Ascensão, mas isso parece nunca ter sido bem estabelecido, pelo menos para Ascensão (Olson, 1977).

De acordo com pescadores da região, a espécie é bastante conhecida e costuma investir contra as iscas utilizadas nas pescarias. De acordo com Efe & Musso (2001), provavelmente, a espécie nidifica nessa ilha desde pelo menos 1970, quando foram encontrados alguns indivíduos pousados sobre a vegetação rasteira existente na ilha.

Com esse registro para o estado do Espírito Santo, no Brasil, pode-se pensar na possibilidade de a espécie estar se dispersando e colonizando novas áreas na região tropical, ou simplesmente, ainda serem desconhecidos outros locais de reprodução da espécie. Isso pode ser explicado pelo fato dessas aves apresentarem hábitos pelágicos e um vôo

muito rápido, o que torna difícil a visualização e identificação da espécie em alto mar. Além disso, as aves dessa espécie costumam chegar na colônia reprodutiva das Ilhas Itatiaia, muito tempo após o anoitecer e saírem antes do dia clarear e, o adulto que fica no ninho durante o dia, cuidando do ovo ou do filhote, permanece no ninho em silêncio, sem dar sinal de sua presença.

O fato de terem sido encontrados nas praias, posteriormente, outros indivíduos jovens sem estarem anilhados, sugere que existam outros ninhos ainda não encontrados na região e estimula a continuidade dos estudos com a espécie, que recentemente foi incluída na lista brasileira de animais ameaçados de extinção (MMA 2003).

Vale registrar, também a reprodução e ocorrência de outras espécies nas ilhas do litoral do Espírito Santo, como o piru-piru, *Haematopus palliatus*, que freqüentemente reproduz-se nas Ilhas Itatiaia, onde já foram observados ninhos, no mês de dezembro, construídos com lascas e pequenos fragmentos de rochas, contendo um ou dois ovos. Na mesma ilha é comum a observação de bandos de vira-pedras, *Arenaria interpres*, alimentando-se, nos meses de primavera e verão. A ilha Escalvada, também é utilizada para o descanso de indivíduos jovens de atobás nascidos na colônia reprodutiva existente no Parque Nacional Marinho dos Abrolhos, Bahia. Em várias oportunidades foram observados e recapturados jovens de atobá-marrom, *Sula leucogaster* e de atobá-mascarado, *Sula dactylatra*. Outro registro importante é a expressiva colônia de garças-brancas (*Casmerodius albus, Egretta thula e Bubulcus ibis*) e do socó-dorminhoco, *Nycticorax nycticorax* existente na Ilha das Garças, em Vila Velha.

CONSERVAÇÃO E EDUCAÇÃO AMBIENTAL

De acordo com Efe *et al.* (2000) as áreas onde foram identificadas atividades reprodutivas do trinta-réis-de-bico-amarelo localizam-se nas regiões sudeste e sul do Brasil e na região patagônica Argentina. Apesar de Magno (1973) citar a existência de reprodução de *Sterna sandvicensis eurygnatha* no norte do Brasil, até hoje não foram encontrados sítios reprodutivos da espécie nessa região. Atualmente, as ilhas do Rio de Janeiro, São Paulo e Santa Catarina não vêm sendo mais utilizadas com sucesso para a reprodução da espécie. Esse fato pode estar ligado principalmente a três fatores: alteração ou perda de habitats, interferência antrópica e forte pressão por predadores naturais.

As ilhas costeiras do litoral sul do Espírito Santo, ao longo dos anos vinham sofrendo uma enorme degradação de seus ecossistemas por estarem próximas ao continente urbanizado e receberem visitas periódicas de pescadores e turistas que ateavam fogo à vegetação insular. Esses incêndios constantes vinham agravando a recomposição natural da vegetação

transformando essas ilhas em ambientes inóspitos tanto para o homem como para a fauna existente nas ilhas, principalmente a colônia reprodutiva dos trinta-réis, que vinha sendo afetada pela constante retirada de ovos feita pelos pescadores locais.

Após a criação do Projeto Andorinhas do Mar em 1988, a situação nas colônias reprodutivas de trinta-réis no litoral do Espírito Santo foi revertida. Através de atividades de controle de desembarque e educação ambiental, as coletas de ovos por parte dos pescadores foram interrompidas. Em 1989, o governo do Estado do Espírito Santo, através da Secretaria Estadual para Assuntos do Meio Ambiente, em reconhecimento à importância dos sítios reprodutivos e ao trabalho de monitoramento e conservação desenvolvido, estabeleceu uma Portaria Normativa (02/89-SEAMA) que proíbe o desembarque de pescadores e visitantes nas Ilhas do Pacote, Itatiaia, Escalvada e Branca, entre os meses de maio e setembro, por ocasião da época reprodutiva dos trinta-réis. A fiscalização do desembarque passou a ser intensificada a partir da instalação e manutenção de bases de apoio nas ilhas.

O trabalho de divulgação e educação ambiental vem sendo realizado pela equipe da Associação Vila-Velhense de Proteção Ambiental – AVIDEPA, através da produção de material educativo (folderes, cartazes e cartilha), apresentação de palestras em escolas da região litorânea, participação em feiras e eventos e recepção dos visitantes nas bases de apoio das ilhas. Durante todas essas oportunidades, a mensagem conservacionista e as atividades do projeto são apresentadas aos ouvintes. Este trabalho tem contribuído na participação cada vez maior da população litorânea nas ações de conservação das ilhas, auxiliando na fiscalização e na obtenção de informações sobre a reprodução das aves, fato este citado como um bom exemplo por Antas (1990).

As atividades de recuperação dos ambientes insulares, também realizado pela equipe da AVIDEPA, tiveram início com a retirada de animais exóticos (Porquinho-da-índia, *Cavia porcellus* e Coelho, *Oryctolagus cuniculus*) introduzidos nas ilhas por pescadores locais, para engorda e posterior captura. Em seguida, procedeu-se a retirada do capim-colonião (*Panicum sp*.) espécie invasora que ameaçava se proliferar e tomar conta de toda a área das ilhas. Paralelamente, foram plantadas espécies nativas rasteiras e arbustivas (p. ex.: *Canavalia rosea*) a fim de manter a qualidade ambiental das ilhas e proporcionar o aumento de área útil para a reprodução das aves.

Iniciativas mais recentes estão investindo na transformação das ilhas de Vila Velha e seu entorno em uma Unidade de Conservação Municipal, na busca de garantias para a conservação dos sítios reprodutivos através de instrumentos legais de proteção.

No que diz respeito à conservação de aves marinhas no Espírito Santo, vale citar, também a preocupação com o grupo formado pela Ilha de Trindade e Martim Vaz, situado à aproximadamente 1.200 km em linha reta da costa

de Vitória, capital do Estado, local com o maior número de espécies de aves oceânicas com problemas de conservação no Brasil. Ali está o único local conhecido de reprodução da endêmica pardela-da-trindade, *Pterodroma arminjoniana*, e das fragatas *Fregata ariel* e *Fregata minor* no Atlântico.

AGRADECIMENTOS

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CAPÍTULO 2

Population Status of Royal and Cayenne Terns Breeding in Argentina and Brazil.

Population Status of Royal and Cayenne Terns Breeding in Argentina and Brazil

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Abstract.—In South America, Royal Terns (*Thalasseus maximus maximus*) and Cayenne Terns (*Thalasseus sandvicensis eurygnathus)* breed mostly in Argentina and Brazil. Royal Terns have been recorded in at least 22 locations (six in Brazil and 14 in Argentina). Cayenne Terns have been recorded in at least 38 locations (15 in Brazil and 23 in Argentina). At 15 locations, mostly located in Argentina, Royal and Cayenne terns breed in association, often with their nests intermingled. Total population size for Royal Terns was estimated in at least 750 pairs in Brazil and less than 5000 in Argentina, while that of Cayenne Tern was estimated in at least 8000 pairs in Brazil and less than 10000 in Argentina. However, lack of counts at some coastal sectors and changes among breeding sites between seasons preclude an accurate estimation of total population size for both species and make spatial management challenging. Main threats faced by their populations in both countries are human disturbance, fisheries, egging, and expanding Kelp Gull (*Larus dominicanus*) populations. Priority research and conservation actions are presented. *Received 20 September 2007, accepted 23 May 2008.*

Key words.—Argentina, Brazil, Cayenne Tern, conservation, population status, Royal Tern, *Thalasseus maximus maximus*, *Thalasseus sandvicensis eurygnathus*.

Royal Terns (*Thalasseus maximus maximus*) and Cayenne Terns (*Thalasseus sandvicensis eurygnathus*) are two widely distributed tern species in the Americas, breeding from southern United States to Argentina (Shealer 1999; Buckley and Buckley 2002). The Cayenne Tern *T. s. eurygnathus* was formerly considered to be a full species (*Sterna eurygnatha*). However, more recent authors (Sibley and Monroe 1990; Shealer 1999; Efe *et al.* 2004, 2005) have considered it as a subspecies, which appears to form a cline and probably hybridize with *T. s. acuflavidus* in overlapping areas of their ranges. Royal Terns breeding in the Western Hemisphere are currently considered *Thalasseus maximus maximus*, although some authors argue that austral populations may be subspecifically or even specifically distinct from those in North America (Buckley and Buckley 2002). In this paper, we treat the Cayenne Tern (*T. s. eurygnathus*) as a subspecies of the Sandwich Tern (*T. sandvicensis*) (Shealer 1999; Bridge *et al.* 2005, Comitê Brasileiro de Registros Ornitológicos 2005) and Royal Tern as *T. maximus* (Bridge *et al.* 2005) until clarification of the taxonomic status of these species.

In the Atlantic coasts of South America, Royal and Cayenne terns breed mostly in Brazil and Argentina (Gochfeld and Burger 1996). Both countries have large coastlines (adding to more than 12,700 km) which provide adequate breeding habitats for terns but which are also subject to growing human activities. Conservation strategies and coordinated management actions at the regional scale require knowledge of tern breeding distribution, the relationships among populations, and of factors affecting them. In this paper we review information on the distribution and abundance of breeding Royal and Cayenne terns in Brazil and Argentina and discuss common problems and threats faced by their populations in both countries. Finally, we present some recommendations on priority actions in relation to research and conservation of both species in Argentina and Brazil.

BREEDING DISTRIBUTION

A review of historical records of nesting distribution, obtained from published and unpublished sources (see references in Tables 1 and 2), indicates that in the last 30

Table 1. Location and size (in breeding pairs) of Royal and Cayenne tern colonies in coastal Brazil. Values presented correspond to the last available census. All locations where terns have bred in the past are listed.

	State	Site	Location	Cayenne Tern			Royal Tern		
				Size (n° nests)	Year	Source	Size (n° nests)	Year	Source
1	Espìrito Santo	Pacotes Is.	20°21'S,40°16'W	NC	1994	1			
2		Escalvada Is.	20°42'S,40°24'W	6500	1996	1			
3		Itatiaia Is.	20°21'S,40°17'W	1500	1996	1			
4		Branca Is.	21°00'S,40°47'W	5.000	1990	1			
5	Rìo de Janeiro	Papagaios Is.	22°24'S,41°48'W	NC	1981	2			
6		Baía de Guanabara Is.	22°47'S,43°08'W	NC		3			
7		Rio-Niteroi Bridge	22°52'S,43°10'W	66	2001	3			
8	São Paulo	Prainha Is.	23°51'S,45°25'W	75	U	4	1	1998	4
9		Apara Is.	23°50'S,45°33'W	25	U	4			
10		Amigos Is.	24°04'S,45°39'W				60	U	4
11		Laje das Trinta-réis Is.	24°05'S,45°41'W				2	1998	4
12		Laje Santos Is.	24°19'S,46°11'W	142	U	4	187	1993	5
13		Gaivotas Is.	24°22'S,46°48'W				7	U	4
14		Lage da Conceição Is.	24°14'S,46°41'W				120	U	4
15		Castilho Is.	25°16'S,47°57'W	40		6			
16	Paraná	Itacolomis Is.	25°50'S,48°24'W	100	1995	7			
17	Santa Catarina	Deserta Is.	27°16'S,48°20'W	65	1999	8			
18		Moleques do sul Is.	27°51'S,48°26'W	200	2000	8			
19		Cardos Is.	27°48'S,48°34'W	76	2002	8			

Notes: NC: present not censused; 1 Efe *et al.* 2000; 2 Antas 1991; 3 Alves *et al.* 2004; 4 Campos *et al.* 2004; 5 T. Neves, unpubl. abstract.; 6 Olmos *et al.* 1995; 7 Krull 2004; 8 Branco 2003.

years Royal Terns have bred in a total of 20 sites, six in Brazil and 14 in Argentina (Tables 1 and 2), while Cayenne Terns have bred in at least 38 breeding sites, 15 in Brazil and 23 in Argentina (Tables 1 and 2). Breeding of *T. sandvicensis* in northern Brazil, as reported by Magno (1971), still needs confirmation. However, both tern species may shift breeding locations among years (Antas 1991; Yorio *et al.* 1999; Efe *et al.* 2000; Branco 2004) and, thus, more than one site listed may have been used by the same population in different years. Based only on the records obtained in the last decade (Branco 2004; Yorio *et al.* 1998a,b; P. Yorio and M. A. Efe, unpubl. data) and on information of shifts in colony location among nearby nesting sites (P. Yorio and M. A. Efe, unpubl. data), the number of breeding sites is estimated at 11 for Royal Terns (five in Brazil and six in Argentina) and 24 for Cayenne Terns (11 in Brazil and 13 in Argentina). Considering these colonies, the estimated total population size is approximately 5,000 pairs for Royal Terns (at least 750 in Brazil and less than 5,000 in Argentina) and approximately 18,000 pairs of Cayenne Terns (at least 8,000 in Brazil and less than 10,000 in Argentina). Lack of counts at some coastal sectors and changes among breeding sites preclude an accurate estimation of total population size.

Despite the extensive coastline in both countries, breeding of both species is concentrated in only a small number of sites. In Brazil, for example, all Royal Terns nest at six islands along the coasts of São Paulo (Table 1; Fig. 1) and more than 80% of Cayenne Terns nest in a given year at two or three islands along the coast of Espírito Santo (Escalvada, Itatiaia, and Branca islands). Other known colonies along the coasts of Rio de Janeiro (Sick 1997; Alves *et al.* 2004), São Paulo (Olmos *et al.* 1995; Campos *et al.* 2004), Paraná (Krull 2004) and Santa Catarina (Branco 2004) rarely exceed the few hundred breeding pairs. Similarly, in Argentina over 75% of Cayenne Terns and 85% of Royal Terns nest in a given year in a few of ten potential breeding sites located at Punta León and a small coastal sector in the north of Golfo San Jorge, Chubut (Table 2; Fig. 2).

Table 2. Location and size (in breeding pairs) of Royal and Cayenne tern colonies in coastal Argentina. Values presented correspond to the last available census. All locations where terns have bred in the past are listed.

	State	Site	Location	Cayenne Tern			Royal Tern		
				Size (n° nests)	Year	Source	Size (n° nests)	Year	Source
1	Buenos Aires	Banco Culebra	40°22'S,61°59'W	695	1990	1	11	1990	1
2		Banco Nordeste	40°32'S,62°09'W	6	2000	2	7	2000	1
3	Río Negro	Islote Redondo	41°26'S,65°61'W	—			24	2002	3
4	Chubut	Islote Notable	42°25'S,64°31'W	97	1970	4	NC	1973	5
5		Playa La Armonía I	42°10'S,64°03'W	55	1996	6	—		
6		Ensenada Medina	42°04'S,63°47'W	3	1979	7	—		
7		Punta Cero	42°30'S,63°36'W	PNC	1970	8	—		
8		Punta Loma	42°49'S,64°53'W	73	2005	9	—		
9		Playa El Pedral	42°57'S,64°23'W	PNC	1998	10	—		
10		Punta León	43°04'S,64°29'W	950	2004	11	450	2004	11
11		Punta Tombo	44°02'S,65°11'W	PNC	1995	12	NC	1967	13
12		Punta Gutiérrez	44°24'S,65°16'W	300	1995	6	—		
13		Isla Aguilón del Norte	45°00'S,65°34'W	(i)	1990	14	(i)	1990	14
14		Isla Valdés	45°03'S,65°43'W	PNC	2005	15	NC	2005	15
15		Islote Luisoni	45°02'S,65°51'W	(ii)	1995	14	(ii)	1995	14
16		Isla Chata	45°03'S,65°58'W	(iii)	1990	14	(iii)	1990	14
17		Isla Gran Robredo	45°08'S,66°03'W	PNC	1998	15	NC	1998	15
18		Isla Ezquerra	45°04'S,66°20'W	(iv)	2003	15	(iv)	2003	15
19		Islas Galiano	45°05'S,66°24'W	PNC	1988	16	NC	1988	16
20		Isla Isabel Norte	45°07'S,66°30'W	5	1993	14	4	1993	14
21		Isla Viana Mayor	45°11'S,66°24'W	PNC	1994	14	—		
22	Santa Cruz	Punta Pájaros	46°57'S,66°51'W	500	1991	17	—		
23		Monte Loayza	47°05'S,66°09'W	80	1996	18	—		
24		Punta Guanaco	47°48'S,65°52'W	30	1994	18	—		

Notes: NC: presen not censused; (i) A total of 633 nests of both species in a mixed-species colony (ii) idem 6500 nests; (iii) idem 3900 nests; (iv) 7135 nests; 1 Yorio and Harris (1997); 2 D. Rábano and P. Yorio, unpubl. data; 3 Bertellotti and Yorio, unpubl. data; 4 Daciuk (1972); 5 De la Peña (1987); 6 Yorio *et al.* (1998a); 7 G. Harris, unpubl. data; 8 Daciuk (1973); 9 A. Gatto and P. Yorio, unpubl. data; 10 P. Yorio, unpubl. data; 11 P. Yorio, F. Quintana and A. Gatto, unpubl. data; 12 D. Boersma, pers. comm.; 13 Korschenewski (1969); 14 Yorio *et al.* (1998b); 15 P. Yorio and F. Quintana, unpubl. data; 16 G. Punta, pers. comm.; 17 Perez *et al.* (1995); 18 Gandini and Frere (1998).

An interesting aspect of their breeding distribution is that, in general, both species nest in association, often with their nestes intermingled (Yorio *et al.* 1999; M. A. Efe, unpubl. data). In fact, except for one site in Argentina (Complejo Islote Lobos, Río Negro) where Royal Terns breed in a monospecific colony, they have always been recorded nesting in mixed-species colonies with Cayenne Terns (Yorio 2005). In Brazil, Royal Terns breed with Cayenne Terns in two of the six recorded locations in the São Paulo State (Campos *et al.* 2004). When not in association with Royal Terns, Cayenne Terns often breed with the South American Tern (*Sterna hirundinacea*), which in Brazil nest in the periphery of the Cayenne Tern dense nest aggregations (Efe *et al.* 2000). In such cases in Argentina, Cayenne Terns nest in small groups within larger South American Tern colonies (Yorio *et al.* 1998a,b; P. Yorio, unpubl. data). Cayenne Terns have not been recorded nesting alone in Argentina, but they do so in locations along the Brazilian coast. Mixed-nesting of Royal and Cayenne terns has also been observed at their only breeding site in Uruguay (J. Lenzi *et al.*, unpubl. data).

Ideally, the identification of conservation priorities requires knowledge of the degree of genetic relationship between populations at a wide regional scale. Studies of morphological and genetic divergence among or within species provide crucial information about the existence of conservation units or patterns within species such as clines, recent
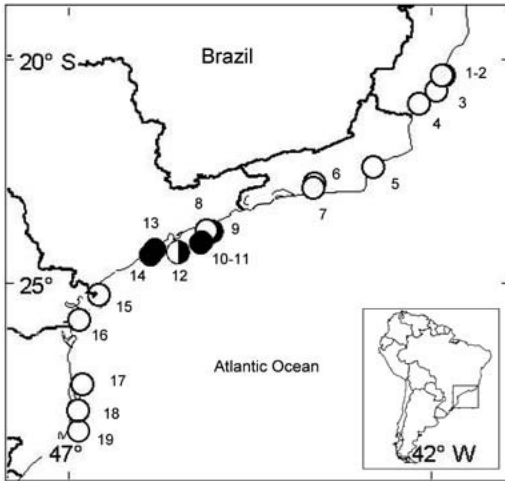
Figure 1. Location of Royal and Cayenne tern colonies along the coasts of Brazil. Black circles: Royal Tern colonies; Open circles: Cayenne Tern colonies; Black and open circles: mixed-species colonies. Numbers correspond to sites in Table 1.

range extensions, or hybrid contacts (Barrowclough 1992). Ongoing genetic analysis among populations on Cayenne/Sandwich Terns in the Americas and Europe is trying to elucidate the relationship of the *sandvicensis /acuflavida /eurygnathus* complex (M. A. Efe, unpubl. data.). Similar studies on Royal Tern populations are still lacking.
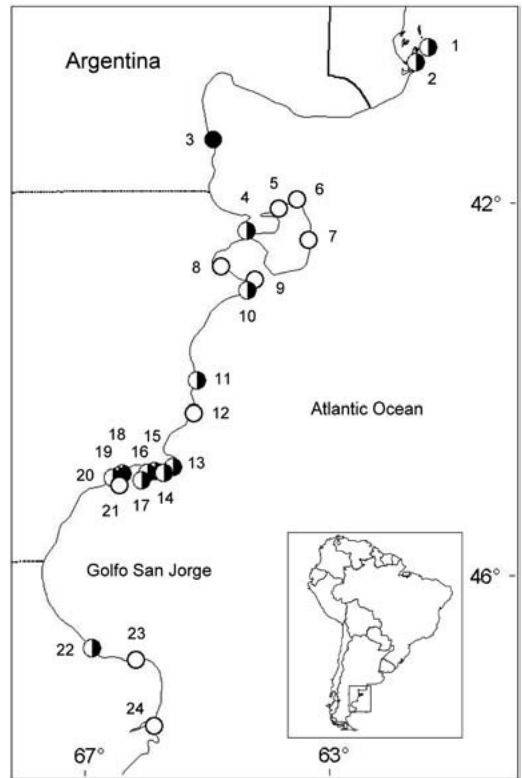

Figure 2. Location of Royal and Cayenne tern colonies along the coasts of Argentina. Black circles: Royal Tern colonies; Open circles: Cayenne Tern colonies; Black and open circles: mixed-species colonies. Numbers correspond to sites in Table 2.

CONSERVATION STATUS AND MAIN THREATS

Royal Terns are listed as Vulnerable at the national level in Brazil (Ministério do Meio Ambiente 2003) and threatened in the State of São Paulo (State Decree N° 42838/98). Antas (1991) identified the Cayenne Tern as the most vulnerable coastal species in Brazil, due to extensive egg collection by fishermen. Since then, this species has been the focus of several studies and conservation initiatives. For example, the Andorinhas do Mar Project begun in 1988 to promote the conservation of Cayenne and South American tern populations and their breeding habitats along the Espírito Santo coast. Since then, facilities built in the Itatiaia and Escalvada islands provide support for different activities, such as research, monitoring, vegetation management, visitation control and environmental educa-

tion. The eradication of exotic species, such as *Cavia porcellus* and *Oryctolagus cuniculus*, the control of the invasive grass *Panicum* sp., and the reintroduction of native plants (e.g., *Canavalia rosea*) have resulted in an increase in the availability and quality of nesting area for the terns. Environmental awareness and education activities implemented throughout the last 17 years have promoted the support of the local community to conservation actions and their participation in project activities such as monitoring and information gathering. Despite of the positive population trend in the study area (estimated at 1.05% increase per year; Efe *et al.* 2005), the recent loss of habitat and population reduction in other Brazilian areas (e.g., some islands in Rio de Janeiro) represent a conservation concern and point out to the importance of protecting and monitoring the Espírito Santo colonies.

Currently, less than half of breeding sites are included in protected areas in both Brazil and Argentina, although protection is afforded mostly to the land where tern nest and not the adjacent waters. Only one site in both Argentina and Brazil also protect part of the tern's potential foraging areas. Even though current protected areas may provide relatively good protection for terns while they are on land, particularly through the control of human visitation to colonies or the prevention of habitat modification, they are clearly not adequate for the long-term conservation of their populations. In addition, the nomadic behaviour of terns highlights the need for addressing metapopulation dynamics and the use of networks of protected areas for regional conservation (Yorio 2000). Adequate protection for terns will also require the inclusion in the reserve network of locations occasionally used for nesting and which do not hold breeding populations during some years (Yorio *et al.* 1999). This is challenging, as government authorities may be reluctant to protect sites which lack birds, particularly for several years. In addition, little is known about their status and ecology outside the breeding season (Favero *et al.* 2001, Silva *et al.* 2005), so future efforts should focus on identifying the wintering grounds of both tern species and developing complementary conservation actions.

Some of the conservation problems of Royal and Cayenne terns in the Atlantic coasts of South America are still the same than those outlined by Daciuk (1973), Escalante (1984, 1985), and Antas (1991). The main threats faced by these two species in both countries are human disturbance, fisheries, egging and expanding Kelp Gull (*Larus dominicanus*) populations.

Human Disturbance

The Brazilian coast has suffered severe environmental degradation in recent decades. Coastal islands are particularly vulnerable to degradation, since they are used by both fishermen and tourists visiting from the mainland. High rates of visitation to beaches

and coastal islands in Brazil have very likely affected tern breeding distribution. In contrast, coastal development in Patagonia, Argentina, has been concentrated at only a few small coastal sectors, although fishing and tourism activities may result in visitation to even remote uninhabited areas. Currently, over 60% of tern colonies in Brazil and 35% of colonies in Argentina are subject to human visitation as a result of recreation, tourism, and fishing activities. This has often resulted in negative effects on breeding success through both egg abandonment and whole colony desertion. Human disturbance at the Rio de Janeiro islands, for example, have often resulted in the loss of tern nest contents (Alves *et al.* 2004). In that same State, Cayenne Terns use the pillars of the Rio-Niterói Bridge for breeding (Alves *et al.* 2004), a site which is highly susceptible to disturbance. Along the São Paulo coast, Cayenne Terns at nesting colonies and roosting sites are threatened by intense use of beaches (Campos *et al.* 2004). Cayenne Tern colonies are also often disturbed by both fishermen and tourists along the Paraná coast (Krull 2004). Branco (2004) has reported that Santa Catarina fishermen occasionally disturb colonies resulting in temporary egg abandonment and induced preation by Kelp Gulls and Black Vultures (*Coragyps atratus*). Terns breeding in Argentina are relatively more sensitive to human disturbance than other seabirds (Yorio *et al.* 2001), and it has been shown that disturbance may result in nest desertion and in induced egg predation by Kelp Gulls due to tern temporary nest abandonment (Yorio and Quintana 1996).

Commercial Fisheries

Terns are considered among seabirds to be one of the most vulnerable groups to commercial fisheries (Furness and Ainley 1984). Interactions among Royal and Cayenne terns in both Brazil and Argentina have been poorly studied. However, available information shows that terns interact in different ways with fishing activities and suggests the existence of some potential conflicts. For example, the diet of Cayenne Terns in Brazil

includes fish species of commercial interest, such as herrings, anchovies and sardines (mainly juveniles) (M. A. Efe, unpubl. data). In Argentina, Royal and Cayenne terns depend on coastal pelagic fish, mainly Anchovy (*Engraulis anchoita*) and Silversides (*Odontesthes* spp.) (Quintana and Yorio 1997; A. Gatto and P. Yorio, unpubl. data). These species are targets of commercial and artisanal fisheries, which often operate close to the coast. Anchovy is not an important commercial fish in most of the Royal and Cayenne tern breeding range, although it supports a growing fishery in northern Argentina with landings in recent years of over 30,000 tons. In 2003, the Federal Fisheries Council of Argentina approved the development of an experimental program to evaluate the viability of opening a small scale trawler fishery on the Anchovy southern stock (south of 41°S) in waters of Chubut. This raises concerns on its effects on tern populations. Negative effects of pelagic clupeid fisheries on seabird populations have been recorded elsewhere (Crawford 2004; Jahncke *et al.* 2004; Skewgar *et al.* 2006).

In Brazil, Royal and Cayenne terns use fish regularly discarded in commercial fisheries as a food source (Branco 2001; Krull 2004; M.A. Efe, unpubl. data). Fish species discarded at fishing vessels operating in Espirito Santo and taken by Cayenne Terns include Dogtooth Herring (*Chirocentrodon bleekerianus*), Sardine (*Pellona* sp.), Weak Fish (*Cynoscion* sp.), Rake Stardrum (*Stellifer rastrifer*), Banded Croaker (*Paralonchurus brasiliensis*) and Barred Grunt (*Conodon nobilis*) (M. A. Efe, unpubl. data). The effect of this interaction is probably beneficial to tern populations, although further studies are needed to confirm this hipothesis. In contrast, these species rarely take advantage of food provided by fisheries in Argentina. Cayenne Terns have been occasionally observed associated in low numbers to the coastal trawl fishery operating in Bahía Engaño (Yorio and Caille 1999) and the high seas hake trawl fishery in Golfo San Jorge, Chubut (González Zevallos and Yorio 2006). Royal Terns have been recorded using discards at coastal trawl fisheries in Golfo San Jorge and in Bahía Grande, Santa Cruz (Yorio and Caille 1999). Both species take advantage of discards thrown overboard, pick small items during towing and obtain food directly from the net during haulback (Yorio and Caille 1999; González Zevallos and Yorio 2006). Much needs to be learned about tern- fishery interactions in both Brazil and Argentina.

Egging

In the past, egg collection together with human disturbance at breeding sites were the main factors limiting reproductive success of CayenneTerns in Brazil (Antas 1991). For example, the Espírito Santo colonies were depopulated by constant egg collection by local fishermen. The Andorinhas do Mar Project (see above) curtailed egg collection in these islands, mainly through inspections and education (Efe *et al.* 2000). Several nesting sites on islands along the Rio de Janeiro coast were also abandoned due to disturbance by fishermen entering colonies to collect eggs (Antas 1991). Egging at Royal and Cayenne tern colonies in Argentina has been rarely observed (Yorio 2005).

Expanding Kelp Gull Populations

Kelp Gulls can be important predators of eggs and chicks of other marine and coastal birds (Malacalza 1987; Yorio and Boersma 1994; Punta *et al.* 1995; Yorio and Quintana 1997; Quintana and Yorio 1998a; Branco 2004; M. A. Efe, unpubl. data). In addition, as it was previously mentioned, they take advantage of tern eggs exposed due to human disturbance (Yorio and Quintana 1996; Yorio *et al.* 2001). The Kelp Gull breeds in association to terns in most of recorded locations and, at least in Argentina, it is one of the most abundant seabird in mixed-species colonies (Yorio *et al.* 1998c). In the last decades, their populations have greatly increased at several coastal sectors of Argentina, probably as a result of the high availability of fishery discards and poor urban waste management (Yorio *et al.* 1998c). For example, 42 of the 51 Patagonian colonies for which data is available show an increase in the number of breeding pairs, with an annual rate of increase of

between one and 64% (Yorio *et al.* 2005). Moreover, at least eight new colonies have been reported in the last decade. This raises concern for the potential negative effects on other coastal species, such as terns, through predation, competition for breeding space, and kleptoparasitism. Unfortunately, information on the population trends of Kelp Gulls in Brazil is still lacking.

On Deserta Island, Santa Catarina, the main threat to Cayenne Terns is predation by Kelp Gulls, which are abundant and breed simultaneously with the terns. At this island, Kelp Gull predation on eggs and nestlings is frequent between the months of June and July, and on occasions has induced birds to abandon the colony site (Branco 2004). Terns breeding along the coast of Rio de Janeiro also suffer predation by Kelp Gulls. In Brazil, Kelp Gulls breed from the coast of Santa Catarina north to the coast of Rio de Janeiro, rarely reaching the Espírito Santo coast (Sick 1997). In fact, the absence of Kelp Gulls, in addition to conservation actions and very likely higher food availability, is considered to be responsible for the relative high breeding success of colonies on the Espírito Santo coast (Efe *et al.* 2000, 2005).

Similarly, Kelp Gulls are the main predator of Royal and Cayenne tern eggs at Punta Leon, Argentina, and can have a significant negative impact on their breeding success (Quintana and Yorio 1997; Yorio and Quintana 1997). Although earlier studies showed that predation was concentrated only on tern eggs, predation on both Royal and Cayenne tern chicks has been also recently recorded (G. García, A. Gatto, and P. Yorio, unpubl. data).

In addition, the flexibility in Kelp Gull habitat requirements (García Borboroglu and Yorio 2004) and its earlier timing of breeding at many sites throughout its range point to the potential competition for breeding space with terns (Yorio *et al.* 1998c). Although no evidence to date indicates the displacement of terns from their breeding sites by Kelp Gulls, studies show that they can affect settlement patters of Royal and Cayenne terns (Quintana and Yorio 1998b). Although the settlement in large dense groups allows

them to obtain breeding space in the presence of gulls, little is known on the effects of higher gull nesting densities. Kelp Gulls also steal prey brought to their chick by parents from both tern species (Quintana and Yorio 1999).

The Kelp Gull is also an important predator of Royal and Cayenne terns nesting in Uruguay (J. Lenzi *et al.*, unpubl. data). Similar predator-prey relationships between both Royal and Cayenne tern species and gulls have been observed throughout the terns' range. Laughing Gulls (*L. atricilla*) prey heavily on breeding Cayenne Terns in Curacao (Gochfeld and Burger 1996) and Aruba (A. del Nevo, pers. comm.) and Royal Terns in eastern United States (Buckley and Buckley 1972). The Yellow footed and Heerman's gulls (*L. livens* and *L. heermanii*, respectively) prey on Royal Terns nesting at Isla Rasa, Mexico (E. Velarde, pers. comm.).

RESEARCH AND CONSERVATION RECOMMENDATIONS

In summary, our review shows that Royal and Cayenne terns have a restricted breeding distribution along the Atlantic coast of South America, with a relatively low number of breeding sites. The total population size is relatively low, particularly for the Royal Tern, and most of the breeding population is concentrated in a few colonies. Recommendations on priority actions in relation to research and conservation of both species in Argentina and Brazil include:

(1) Update and improve population estimates of both tern species, particularly at coastal sectors that appear to concentrate most of the population. The frequent changes in breeding location between years indicate the need for simultaneous surveys at a relatively large geographical scale. (2) Evaluate factors determining changes in colony sites, including natural and human induced factors such as predation, food availability and human disturbance. (3) Analyze genetic structure and relationships of Royal Terns breeding in both countries, and their relationship with North American populations. (4) Improve the knowledge on tern

feeding ecology and their interaction with fisheries. (5) Improve the knowledge of the negative impact of Kelp Gulls on terns, as species interactions have been quantified at only one site in Argentina. Explore mechanisms to minimize negative effects. (6) Improve the protection of tern breeding populations through the designation of new marine protected areas and the seaward extension of existing reserves, including the development of spatial zoning schemes of waters adjacent to colonies. Among other things, promote and improve the legal protection of northern Golfo San Jorge, Argentina, and the four coastal islands in Espirito Santo, Brazil. In addition, promote the inclusion in the protected area systems of both countries of coastal sites which have been used for breeding in the past.

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CAPÍTULO 3

Multigene phylogeny and DNA barcoding indicate that the Sandwich tern complex (*Thalasseus sandvicensis,* Laridae, Sternini) comprises two species.

<u>**Aceito**</u> como nota à ***Molecular Phylogenetics Evolution***.

Multigene phylogeny and DNA barcoding indicate that the Sandwich tern complex (*Thalasseus sandvicensis*, Laridae, Sternini) comprises two species.

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1. **Introduction**

The crested terns are a group of six species of seabirds with a world-wide distribution closely allied to but larger than typical *Sterna* species. They are black-capped with elongated crest feathers and most have a bright yellow or orange to orange-red bill (Gochfeld and Burger, 1996). The taxonomic status of this group as a separate genus, *Thalasseus*, is gaining increasing acceptance following the publication of a molecular phylogeny demonstrating they form a strongly supported monophyletic clade (Bridge et al, 2005).

One of the remaining taxonomic uncertainties in the Sternini is in the classification of the species complex of the Sandwich tern (*Thalasseus sandvicensis*), a taxon whose definition and limits have been controversial over the last century. Within this complex, there are three forms that have been classified either as subspecies or species. The most frequent treatment is to consider them as three subspecies: the Sandwich tern (*T. s. sandvicensis*) that breeds on the Atlantic and Mediterranean coasts of Europe, Cabot´s tern (*T. s. acuflavidus*) that breeds on the Atlantic coasts of North America and the Caribbean, and Cayenne tern (*T. s. eurygnathus*) that breeds on the Atlantic coast of South America from Argentina north to the Caribbean. The three races of Sandwich tern were originally described as distinct species (Latham, 1787; Cabot, 1847; Baird, 1884), and due to their morphological and behavioral similarities were later suggested to be part of the same species complex (Baird et al., 1884; Junge and Voous, 1955), an issue that is still controversial (Gochfeld and Burger, 1996; Hayes, 2004).

These taxa are morphologically very similar, with a few distinctions: the Sandwich tern is slightly larger with wider white margin on outer primaries, shorter bill and, paler upperparts (Olsen and Larsson, 1995). Cabot's and Cayenne terns are virtually identical in plumage, although the Cayenne terns possess, on average, a slightly longer, shaggier nuchal crest and slightly darker gray

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upperparts (Shealer, 1999). The chief distinction between these taxa is in bill coloration. In Sandwich and Cabot's terns the bill is always black with a yellow tip; in the Cayenne tern it is much more variable, typically pale yellow but often with black markings that may be extensive, and rarely orange or even reddish (Hayes, 2004). Cabot's and Cayenne terns often hybridize in Caribbean region (Hayes, 2004).

Breeding habitats of these terns also differ. Sandwich terns nest in open areas with little or no vegetation: bare sand or sand-shell substrates, sandflats, dredge spoil islands and coral cayes (Shealer, 1999). In Europe, they breed in the Ebro Delta in open and sandy beaches and dikes in salinas (Oro et al., 2004). In the Caribbean, Cayenne and Cabot's terns breed in flat islands situated in extensive saline lagoons or on patches of coral debris and sand and a few elevated rocks locally covered with thorny scrub and opuntias (Junge and Voous, 1955). In South America, Cayenne terns nest on islands in Brazil covered by low shrub vegetation, cactus and grasses (Efe et al., 2000), and on coasts characterized by extensive cliffs 30-100 m high and gravel beaches in Argentina (Quintana and Yorio, 1997).

A recent thorough mtDNA analysis of the Sternini species (Bridge et al., 2005) has helped to clarify the phylogenetic relationships of most of the species. However, the relationships of taxa in the *T. sandvicensis* complex was not resolved, as few representatives of Cabot's and Cayenne terns were included and Sandwich terns of the Old World were not examined. Therefore the aim of this study is to clarify the relationships among the Sandwich, Cayenne, and Cabot's terns based on nuclear and mtDNA sequences.

2. Materials and Methods

Material was collected for this study by the authors and collaborators, from a wide range of geographic locations, as follows: the Sandwich tern, *T. s. sandvicensis*, on Ebro Delta, Spain, 40º

37'N/ 00º 35'E, (Code ESP, 2004, *n*=3); the Cabot's tern, *T. s. acuflavidus*, in North Carolina, USA, 35º 32'N/ 75º 59'W (Code USA, 2005, *n*=2); the Cayenne tern *T. s. eurygnathus*, in Escalvada Is., Brazil, 20° 41'S/ 40°24'W (Code ES, 2002, *n*=3) and Punta León, Argentina, 43º03'S/ 64º27'W (Code ARG, 2002, *n*=2). We have also obtained samples from another European population of Sandwich tern (Griend Is., Wadden Sea, The Netherlands) that unfortunately could not be fully sequenced due to sample conservation problems, but we managed to obtain partial sequences from some genes (MyO, COI, cyt *b*, see below) in a few individuals and all resulted in sequences that were indistinguishable from those from Spain (results not shown). Blood samples of breeding birds (adults and nestlings) were taken in the field from the brachial or jugular vein. Samples were preserved in EDTA/Tris-buffer (Dutton, 1995). One additional sample of the Royal tern (*T. maximus*) was from São Paulo, Brazil (provided by P.J. Faria) and another of Trudeau's Tern (*S. trudeaui*) was from Lagoa do Peixe, RS, Brazil. All other samples were described in Bridge et al. (2005).

Total DNA was extracted from the blood samples by a standard phenol/chloroform extraction (Sambrook et al., 1989). DNA was precipitated with cold isopropanol, centrifuged, washed, dried and resuspended in TE buffer. Polymerase chain reaction (PCR) amplifications of the mitochondrial genes cytochrome *b* (cyt *b*), NADH 2 (ND2), and cytochrome oxidase I (COI), and the nuclear genes β-fibrinogen intron 7 (FIB) and Myoglobin intron 2 (MyO) were in 20 µL reactions containing 1 µl DNA, 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 µM of each primer, 1U *Taq* DNA polymerase (Invitrogen) and 1X buffer (Invitrogen). Primers and PCR conditions for cyt *b* and ND2 were as described in Sorenson et al. (1999), for COI as in Hebert et al. (2003), for FIB as in Prychitko and Moore (1997), and for MyO as in Heslewood et al. (1998). Sequencing of *T. sandvicensis* genes was performed as described in Grazziotin et al. (2006) and of nuclear genes from additional Sternini was performed as described by Bridge et al. (2005). Sequences were deposited in GenBank (Genbank accession Nos. FJ356177 - FJ356229).Other mitochondrial

sequences used in this study were obtained from GenBank (Accession numbers AY631284–AY631390, Bridge et al. 2005).

Traces and sequences were checked manually for ambiguities and aligned using the ClustalW algorithm of MEGA 4.0 (Tamura et al., 2007), with further adjustment by eye. Phylogenies were estimated by maximum parsimony (MP) and maximum likelihood (ML) using PAUP* (Swofford, 2003), and by Bayesian Inference (BI) using MrBayes 3.1 (Ronquist and Huelsenbeck, 2003). Tree topologies were rooted with the Inca tern (*Larosterna inca*) as an outgroup. The Akaike Information Criterion (AIC) was used in Modeltest v3.7 (Posada and Crandall, 1998) and MrModeltest (Nylander, 2004) to select the best-fit substitution model for use with PAUP* and MrBayes, respectively. MP and ML heuristic searches for optimal trees were conducted using Tree Bisection Reconnection branch-swapping with 100 random addition replicates. Non-parametric bootstrapping was used to assess support for nodes in the MP (1000 replicates) and ML (200 replicates). Random starting trees were used in BI, and four Markov chains were run for one million generations with rate variation among sites modeled as a gamma distribution. Phylogenetic analyses were performed for three datasets: the mtDNA segments combined, the nuclear introns combined, and for all segments concatenated. All analyses were conducted with a partitioned approach (one partition per gene), where the model parameters were estimated independently for each partition.

DNA barcode comparisons using COI sequences of the *T. sandvicensis* complex were performed in a subclade of the main phylogeny, including all the species of *Thalasseus* with additional sequences of *T. s. acuflavidus* (n=10), *T. s. eurygnathus* (n=2), and *T. elegans* (5) detailed in previous papers (DQ433214-DQ433218, DQ434157-DQ434171, Kerr et al., 2007; and EU525544-EU525547, Tavares and Baker, 2008). The best-fit model (HKY with gamma) was selected by AIC in Modeltest v3.7 (Posada and Crandall, 1998). To check for monophyletic sequence clusters a Neighbor-joining (NJ) tree with the best-fit model parameters was constructed

in PAUP*. BI analysis of the barcodes were performed in MrBayes v3.1.2 with four Markov chains (average standard deviation of split frequencies = 0.005633) of 2 million generations, with one cold and four heated chains each, sampling once every 1000 trees and with the burnin time determined after the convergence of likelihood scores (burnin=200). COI phylogenetically informative characters were mapped on the BI tree in MacClade v4.08 (Maddison and Maddison, 2005), and a test of the chance occurrence of reciprocal monophyly were performed using the coalescent method in Rosenberg (2007) with level of significance α=1%.

Divergence times were estimated using Markov chain Monte Carlo (MCMC) sampling and a relaxed molecular clock as implemented in Beast v1.4.7 (Drummond and Rambaut, 2006). A root age of 24.4 million years before the present (MYBP) from Paton et al. (2003) and adopted in Bridge et al. (2005) was used. The main parameters and priors used were the uncorrelated log-normal relaxed molecular clock, Yule model of speciation and HKY substitution model with gamma distribution of rates among sites. Samples were drawn every 1,000 MCMC steps from a total of 10,000,000 steps, following a discarded burn-in of 1,000,000 steps. Pairwise distances were estimated with MEGA 4.0 using Kimura's two-parameter correction for multiple hits.

3. Results

The size of the alignments for each segment were 420 bp of cyt *b*, 1015 bp of ND2, 684 bp of COI, 730 bp of MyO, and 983 bp of FIB. The number and percentage of variable sites were: cyt *b* (86/20.5%), ND2 (292/28.8%), COI (160/23.4%), MyO (26/3.6%), and FIB (42/4.3%).

The mean Kimura two-parameter (K2P) distance between the *T. s. eurygnathus* and *T. s. acuflavidus* was very small, 0.25% for mtDNA and 0.09% for nuclear genes. However, between *T. s. sandvicensis* and the *T. s. eurygnathus/acuflavidus* sequence divergence was similar to among-

species distances observed within genera (Table 1). The K2P distances between *T. elegans* and *T. s. eurygnathus/acuflavidus* were only 1.08% and 0.2% for mtDNA and nuclear genes, respectively.

Table 1. Mean K2P pairwise percent distances among species of terns. MtDNA distances are in the lower triangle and nuclear DNA distances are in the upper triangle.

	1	2	3	4	5	6	7	8	9
1- *T. s. eurygnathus*	-	0.09	0.52	0.27	0.73	0.17	0.27	0.67	0.87
2- *T. s. acuflavidus*	0.25	-	0.55	0.30	0.77	0.23	0.30	0.70	0.90
3- *T. s. sandvicensis*	2.72	2.72	-	0.50	0.96	0.39	0.50	0.81	1.01
4- *T. maximus*	2.87	2.80	2.94	-	0.59	0.21	0.13	0.65	0.85
5- *T. bengalensis*	2.64	2.73	2.67	1.16	-	0.73	0.59	0.72	0.92
6- *T. elegans*	1.06	1.09	2.94	3.01	2.99	-	0.21	0.55	0.83
7- *T. bergii*	2.71	2.63	3.03	1.75	1.83	3.13	-	0.65	0.85
8- *S. sumatrana*	7.58	7.95	7.66	7.72	7.53	7.92	7.39	-	0.59
9- *S. hirundinacea*	8.10	8.26	7.82	7.63	7.27	8.35	7.18	5.69	-
10- *S. albostriatus*	10.05	10.04	9.39	9.59	9.52	10.07	9.54	8.57	9.35
11- *S. dougallii*	8.21	8.36	8.17	7.98	7.67	8.29	7.99	4.38	5.51
12- *S. hirundo*	7.71	7.82	7.43	7.17	7.08	7.93	7.08	5.06	4.90
13- *S. striata*	8.29	8.48	8.31	7.83	7.59	8.33	7.44	4.34	5.12
14- *S. vittata*	8.20	8.38	8.04	7.85	7.60	8.35	7.58	6.21	1.25
15- *S. forsteri*	7.41	7.93	7.61	7.49	7.49	7.34	7.16	7.43	7.51
16- *S. trudeaui*	10.51	10.84	10.60	10.16	10.14	10.28	9.87	9.71	9.99
17- *C. hybridus*	8.71	8.74	8.73	8.63	8.88	8.68	8.95	9.12	9.55
18- *L. inca*	9.56	10.21	9.83	9.26	9.59	9.23	9.37	9.94	9.60
	10	11	12	13	14	15	16	17	18
1- *T. s. eurygnathus*	1.00	0.73	0.54	0.73	0.86	0.86	0.86	0.54	1.33
2- *T. s. acuflavidus*	1.03	0.76	0.57	0.76	0.89	0.89	0.90	0.57	1.36
3- *T. s. sandvicensis*	1.14	0.87	0.67	0.87	1.00	1.09	1.09	0.68	1.47
4- *T. maximus*	0.98	0.72	0.52	0.72	0.85	0.85	0.85	0.52	1.25
5- *T. bengalensis*	1.18	0.79	0.59	0.79	0.92	0.92	0.92	0.79	1.58
6- *T. elegans*	0.90	0.62	0.48	0.62	0.76	0.83	0.83	0.48	1.25
7- *T. bergii*	0.98	0.72	0.52	0.72	0.85	0.85	0.85	0.52	1.31
8- *S. sumatrana*	0.85	0.20	0.26	0.20	0.46	0.85	0.85	0.39	1.38
9- *S. hirundinacea*	0.92	0.52	0.46	0.52	0.33	1.05	1.05	0.52	1.51
10- *C. albostriatus*	-	0.92	0.72	0.92	1.05	1.25	1.18	0.52	1.58
11- *S. dougallii*	9.56	-	0.33	0.13	0.39	0.92	0.92	0.46	1.44
12- *S. hirundo*	8.88	5.39	-	0.33	0.46	0.72	0.72	0.26	1.25
13- *S. striata*	9.45	4.16	5.27	-	0.39	0.92	0.92	0.46	1.44
14- *S. vittata*	9.25	5.97	5.48	5.80	-	1.05	1.05	0.59	1.58
15- *S. forsteri*	10.18	7.92	7.55	7.71	7.49	-	0.39	0.79	1.58
16- *S. trudeaui*	11.62	10.57	10.22	10.44	9.87	6.59	-	0.79	1.58
17- *C. hybridus*	6.12	9.76	9.55	9.89	9.63	9.84	11.90	-	0.98
18- *L. inca*	11.02	10.61	10.26	10.30	9.68	9.95	11.68	10.37	-

Phylogenetic relationships estimated by the different methods and sequence partitions (mtDNA, nuclear, and mtDNA+nuclear) were similar (Figure 1), with just a few differences due to the low number of informative sites and thus many poorly supported nodes in the nuclear gene tree. However, in all trees individuals of *T. s. sandvicensis* and *T. s. eurygnathus/acuflavidus* grouped into distinct monophyletic clades that branched basally in the *Thalasseus* clade. *T. sandvicensis* as currently recognized was paraphyletic, with *T. s. eurygnathus/acuflavidus* forming the sister group to the Elegant Tern (*T. elegans*) both from the Americas, rather than to European *T. s. sandvicensis*. *Thalasseus* form a well-supported monophyletic clade separated from other terns by a long branch. Although the *Thalasseus* clade is sister to two species of *Sterna* (*S. forsteri* and *S. Trudeaui*), thus making *Sterna* paraphyletic, support values at this node are very weak, and further work is required to test the monophyly of this genus.
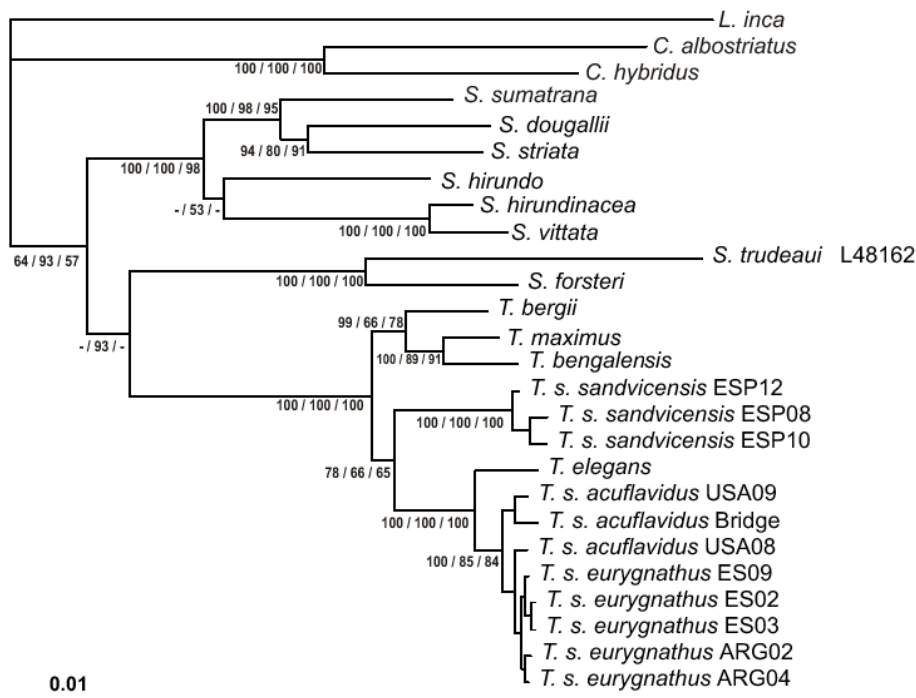
Fig. 1. Phylogenetic tree inferred from Bayesian analysis of 3832 bp from mtDNA+nuclear sequences. Support values are indicated at nodes (Bayesian posterior probabilities, ML and MP bootstrap values, respectively). (-) indicates values lower than 50%.

Trees recovered with Neighbor-Joining and BI analysis of COI barcodes were congruent: the three individuals of *T. sandvicensis* were monophyletic and were sister to a clade including *T. elegans*, *T. s. eurygnathus*, and *T. s. acuflavidus*, with *T. elegans* as a monophyletic group with all clades supported by posterior probability of 1 (Figure 2). Individuals of *T. s. acuflavidus* and *T. s. eurygnathus* were not reciprocally monophyletic. The European terns differed from Cabot's, Cayenne and Elegant terns by 4.2% (HKY+gamma distance) and 3.2% (K2P distance). There are 15 characters that distinguish these clades, 7 on the branch to *T. s. sandvicensis* and 8 on the branch to the other subspecies of the Sandwich tern and the Elegant tern. Chance occurrence of reciprocal monophyly of these two clades was rejected (p= 2.26 x10^{-5}, α= 1%).
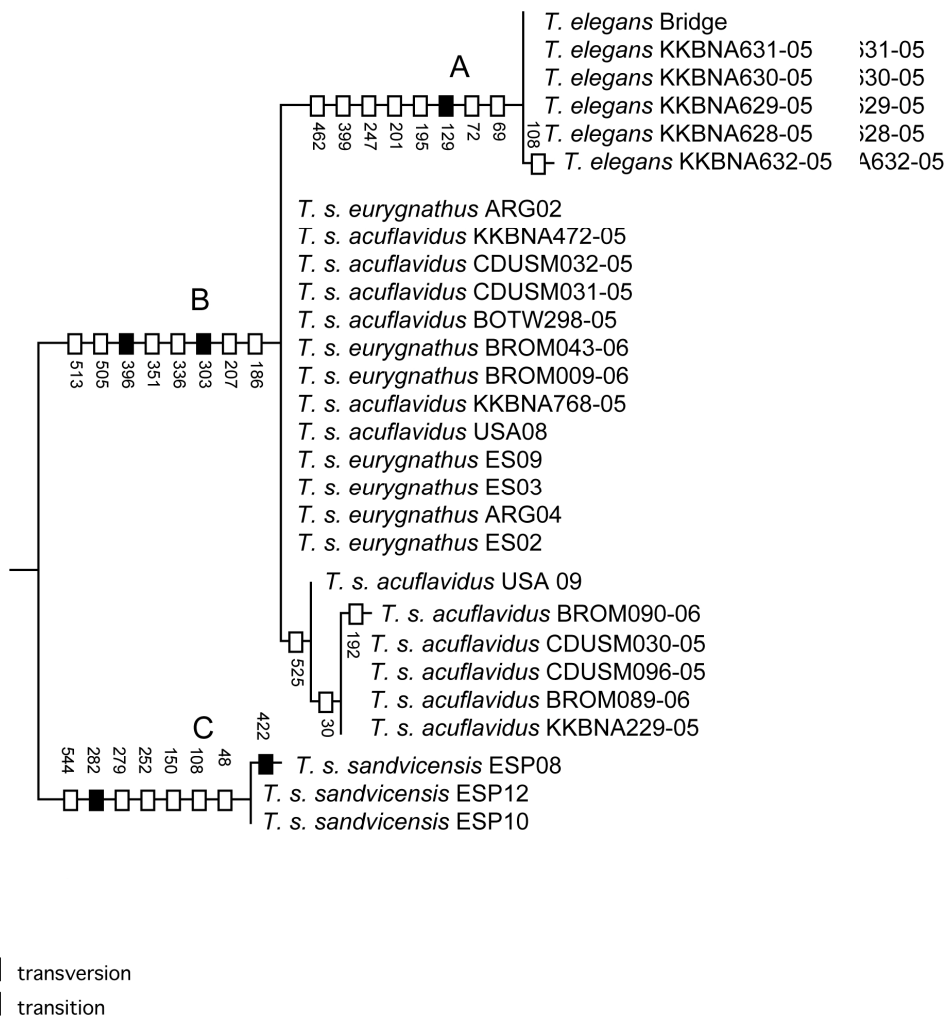
Fig. 2. Diagnostic substitutions in COI mapped on the Bayesian tree topology of the barcode sequences of 630 bp. Substitutions are numbered according to their position in the sequences. The branch lengths are proportional to the characters that change unambiguously on the branches. Nodes A, B, and C have posterior probabilities of 1.0.

The divergence times estimated with the MCMC Bayesian approach using only mtDNA sequences agree with dates presented by Bridge et al. (2005), e.g. the separation between the *T. sandvicensis/maximus/bengalensis/bergii* and the *T. s. eurygnatus/acuflavidus/T. elegans* clades were about 2.7 MYBP, however using the nuclear+mtDNA dataset the separation between the *T. sandvicensis* and the *T. s. eurygnatus/acuflavidus/T. elegans* clades were older, dated around 3.6 MYBP.

4. Discussion

Our analysis indicates that the Old World (*T. s. sandvicensis*) and the New World (*T. s. acuflavidus/eurygnathus*) tern populations are genetically as divergent as different species in the genus, and do not form a monophyletic group. Instead, the latter are sister to the Elegant tern (*T. elegans*). These results strongly suggest that the current taxonomic treatment of the *T. s. sandvicensis/acuflavidus/eurygnathus* complex as subspecies within a single species or as a northern hemisphere (*T. s. sandvicensis*) and a southern hemisphere species (*T. s. eurygnathus*) are phylogenetically inappropriate. The new arrangement should be one in which the Old World (Sandwich) tern *T. s. sandvicensis* and the New World (Cayenne and Cabot's) terns *T. s. acuflavidus/eurygnathus* are considered two different species. COI barcodes of a larger sample of individuals of the Sandwich tern complex also supported the multigene phylogeny, splitting with high statistical support the European group (*T. s. sandvicensis*) from the other two subspecies of the complex (*T. s. acuflavidus*, and *T. s. eurygnathus*) and illustrating the efficacy of DNA barcoding in discovering potential new taxa of birds (Hebert et al, 2003). The advantage of complementing multigene phylogenetic evidence with DNA barcoding of the complex is that diagnostic substitutions characteristic of other well known sister species of birds are clearly revealed (Figure

2), and can be tested statistically for taxonomic distinctiveness (Tavares and Baker, 2008, Rosenberg, 2007).

Our study shows that the North American/ Caribbean (Cabot´s tern) and the Caribbean/South American (Cayenne tern) populations are very similar genetically (Table 1). In a more extensive study on the genetic structure of the New World populations based on DNA sequence and microsatellite variability (M.A.E, S.L.B. unpublished results), populations from these two taxa share mtDNA and nuclear haplotypes and present low microsatellite differentiation with a complex genetic structure, with no evidence of complete reproductive isolation,. Therefore, our preliminary genetic results do not support the existence of subspecies in this taxon. We propose that the appropriate taxonomic treatment for the New World terns (*acuflavidus/eurygnathus* complex) should be as Cabot's Tern, *Thalasseus acuflavidus*, since *S. acuflavida* was nominated by Cabot in 1847 and *S. eurygnatha* by Sanders in 1876, in agreement with the grammatical arrangement suggested by David and Gosselin (2002).

What are the consequences of this new taxonomic treatment for the conservation efforts of these taxa? The IUCN currently classifies the *T. sandvicensis* complex as of Least Concern (LC) because of its large geographic range, with an estimated global extent of occurrence of 100,000–1,000,000 km² and a large global population (BirdLife International, 2008). Global population trends have not been quantified, but the species (s.l.) was not believed to approach the thresholds for the population decline criterion of the IUCN Red List (BirdLife International, 2008). A taxon is of Least Concern when it has been evaluated against these criteria and does not qualify for other categories (IUCN, 2001), but requires the same degree of attention that a more threatened taxon. The UK Joint Nature Conservation Committee considers the conservation status of *T. sandvicensis* in the UK to be unfavourable, and recommends general protection of breeding grounds (JNCC, 2008). In view of the new arrangement suggested here, in which the Old World and the New World

populations are two distinct species, the conservation status of both *T. sandvicensis* and *T. acuflavidus* need to be revised.

Acknowledgments

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CAPÍTULO 4

Phylogeography and genetic structure of populations of the Cabot's Terns, *Thalasseus acuflavidus* (Laridae, Sternini), based on DNA and microsatellites variation.

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Phylogeography and genetic structure of populations of the Cabot's Terns, *Thalasseus acuflavidus* (Laridae, Sternini), based on DNA and microsatellites variation

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Abstract. In spite of the great amount of ecological studies developed for more than 40 years with populations of the *T. acuflavidus*, the relationship between the North and South American populations as well as between the main South American populations remain poorly understood, including their degree of genetic isolation. In this work we present the first genetic study of this species using mitochondrial and nuclear sequences as well as microsatellites data from one population form North America, one from Brazil, and one from Argentina. MtDNA diversity is low in the species as a whole, with the USA population divergent from both Brazil and Argentina, which are not differentiated. All three populations present signals of bottleneck and population expansion, with the South American populations presenting an expansion estimated around 30,000 years ago. On the other hand, microsatellites data support a high recent gene flow among the populations of Brazil and USA, and a low gene flow among the populations of Brazil and Argentina. These results suggest the occurrence of a hybridization zone between Brazil and North America. The different breeding periods in Brazil and Argentina could be important in the recent isolation of these coastal birds, suggesting that allochrony may be a neglected process in the formation of the biodiversity in the Neotropics.

Introduction

Cabot's tern is distributed for almost the whole Atlantic coast of the American continent, with several isolated breeding populations at different sites, and moving periodically among sites. Historically, the populations that breed on the Atlantic coasts of North America and the Caribbean was named Cabot's tern (*Thalaseus sandvicensis acuflavidus*), and those that breed on the Atlantic coast of South America from Argentina north to the Caribbean was termed Cayenne tern (*T. s. eurygnathus*). Both were considered subspecies or races of the Sandwich tern (*T. s. sandvicensis*) that breeds on the coasts of Europe (Shealer, 1999). However, Efe et al. (Submitted) showed that the European and the American populations represent distinct non-sister species, supporting the treatment of the *acuflavidus/eurygnathus* complex as Cabot's tern, *Thalasseus acuflavidus*.

The ecological variability is common, nests are in open areas with little or no vegetation: bare sand or sand-shell substrates, sandflats, dredge spoil islands and coral cayes (Shealer, 1999). In the U.S. and Gulf coasts, they typically nests on low, sandy, flat islands close to shore (Oberholser, 1974, Visser and Peterson, 1994). In the Caribean region the breeding place of the *T. acuflavidus* are flat islands situated in an extensive saline lagoon of shallow water or on bare coral rock and patches of coral debris and sand and a few elevated rocks locally covered with thorny scrub and opuntias. The nests were mere shallow depressions in the coral sand or in the sparse vegetation of *Sesuvium portulacastrum*, in some cases in the shade of single plants of *Opuntia wentiana* (Junge and Voous, 1955). In Brazil breeding grounds have low shrub vegetation with cactaceans predominating (Efe et al., 2000). In Argentina colonies are characterized by extensive cliffs 30-100 m high and gravel beaches along the shoreline. About 700 m of shoreline are separated from the cliffs by a silt platform covered by vegetation consisting mainly of *Suaeda divaricata*, *Atriplex*

lampa, and *Lycium chilense*, and is used as nesting substrate by several seabird species (Yorio et al., 1998).

In the United States, Cabot's Tern usually nests in dense groups among Royal Tern, *T. maximus*, Laughing Gulls, *Larus atricilla*, and sometimes Black Skimmers, *Rynchops niger* (Shealer, 1999). In North Carolina and Caribe it breeds with the Royal Tern (McGinnis and Emslie, 2001, Hayes, 2004) and Roseate Tern, *Sterna dougallii*. In Brazilian coast breeding occurs mixed colonies with the South American Tern, *S. hirundinacea* (Efe et al., 2004). The colonies of Argentina are located within the Kelp Gull colony in mixed colonies with the Royal Tern (Quintana and Yorio, 1997).

Breeding season also differs between colonies, in most U.S. and Caribbean colonies, first adults arrive in late April or early May (Shealer, 1999); Netherlands Antilles breeding activity has been observed from May to August (Junge and Voous, 1955). The terns in Brazilian colonies began to arrive in mid-April and the settlement at the colony site occurs from May onwards. The first chicks hatch in early June. In mid-September birds begin to leave the colonies and after the end of October they are rarely found on the coast of the State of Espírito Santo (Efe, 2004). However, in Argentina, Cabot's terns started to arrive in mid-September and remained courting and mating on the beach for up to a month before finally settling in the colony site. The settlement at the colony site occurred during the second or third week of October (Quintana and Yorio, 1997) and the breeding activity has been registred until January (Escalante, 1970).

The morphological variability among North and South American specimens of *T. acuflavidus* was presented by Shealer (1999) and the variety of colors in eggs, legs and culmen of the *T. acuflavidus* in Curaçao was described and quantified by Ansingh et al. (1960). The populations differ in overall size (North American specimens of may have a slightly weaker bill; Junge and Voous 1955) and plumage (South American specimens is also slightly darker above than North American specimens; Junge and Voous, 1955), but the main distinction between the populations is bill coloration. In North populations the bill is always black with a yellow tip (Hayes, 2004); in Caribbean and Brazilian it is much more variable, black with yellow patches or yellow with black patches (Efe, 2000, Hayes, 2004) and, in Argentinean populations typically pale yellow and rarely yellow-orange (P. Yorio, pers. comm.).

The taxonomic relationship between North and South populations is poorly understood, which appears to form a cline, with frequent hybridiztion (Hayes, 2004). A major motivation for mtDNA surveys of birds has been the need for critical reassessment of the processes governing genetic differentiation among conspecific populations (Avise and Ball, 1994) and, information on

connectivity between breeding populations is crucial for developing adequate plans for conservation and management species. Recently, nucleotide sequences were used to study the phylogeography of some seabirds (e.g., Avise et al., 2000, Liebers and Helbig, 2002, Steeves et al., 2003, Gómez-Díaz et al., 2006). MtDNA analysis is being used with increasing frequency to document genetic variation within avian species and the development of population markers that can be used to recognize birds from different breeding populations (Wennerberg et al., 2002). A large number of microsatellites also have already been characterized for seabirds (Burg and Croxall, 2001, Tirard et al., 2002, Abbott and Double, 2003, Genovart et al., 2003). Scarce population genetic information is available for any tern species (see Randi and Spina, 1987, Hackett, 1989, Avise, 2000, Peck and Congdon, 2004, Szczys et al., 2005).

Using samples from geographically divergent areas throughout the species breeding range, we describe the intraspecific variation in the populations of the *T. acuflavidus* based on nucleotide sequences of nuclear and mtDNA, and genotypes at microsatellite loci. We also discuss the evolutionary history, morphological and ecological variability, and taxonomic and conservations implications for their genetic structure.

Material and Methods

Sampling

Samples of the Cabot's Tern were specifically collected for this study, by the authors and collaborators, from three different geographic locations comprising the most distant populations: 1) The North American populations in North Carolina, USA, 35º32'N/75º59'W (Code USA, 2005, *n*=10); 2); the South American populations in Escalvada Island, Espírito Santo state, Brazil, 20°41'S/ 40°24'W (Code ES, 2002, *n*=10); and 3) and in Punta León, Argentina, 43º03'S/ 64º27'W (Code ARG, 2002, *n*=10). Blood samples of breeding birds (adults and nestlings) were taken in the field from the brachial or jugular vein and preserved in EDTA/Tris-buffer (Dutton, 1995).

Molecular methods

Total DNA was extracted from the blood samples by a standard phenol/chloroform extraction protocol (Sambrook et al., 1989). DNA was precipitated with cold isopropanol, centrifuged, washed, dried, and resuspended in TE buffer. Three mitochondrial and two nuclear fragments were amplified by polymerase chain reaction (PCR) as follows: 422 bp from the cytochrome *b* gene (CyB), 678 bp from the NADH enzyme subunit 2 gene (ND2), 632 bp from the cytochrome-oxidase *c* enzyme subunit 1 gene (COI), 983 bp from the intron 7 of the β-fibrinogen

gene (FIB) and 690 bp from the intron 2 of the Myoglobin gene (MyO). Amplifications were conducted in 20 µl reactions containing 1 µl DNA, 1.5 mM MgCl^2, 0.2 mM dNTPs, 0.4 µM of each primer, 1U *Taq* DNA polymerase and 1X polymerase buffer. Primers and reaction conditions used were described in Sorenson *et al*. (1999) for CyB and ND2, Hebert *et al*. (2003) for COI, Prychitko and Moore (1997) for FIB, and Heslewood *et al*. (1998) for MyO. Sequencing was performed as described in Grazziotin *et al*. (2006). Traces and sequences were manually checked and aligned using ClustalW algorithm implemented in the software MEGA 4.0 (Tamura *et al*., 2007).

Nine microsatellites loci were used for genotyping and yielded polymorphic amplification products for the *T. acuflavidus*. Three of these (RBG13, RBG18 and RBG27) were designed originally for the red-billed gull (*Larus novaehollandiae scopulinus*; Given et al., 2002). The other six (LARZAP11, LARZAP12, LARZAP14, LARZAP26, LARSNX10B and LARSNX24) were designed originally for the herring gull (*Larus argentatus*, Gregory and Quinn, 2006). Forward primers were 5'-tailed with the M13 sequence (5'-CACGACGTTGTAAAACGAC-3') that is used in combination with an M13 primer marked with fluorescence (FAM, HEX, NED) (Boutin-Ganache et al., 2001). All loci were amplified in separate reactions following the published protocol profiles, with few adjustments. Genotyping was performed on an automated sequencer MegaBACE 1000 DNA Analysis Systems (GE Health Care) following recommended procedures. Genotypes were checked using the Genetic Profiler v2.2 (GE Health Care), and alelle sizing was checked by hand.

Not all individuals could be successfully screened with all marker systems (nuclear and mitochondrial sequences; and microsatellites loci); thus, the respective data sets differ in size (mtDNA n=30, ncDNA n=30, and microsatellites n=45).

DNA sequences analyses

Basic DNA diversity statistics, including nucleotide (π) and haplotype diversity (Hd), as well as Tajima's D (Tajima, 1983) and Fu's FS (Fu, 1997) neutrality tests, and mismatch distribution analyses (Rogers and Harpending, 1992) were estimated for each population using the ARLEQUIN 3.1 program (Excoffier and Schneider, 2005).

The degree of genetic structure was inferred using the F_{ST} values (based on pairwise sequence divergence and haplotypic frequency) calculated between populations in ARLEQUIN 3.1, and with the statistic significance for the F_{ST} calculated by permutation test (α=0.05). An analysis of molecular variance (AMOVA; Excoffier et al., 1992) was used to estimate the level of genetic variation within and among groups of populations with ARLEQUIN 3.1. These genetic structure

statistics were contrasted with haplotype networks, which were inferred by the Median Joining method (MJN) (Bandelt et al., 1999) using the program Network 4.1.0.8 (www.fluxus-engineering.com).

Demographic parameters such as effective population size (N_e), fluctuation in population size (G), and migration rates (Nm) were estimated, based on mtDNA sequences, for each population using the Markov Chain Monte Carlo (MCMC) approach implemented in the package LAMARC 2.1.3 (Kuhner, 2006). The parameters of the searches were set to perform a Bayesian analysis with one initial chain of length 50,000, followed by one final chain of length 1,000,000, and sampling trees every 100 steps in each case. For both, short and long chains, 1,000 steps were discarded as initial "burn-in" and four chain temperatures were set (1, 1.1, 3, and 6) to perform multiple simultaneous searches with adaptive heating. Each search was replicated twice and the confidence interval for the parameters θ (theta = $2N_e\mu$ for haploid data or $4N_e\mu$ for diploid data, μ = substitution rate), M (m/μ, m=migration rate) and G (growth rate) were calculated by percentile profiles. The posterior probability for each parameter was checked using the Trace v1.4 (Rambaut and Drummond, 2007) software. The mean substitution rate used was 8 x 10^{-9} substitutions per site per year, based on the results for CyB found by Fleischer *et al*. (1998) for Drepanididae species in Hawaii; and used by Liebers and Helbig (2002) in a phylogeographic study in Lesser Black-backed Gulls (*Larus fuscus*), and Peck and Congdon (2004) to infer historical processes in the Sooty Tern (*Onychoprion fuscatus*), both based on adjustments of this rate to control region sequences (but see Crochet and Desmarais, 2000 for differences in rate of evolution of mitochondrial segments in Gulls).

The generation time for this species was calculated as 11.5 years using the substantial amount of information available for Laridae as follows. The birds from this family are characterized for its long life spans (frequently more than 30 years) and are the subject of several studies involving longevity, senescence and fitness (Monaghan and Metcalfe, 2000, Haussmann et al., 2007, Møller, 2006). Moreover, several studies had shown that the breeding success increase with age in Sternini as a direct product of breeding experience (Limmer and Becker 2007), best energetic efficiency (Galbraith et al., 1999), less stress susceptibly (Heidinger et al., 2006), reduced nest defense behavior (Pearson *et al*., 2005), and early arrival at the breeding site (Arnold et al., 2006; Becker et al., 2008). The generation time was then estimated as the weighted mean among all breeding ages, taking in account the mean frequency of each age in the breeding site and differences in breeding success. To perform this, the mean was weighted by the product between the cumulative frequency for each age and the efficiency of breeding (measured as the percentage of

survival of chicks until the fledging at 24 days, see Nisbet, 2002). Since there is no complete life-table available for *T. acuflavidus* we used the very detailed information presented for a species in the sister genus, *Sterna hirundo* (Nisbet, 2001; Nisbet, 2002; Nisbet et al., 2002). We consider that *S. hirundo* life table could be a reasonable approximation (see Table A1 in Appendix for the life-table) since *T. sandviciensis* (the former epithet to *T. acuflavidus*) has been reported as living up to 30.8 years and *S. hirundo* up to 33 years (AnAge databank – The Animal Ageing and Longevity Database, http://genomics. senescence.info/species/) and they have similar breeding behavior.

Times to the most recent common ancestor (TMRCAs) were estimated using a Bayesian MCMC approach with the uncorrelated lognormal relaxed molecular clock as implemented in Beast v1.4.7 (Drummond and Rambaut 2007). Other parameters used were the HKY substitution model with four categories of gamma distribution among sites, the coalescence tree prior, and the substitution rate presented above. Samples were drawn every 1,000 MCMC steps from a total of 10,000,000 steps, following a discarded burn-in of 1,000,000 steps after checked the convergence of the parameters values in the software Trace v1.4.

Microsatellite analyses

For the microsatellite loci, we calculated the number of alleles per locus (na), expected (H_e) and observed heterozygosity (H_o), using ARLEQUIN 3.1. As carried out for DNA sequences, the F_{ST} value between populations was used to infer the degree of genetic structure. F_{ST} was estimated using the number of different alleles (F_{ST}-like) and using summed of squared differences (R_{ST}-like); their statistic significance were calculated by permutation test ($\alpha=0.05$).

The demographic parameters N_e and Nm for each population were estimated using the package LAMARC 2.1.3. The same search strategy applied to DNA sequences was set to microsatellite data. However, here the search was replicated three times, with the final chain set to 50,000,000 and only two chain temperatures were set (1 and 1.3). As a result of the complexity of microsatellite data set only two parameters, θ ($4N_e\mu$) and M, could be estimated, as well as, its confidence intervals (see the LAMARC manual). To estimate the N_e and Nm for the three population of *T. acuflavidus* the same approach implemented to mtDNA sequences was used, adjusted for diploid data. The mean evolutionary rate was set to 5.5×10^{-5} mutations per generation, estimated as a mean between the two most frequent mutation rates for microsatellites data in literature (1×10^{-4} and 1×10^{-5}; Ellegren, 2004).

To test if the number of populations was correctly obtained by the inferences made using F_{ST} and by biological evidences the program STRUCTURAMA (Huelsenbeck et. al., in press) was

used to infer population genetic structure from microsatellites data by allowing the number of populations to be a random variable that follows a Dirichlet distribution prior. To choose an appropriate value of population (K) for modelling the data, 10 runs of MCMC approach were ran, set to 10,000 cycles. After that, the program STRUCTURE (Pritchard et al., 2000) was used to identify clusters of related individuals from the multilocus genotypes using the defined K. Results are based on 50,000 MCMC iterations following a burn-in period of 20,000 iterations. Simulations were conducted using an admixture model and correlated allele frequencies between populations, as suggested by Falush *et al*. (2003) in cases of subtle population structure. The results were displayed using the program Distruct 1.1 (Rosenberg, 2004).

To detect possible recent bottlenecks in the populations, the approach implemented in the BOTTLENECK 1.2.02 software (Cornuet and Luikart, 1996) was applied. It is based on the difference between the expected and observed heterozygosis value, and argue that significant heterozygote excess represents a signal of a recent bottleneck, since the number of alleles decrease faster than the heterozygosis in a bottlenecked population. The three models of molecular evolution implemented in the program were tested: Stepwise Mutation Model (SMM), Infinite Allele Model (IAM), and Two-Phased Model of Mutation (TPM), and the significance of the results were based on Wilcoxon sign-rank test and the mode-shift analysis.

Results

DNA sequence variation

The alignments obtained for the mtDNA genes and for the FIB and MyO introns did not present any indel and in 3,406 bp only 27 substitution were found, as follows: 17 variable sites in mtDNA (10 transitions, *ti* and seven transversions, *tv*), three in MyO (two *ti* and one *tv*), and seven in FIB (two *ti* and five *tv*). No stop codon or any /unusual/ non-synonymous substitutions were observed in the mtDNA sequences suggesting they are of mitochondrial origin. The sequences were submitted to GenBank..

The three populations present very different diversity patterns (Table 1). The results for FIB and mtDNA are concordant and reveal that the USA population has the highest haplotype and nucleotide diversity, followed by BRA population, but the latter showing 30% less haplotype and almost 70% less nucleotide diversity. For these DNA fragments, the ARG population showed the lowest diversity within the *T. acuflavidus* (H_d and π about three and six times less than the USA, respectively). However, in the MyO sequences the Argentinean population has the highest values for H_d and π, while in the BRA did not show any variable site.

The mtDNA haplotype network (Figure 1) shows a typical star-like shape, where the majority of rare haplotypes diverge by a single mutation from the most frequent haplotype. Besides the central shared haplotype, all others are singleton exclusive, with the USA population presenting more diverse haplotypes than ARG and BRA. A similar haplotype network can be seen in FIB network, where only USA has one exclusive haplotype. The MyO network, although also star like, contrasts with other results in that ARG is the more diverse population, with two exclusive haplotypes, then USA with one and BRA with only the most common haplotype.

Table 1. Summary statistics observed in Brazilian (BRA), Argentinean (ARG) and North American (USA) populations of *T. acuflavidus* based on DNA sequences and microsatellites.

		ARG	BRA	USA	Total
	Fib				
n		20	16	16	52
h		2	2	3	3
S		3	3	4	4
H_d		0.479 ± 0.072	0.533 ± 0.0456	0.692 ± 0.058	
π (%)		0.0015 ± 0.0010	0.0016 ± 0.0011	0.0018 ± 0.0012	
	Myo				
n		20	20	20	60
h		3	1	2	3
S		2	0	1	3
H_d		0.358 ± 0.127	0	0.189 ± 0.108	
π (%)		0.0005 ± 0.0006	0	0.0003 ± 0.0004	
	mtDNA				
n		10	10	10	30
h		3	5	7	13
S		2	4	7	13
H_d		0.378 ± 0.181	0.667 ± 0.163	0.933 ± 0.062	
π (%)		0.0231 ± 0.0263	0.0449 ± 0.0402	1.373 ± 0.0921	
	STR				
n		32	34	24	90
mn		27.3	29.3	21.6	78.2
na		4.4 ± 1.9	4.1 ± 2.5	3.2 ± 1.2	3.5 ± 2.2
G_d		0.404 ± 0.230	0.366 ± 0.211	0.420 ± 0.241	
H_o		0.362 ± 0.264	0.423 ± 0.356	0.283 ± 0.291	
H_e		0.617 ± 0.172	0.546 ± 0.219	0.575 ± 0.130	

n, number of genes copies; S, number of variable sites; h, number of haplotypes; Hd, haplotype diversity; π, nucleotide diversity; mn, mean number of genes copies (discounting missing data); Gd, gene diversity; na, mean number of alleles per locus; H_e, expected heterozygosity; H_o, observed heterozygosity.
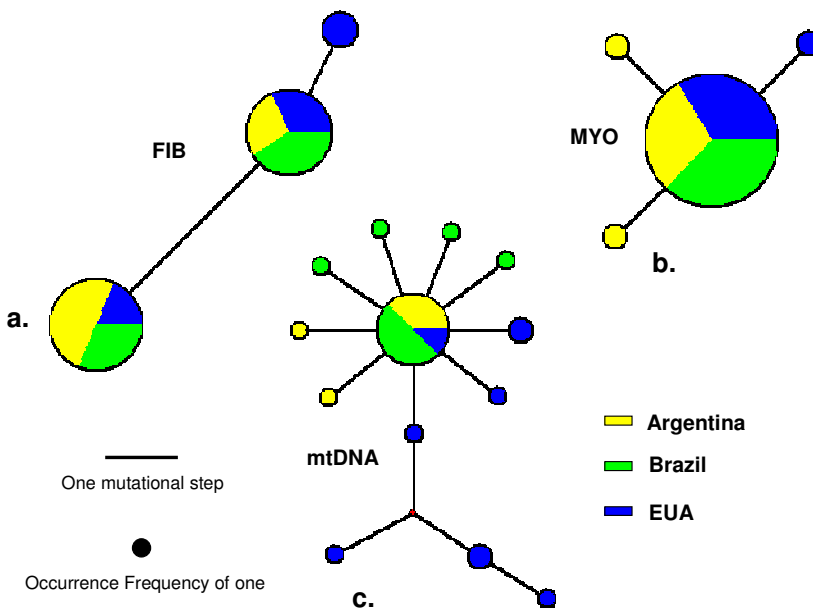
Figure 1. Median-joining networks for mitochondrial haplotypes and intronic alleles, from three populations of *T. acuflavidus*. a. β-fibrinogen gene (FIB); b. Myoglobin gene (MyO) and c. mtDNA with cytochrome *b* gene (CyB), NADH enzyme subunit 2 gene (ND2) and cytochrome-oxidase *c* enzyme subunit 1 gene (COI) concatenated.

The population pairwise F_{ST} values indicates a low genetic differentiation and a consequently high level of gene flow between ARG and BRA and, conversely, between USA and the other two South American populations a high F_{ST} indicating a restricted gene flow, in special with mtDNA data. (Table 2). There is a tendency of higher F_{ST}s in the comparisons of USA population with ARG than BRA. Most comparisons using the nuclear sequences were not significant, probably due to very low diversity.

Table 2. Genetic differentiation among populations of *T. acuflavidus* based on DNA sequences and microsatellites.

	mtDNA F_{ST}	mtDNA ΦST	R_{ST}	STR F_{ST}
ARG X BRA	0	0.0019	**0.1556**	**0.1067**
ARG X USA	**0.2196**	**0.2612**	**0.1068**	0.0166
BRA X USA	**0.0909**	**0.2228**	**0.0428**	**0.1233**

Upper-right values of F_{ST} were estimated using a distance matrix computed by pairwise difference (for STR using summed of squared differences - R_{ST}-like); Lower-left values of F_{ST} were estimated using haplotype frequency only. Values in bold are statistically significant at p<0.05.

Based on the F_{ST} results, the LAMARC search using DNA sequences was carried out only with the mtDNA sequences, and two sets of populations: samples from USA, and samples from ARG and BRA pooled. To balance the analysis, the same number of sequences was used for both populations, so 10 sequences were randomly chosen from ARG and BRA sequences. The LAMARC results showed a consistent pattern of higher genetic diversity in USA population (θ =

0.0071) than South American populations (θ = 0.0041), in accordance with the summary statistics. This also directly reflects in the estimated N_{ef} (female effective population size), that was almost twice for USA than for the for ARG and BRA pooled (Table 3), although its confidence interval is large. The gene flow between the two populations was estimated as Nm = 0.811 from USA into ARG-BRA and Nm = 0.862 from ARG-BRA into USA and they are in agreement with the relatively high F_{ST} values estimated between these populations. However, the Nm based on F_{ST} using the equation F_{ST} = 1 / (1 + 2Nm) is about twice the Nm estimated with LAMARC (1.4 between ARG and USA and 1.7 between BRA and USA). Both population sets showed a strong signal of population growth and the South American populations showed a higher signal (G=8230.531) than USA (G=3781.625) as could be expected by the network shape.

Table 3. Comparison between STR and mtDNA signals of effective population size (N_e) and female effective population size (N_{ef}) estimated by Bayesian approach using LAMARC.

|	STR		mtDNA
Population	N_e*	Population	N_{ef}*
ARG	**7,461** (5,518-9,524)	BRA-ARG	**22,359** (2,011-341,940)
BRA	**6,653** (5,243- 8,183)		
USA	**5,552** (3,858- 7,278)	USA	**38,652** (7,505- 406,348)

* See appendix for posterior distribution graphics of estimated θ values.

Neutrality tests for the three populations gave negative values for MyO and mtDNA sequences, but positive for FIB sequences (Table 4). However, only mtDNA data showed significant values, such as the significantly negative Fu's F_S for the three populations. The mtDNA graphs of mismatch distribution were unimodal for ARG and BRA and bimodal for USA (Figure 2).

Table 4. Neutrality tests based on DNA sequences for populations of *T. acuflavidus*.

Marker	Test	ARG	BRA	USA
mtDNA	Tajima's D	-0.0364	**-1.7274**	-0.1654
	Fu's FS	**-1.1639**	**-2.9237**	**-2.7275**
FIB	Tajima's D	1.4781	1.8674	2.2314
	Fu's FS	2.4550	4.0615	4.0915
MYO	Tajima's D	-0.76857	n.a.	-0.59155
	Fu's FS	-0.72368	n.a.	-0.0966

Values in bold are statistically significant (p<0.05).
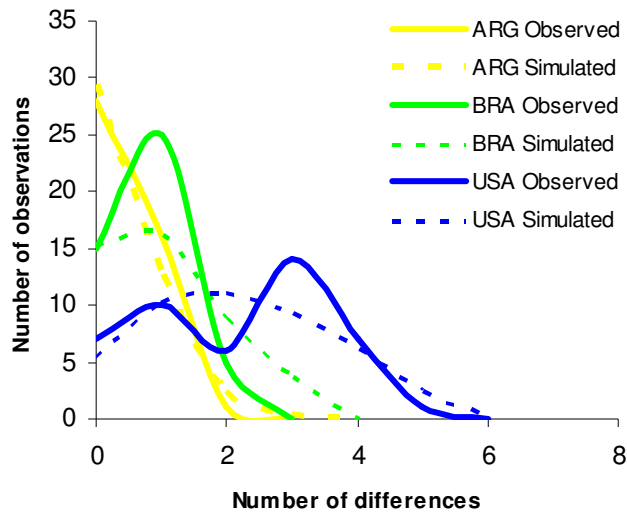
Figure 2. Mismatch distribution for three populations of *T. acuflavidus*.

The time to most recent common ancestor (TMRCA) for the whole species based on the mtDNA sequences was estimated around 100,000 years ago (YA; confidence interval of 95% (CI) of 39-220 kYA) and was similar to the TMRCA for USA population estimated at 97,000 YA (CI=38-215 kYA). For the two populations from South America the TMRCA was almost the same: 82,500 YA (CI=36,8-181 kYA) for ARG and 82,000 YA (CI=37.1-181 kYA) for BRA. The posterior distribution density for the parameters estimated and for likelihood of search can be seen in the Appendix.

Microsatellite sequence variation

Results of the summary statistics of the STRs showed a different pattern from that of the DNA sequences (Table 1). The ARG population showed the largest number of alleles per locus (4.4) and the USA population had the smallest (3.2). On the other hand, gene diversity was highest in USA and lowest in BRA population. The level of observed heterozygosity showed highest values in BRA and lowest in USA populations.

The analysis of genetic structure based on the STRs differs of the mtDNA (Table 2). The pairwise F_{ST} values showed some degree of genetic structure and were significant for all comparisons, with exception of the ARG x USA under classical F_{ST}. On the other hand, ARG and BRA present higher differentiation, in special in the R_{ST} approach. The bidirectional migration estimated using LAMARC software (Table 5) corroborates results of the R_{ST} analyses showing a

higher gene flow between BRA and USA and lower between BRA and ARG. The contribution to gene flow seems to be similar in both directions for all comparisons.

**Table 5**. Bidirectional migration between populations of *T. acuflavidus* based on STR data.

Nm direction	MPEs	95%L	95%U
ARG into BRA	**0.245**	0.158	1.044
BRA into ARG	**0.351**	0.116	0.882
BRA into USA	**0.568**	0.184	1.148
USA into BRA	**0.618**	0.203	1.265
ARG into USA	**0.450**	0.123	1.058
USA into ARG	**0.302**	0.094	0.955

MPEs, Most probable estimates

Bayesian inference under a Dirichlet process prior on STRUCTURAMA estimated K = 3 as number of clusters with the highest posterior probability for the individuals sampled. Assuming that K was correctly estimated, STRUCTURE obtained correct groupings of individuals with low accuracy (Table 6). Our clustering results showed that the three main geographic groups in the terns' data set represent three or two genetically distinct populations (Figure 3a), with the major difference between ARG and the other two populations. In Fig. 3b the triangle plot shows that ARG individuals are frequently clustered apart of the others and several individuals of USA and BRA are mixed.

Table 6. Proportion of membership of each pre-defined population in each of the 3 clusters of the STRUCTURE software

Population	Inferred Cluster			n
	1	2	3	
ARG	0.413	0.466	0.121	16
BRA	0.288	0.059	0.653	17
USA	0.310	0.158	0.532	12

a.   b.
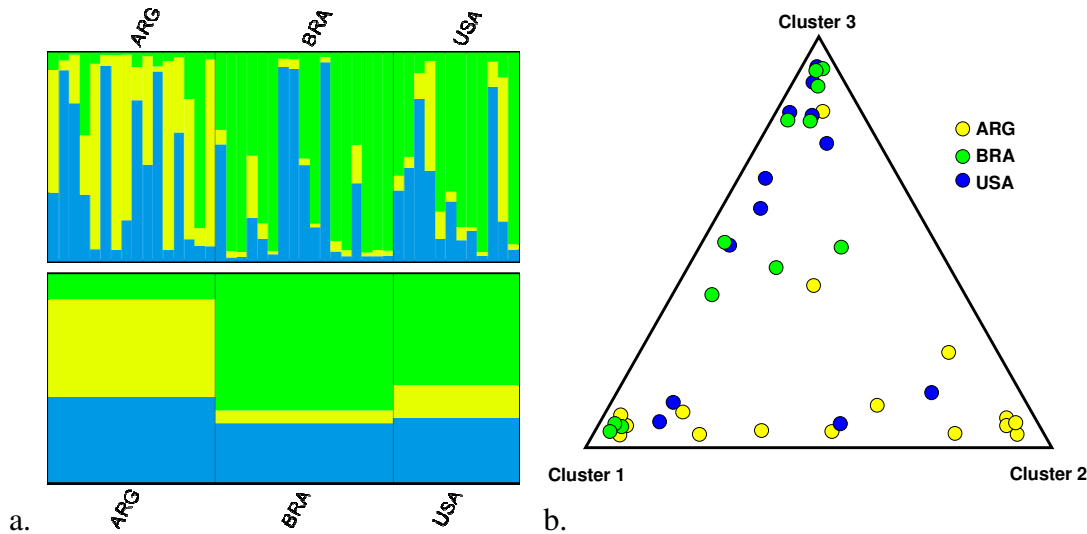
Figure 3. Population structure and population-of-origin assignment based on STR data for 45 individuals of *T. acuflavidus*. a. Bar plot showing the genetic structure where each individual is represented by a line partitioned into three segments corresponding to its membership coefficients in three inferred clusters (colors make reference to the principal cluster membership for each population). Upper box, plotted by individuals; Lower box, plotted by population; b. Triangle plot showing the assignment of each individual among three clusters.

Corroborating the LAMARC results for N_e estimations, all mutation models and all statistical tests implemented in Bottleneck software showed significant results for heterozygosity excess for the USA population and therefore, a signal of recent bottleneck (Table 7). The other two populations showed significant results (for α=0.05) only using the IAM model, with all the other results non-significant. The strict SMM model showed the larger p-value for all populations and USA had significant results only under α=0.05 for this model. However, the Mode shift was completely shifted by an excess of heterozygosity for this population, reinforcing the significant result for a bottleneck signal.

Table 7. Signals of heterozygosity excess inferred for populations of *T. acuflavidus* by the Bottleneck software.

Mutation Model		Population		
		ARG	BRA	USA
IAM	N	9(8)	9(7)	9(9)
	one tail	**0.01367**	**0.01855**	**0.00098**
	two tail	**0.02734**	**0.03711**	**0.00195**
SMM	N	9(5)	9(3)	9(8)
	one tail	0.36719	0.75195	**0.01855**
	two tail	0.73438	0.57031	**0.03711**
TPM	N			
	one tail	0.08203	0.15039	**0.00098**
	two tail	0.16406	0.30078	**0.00195**
Mode shift		L-shaped	L-shaped	Shifted

N, number of loci analyzed (values in parentheses are loci showing heterozygosity excess). IAM, p-value for Infinite Allele Model; SMM, p-value for Stepwise Mutation Model; TPM, p-value for Two-phase Model of Mutation. Values in bold are statistically significant (0.05).

Discussion

Genetic diversity and structure

The genetic diversity found in three different molecular markers (mtDNA, ncDNA and STR), presents a complex scenario for the evolutionary history of *T. acuflavidus*, including some apparently conflicting patterns.

If *T. acuflavidus* would follow isolation by distance process, gene flow would be more frequent among closer groups (ARG and BRA) and less frequent among distant groups (ARG and USA, BRA and USA). Signal of this pattern was found in the results of DNA sequences analyses, where ARG and BRA were genetically closer than ARG or BRA with USA. However, for the STR analyses the results were discordant with these expectations, suggesting a low gene flow between populations of terns from Brazil and Argentina as well as between populations of Argentina and USA.

Partial reproductive isolation could explain some cases of dramatic differences between population subdivisions revealed by mitochondrial and nuclear markers. Other examples of highly structured mtDNA in spite of poorly differentiated nuclear markers have been documented between bird species. Several large white-headed gull species are weakly differentiated in allozyme frequencies (Snell, 1991), but differ markedly in terms of mtDNA haplotypes (Crochet, 1998). Different dispersal behaviour of males and females and faster response of mitochondrial genes to drift effect in case of gene flow reduction will affect unequally intraspecific population structures of mitochondrial and nuclear genes. Imperfect reproductive isolation might also prevent exchange of mitochondrial lineages before the evolution of barriers to nuclear gene flow. According to Crochet (2000) even in a population with neither sex-biased dispersal nor skewed sex-ratio, expected values of F-statistics are higher when using mtDNA than nuclear markers, particularly when F_{ST} is small. Avise et al. (2000) analyzed mtDNA control region variation from Sooty tern and found that colonies within an ocean basin are only weakly differentiated in matrilineal composition with similar or identical mtDNA haplotypes shared across nesting sites separated by 16,000 kilometers.

Divergence times and the arising of South American population

Based on the TMRCA for mtDNA sequences both South and North American populations seem arose simultaneously. However, a molecular phylogenetic tree of *T. acuflavidus* and several outgroups showed that the root is within the EUA population (Efe *et al*., submitted), rendering the

South America population a peripheral isolation of an older North American linage. This is also a plausible interpretation for the mtDNA network (Fig. 1). The Fs statistics for the South American populations and their mismatch distributions can be interpreted as a signal of a bottleneck followed by a population expansion for the South American population. Given they present a peak around 1 difference and using the standard 2% divergence for mtDNA coding region, this translate to an expansion time around 30,000 years ago for their single ancestral population.

The low level of divergence between ARG and BRA for the DNA sequences could be interpreted as a recent differentiation with incomplete lineage sorting, assuming that the first divergence occurred between USA and South American populations. Two main hypotheses could be proposed to explain the second and more recent split between BRA and ARG populations and their genetic diversity characteristics.

One scenario is that the ARG population is a recent and peripheral isolated from some Brazilian lineages, which could explain the lower diversity of DNA sequences in Argentinean population. An alternative hypothesis is a basal divergence between them, but that the ARG population experienced a later population bottleneck due to glacial periods. As Cabot's terns in Argentina is distributed in higher latitudes, the consequences of the glacial cycles could be enough to decrease the population size substantially.

Demographic scenarios

The estimated effective population sizes for the studied populations of *T. acuflavidus* were similar to the current population census. As the biggest colonies in each geographic group were sampled (North Carolina, in North America, Espírito Santo in Brazil and Punta León in Argentina) and the migration between nearby colonies was demonstrated (Shealer 1999, Efe et al., 2000), the sampling used in this study was assumed as enough to sample haplotypes from different colonies from each major geographic group. Therefore, the estimated N_e for each populations based on mtDNA sequences were took here as an estimative of the N_e for the major geographic groups.

For the whole North American breeding colonies the estimative of the population census ranges from 37,530 to 46,945 pairs (Clapp et al., 1983, Shealer, 1999), a mean of 84,475 individuals. Based on a sexual ratio of 1:1 (Shealer, 1999) the population census is only 1.9 times larger than the N_e estimated using mtDNA sequences. For South American breeding colonies the population census is not so well known and there are only estimates for individual breeding sites: 1,700-3,470 pairs for Aruba, 1,200 pairs for Guiana (data compiled by Shealer, 1999), less than 10,000 pairs in Argentina (Yorio and Efe, 2009) and 8,000 pairs in Brazil (Yorio and Efe, 2009).

An approximation of the total number of Cabot's terns in South America results in 45,340 individuals, wich represents 0.59 times the N_e estimated based on mtDNA sequences. On the other hand, the N_e estimated based on STR markers are much lower (Table 3). However, as the mutation rates presented for microsatellite loci have at least an order of magnitude of difference, and we have used a mean rate, just using another value within the accepted interval is enough to bring the N_e from STR dataset to the values compatible with mtDNA values. An additional explanation is that, given the faster mutation rate of mtDNA than STR markers the N_e estimated from mtDNA represents a long-term estimate while the STR represents a much more recent and more local demography (see Webster et al., 2002, Ellegren, 2004). This view is supported by the estimated gene flow, which shows stronger genetic structure using STR than using mtDNA sequences. Therefore, almost all N_e estimations matched with census estimation indicating that the time and strength of the historical demographic process undergone by these populations were enough to imprint very clear signals in the studied markers, which could be recovered by population genetic statistics.

The North American bottleneck

The very clear signal for excess to heterozigosity in EUA populations suggests that this population went through a historical but relatively recent size reduction. This population suffered the major known decline during nineteenth century, due mostly to millinery trade and egg collecting (Shealer, 1999). However, the colony sampled in North Carolina has been reported as an example of the recent population increase (Shealer, 1999). Clapp and Buckley (1984) suggest that North Carolina colonies were the largest there have ever been before. Number of breeding pairs in these colonies more than doubled between 1977 (1,190 nests) and 1995 (2,905 nests; Parnell et al., 1997). Given the very recent timeframe for human influences, it is unlikely that they left any significant signal in the genetic makeup of this population. Both the older expansion found in the mtDNA data and the recent bottleneck were more likely consequences of natural events as discussed herein.

Taxonomic implications

South and North American populations are commonly classified as two distinct entities, Cayenne terns, *T. sandvicencis eurygnathus* and Cabot's tern, *T. sandvicencis acuflavidus* (Shealer, 1999) and have some clear distinctions in the mtDNA variability (Fig. 1, Table 2) that could be used as an argument to keep the sub-specific taxonomy rank. However, it was showed here that these two populations present high levels of recent gene flow based on STR analyses (Fig. 3, Table

2 and 4). This low level of genetic differentiation between South and North American colonies are consistent with expectations based on the large, transient nature of the populations and the potential for population mixing. The geographical extent of possible hybridization sites between North and South American populations has been documented quantitatively (see Hayes, 2004). Norton (1984) argued that the winter area of North populations, which has black culmem with yellows tip, is overlapped with the reproduction area of South populations, which has complete yellow culmem, in the north coast of South America, where social pairs could be formed for next breeding season, influencing extra-limited movements and recruitment of immature birds seeking reproduction habitats.

Evidence of allochronic barriers

The *T. acuflavidus* complex has now been defined as a single entity (Efe et al., submitted). However several studies had shown the idiosyncratic characteristics of populations from the extreme southern South America (see introduction, Junge and Voous, 1955, Escalante, 1970, 1973). Results in this study show a recent barrier to gene flow between ARG and BRA populations (Fig. 3, Table 3, 5 and 6). Interestingly, these two populations are in close contact in some localities in Brazil (i.e. Lagoa do Peixe, RS), which is constantly used for both populations as a feeding site (Efe et al., 2000, Bugoni e Vooren, 2005). Individuals banded in Brazil were captured in Argentinean and Uruguay feeding sites and vice-versa (Efe et al., 2000). However, they have asynchronous reproduction: the Brazilian population breeds between April and September and population from Argentina between September and January (Escalante, 1970). This difference in breeding season could explain the low level of gene flow between these two populations. Reproductive phenology can be governed by a combination of genetic and environmental factors (Gwinner, 2003; Lambrechts et al., 1999) and has been described as the main cause of allochronic speciation (Cooley et al., 2001, 2003). Moore et al. (2005) have showed that the reproductive asynchrony causes genetic divergence between populations of Rufous-collared sparrows (*Zonotrichia capensis*) as a consequence of limited gene flow, and argued that greater genetic diversity in tropical populations can be associated with locally adapted reproductive phenologies. Sympatric speciation has been a polemic issue in speciation debate (Friesen et al., 2007) and within all possible hypotheses causing sympatric speciation the allochrony is one of the least supported by empirical data. The most common examples of speciation by allochrony were given by insects (Abbot and Withgott, 2004), but in this case they are strongly supported by evidences, such as speciation of cicadas in eastern North America (Cooley et al., 2001). Nevertheless, the examples for vertebrates are scarce and

sometimes not evident, despite a recent paper (Friesen et al., 2007) describing the first example of allochronic speciation for a tetrapoda. They studied a small seabird that nests in islands throughout the Atlantic and Pacific Oceans, the Madeiran or bandrumped storm-petrel (*Oceanodroma castro*). In this storm-petrel different individuals breed in different season, but in the same islands, and in all archipelago they are very different genetically, and in two of then they ceased to exchange genes.

The results for South populations of Cabot's tern showed a clear tendency of genetic isolation in southern South America, and considering the DNA sequence and STR results showed herein, it is plausible to argue that, as the Argentinean population may represents a peripheral branch of the Brazilian, the STR divergence between BRA and ARG can be explained by allochrony. The Brazilian and Argentinean populations probably are in the early stages of genetic divergence, sharing mitochondrial haplotypes and STR alleles. It is possible to argue that the STR divergence showed by ARG is a signal of genetic drift that fixed different alleles or frequencies of the ancestral population pool and not a clear effect of allochrony. Although this hypothesis is certainly true concerning the lower mtDNA diversity in ARG, it does not represent a conflict with the allochrony hypothesis, because even though the fixed alleles were a result of genetic drift it does not explain why the gene flow signal is so low between the ARG and BRA populations.

Therefore, the small size of the most colonies of North and South populations of Cabot's tern coupled with the phylopatric nature of the species suggest that inbreeding and allochronic isolation (phenological barriers for breeding between the Argentinean and Brazilian populations) may be significant factors in the dynamics of this species in the Atlantic region and an issue of particular interest for studies concerning patterns of gene flow in hybrid zones and behavioral barriers.

Conservation implications

The population structure of Cabot's Tern in America presented here fits very well with the metapopulation concept. Metapopulation ecology is expected to make predictions about the biological and ecological consequences of habitat destruction and its effects on loss of biodiversity. In some areas of high human development such as the coastal region, these predictions will be crucial for the future of the terns and the landscapes they inhabit. This terns nest on small coastal islands susceptible to environmental disturbance (Gochfeld and Burger, 1996, Shealer, 1999) and its colonies have historically suffered extensive egg collection which has severely decreased its reproductive success. Therefore, these peripheral populations are typically small and subject to colonization and founder events, increasing the potential for genetic drift and inbreeding (Bouzat

and Johnson, 2004). These populations are valuable for conservation, because they may preserve rare alleles and gene combinations important for local adaptation (Lesica and Allendorf, 1995).

Co-specific populations not clearly defined by large phylogenetic gaps, as the population of Cabot's Tern studied here, which showed weak levels of matrilinean subdivisions, may be relevant for conservation efforts, because if over exploited or excised they are probably sunk. If we define our goal as protecting intraspecific biological diversity there are many potential ways to elect the Argentinean population as a Management Unit, for example distinct phenotypes, such as "pure" yellow billed and different breeding regime, such as temporal breeding isolation, which produce distinct life histories. Even geographic distance, in the absence of clear isolation could be used to assign conservation unit status, protecting extremes of a species' geographic range.

Acknowledgments

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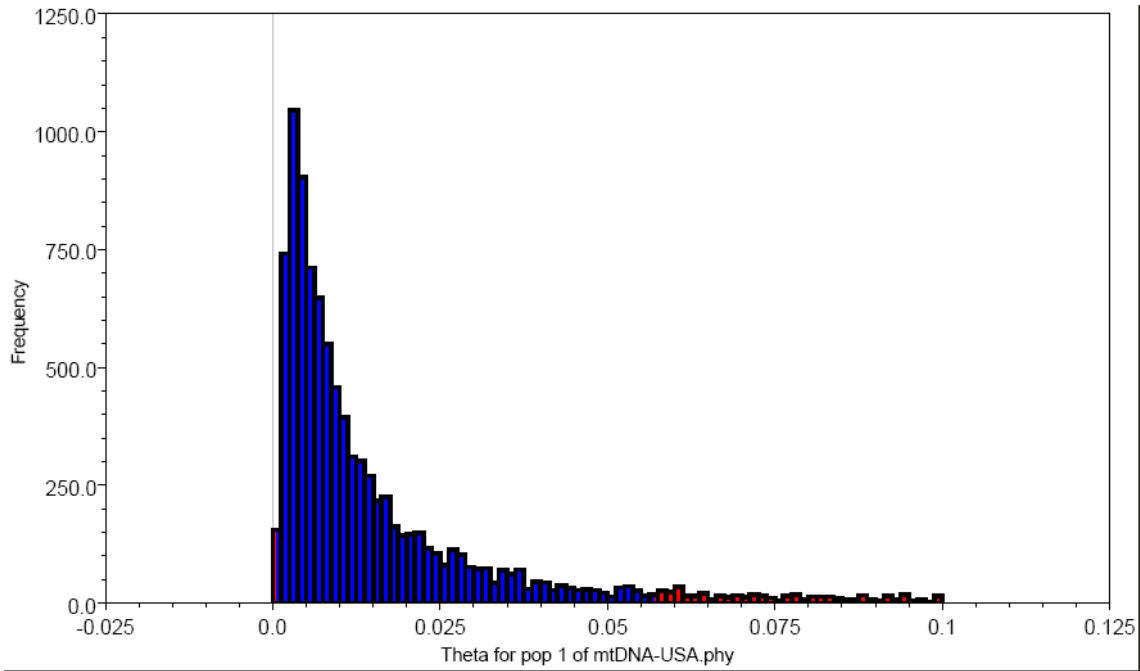
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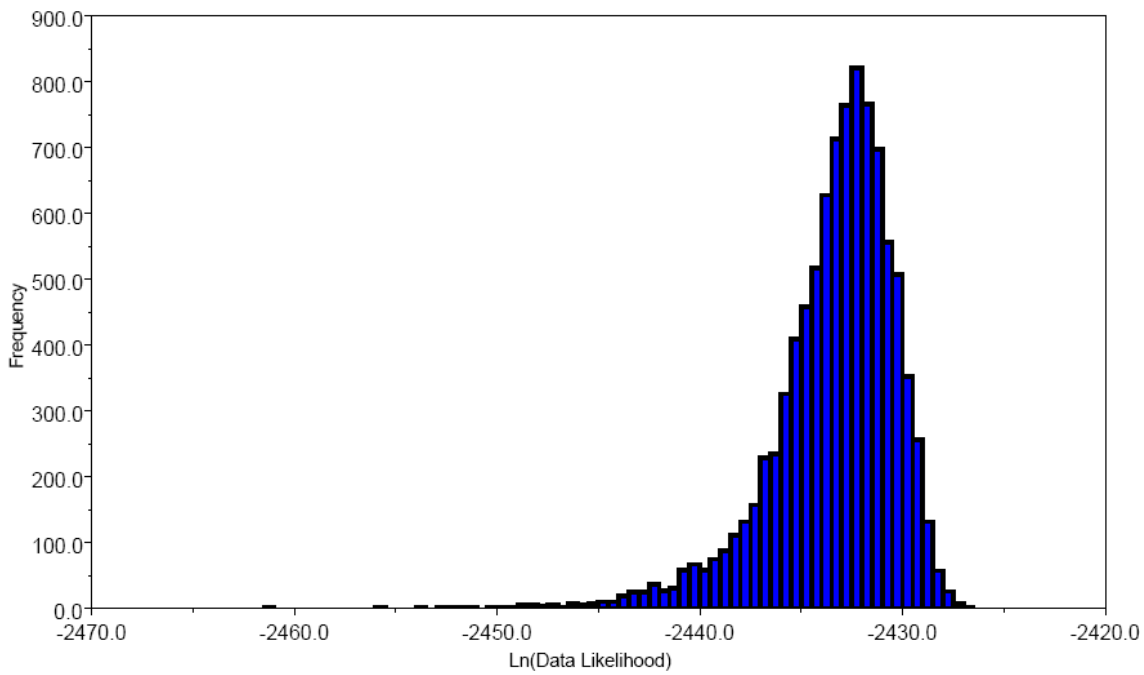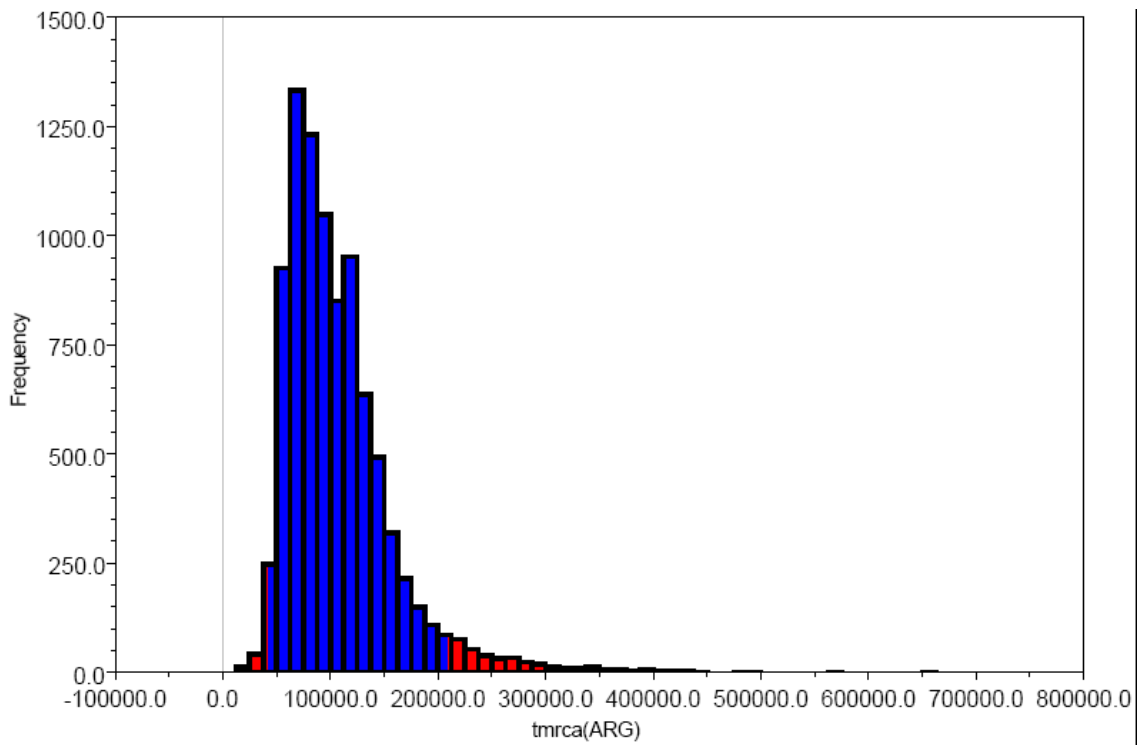

Fig. A1. Distribution density for likelihood value estimated in BEAST.
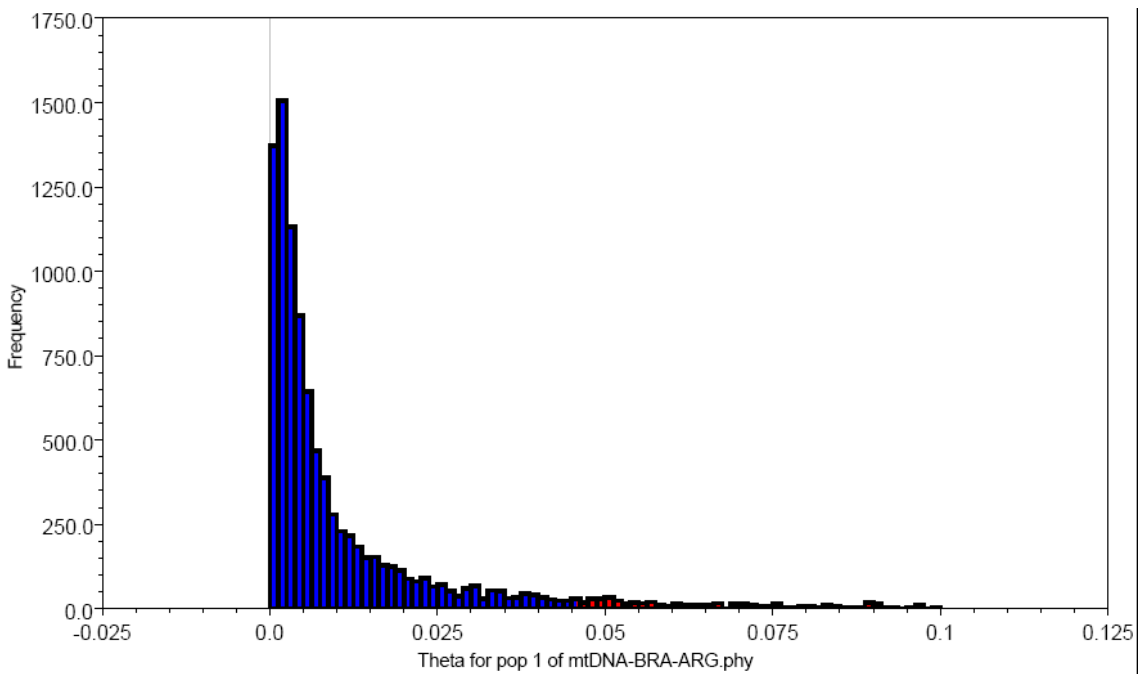
Fig. A2. Distribution density for θ value estimated for population from South America (BRA-ARG) using BEAST.

Fig. A3. Distribution density for θ value estimated for population USA using BEAST.
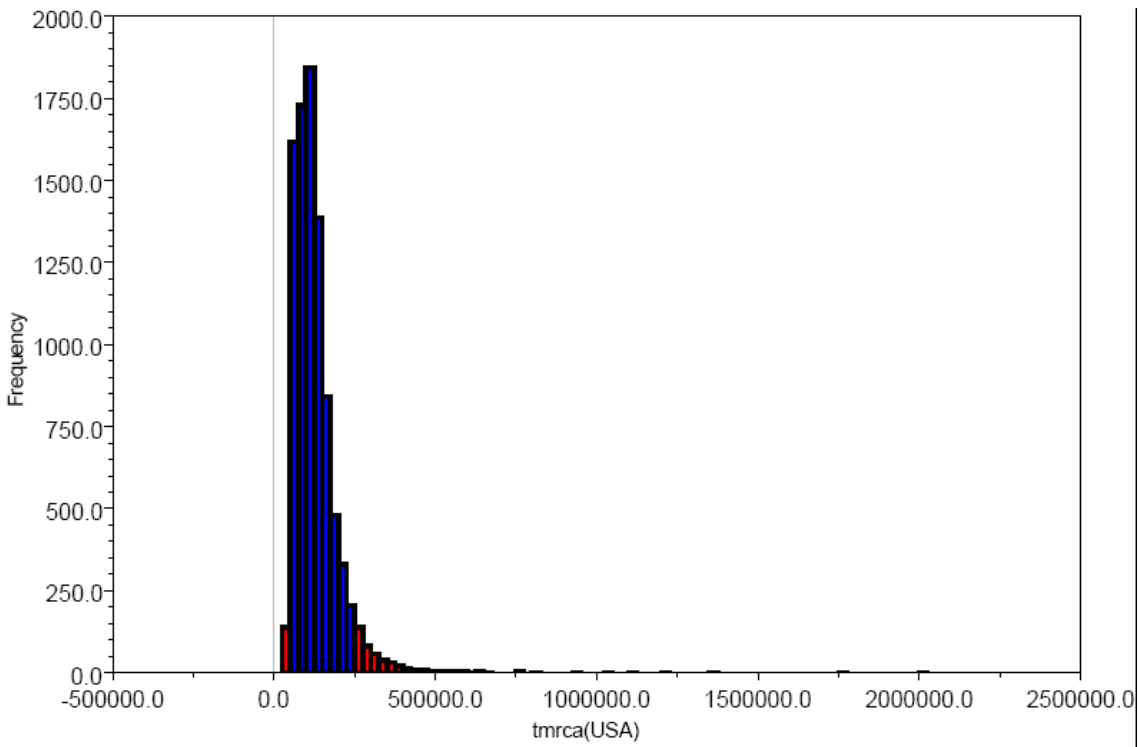

Fig. A4. Distribution density for T_{MRCA} value estimated for ARG population using BEAST.
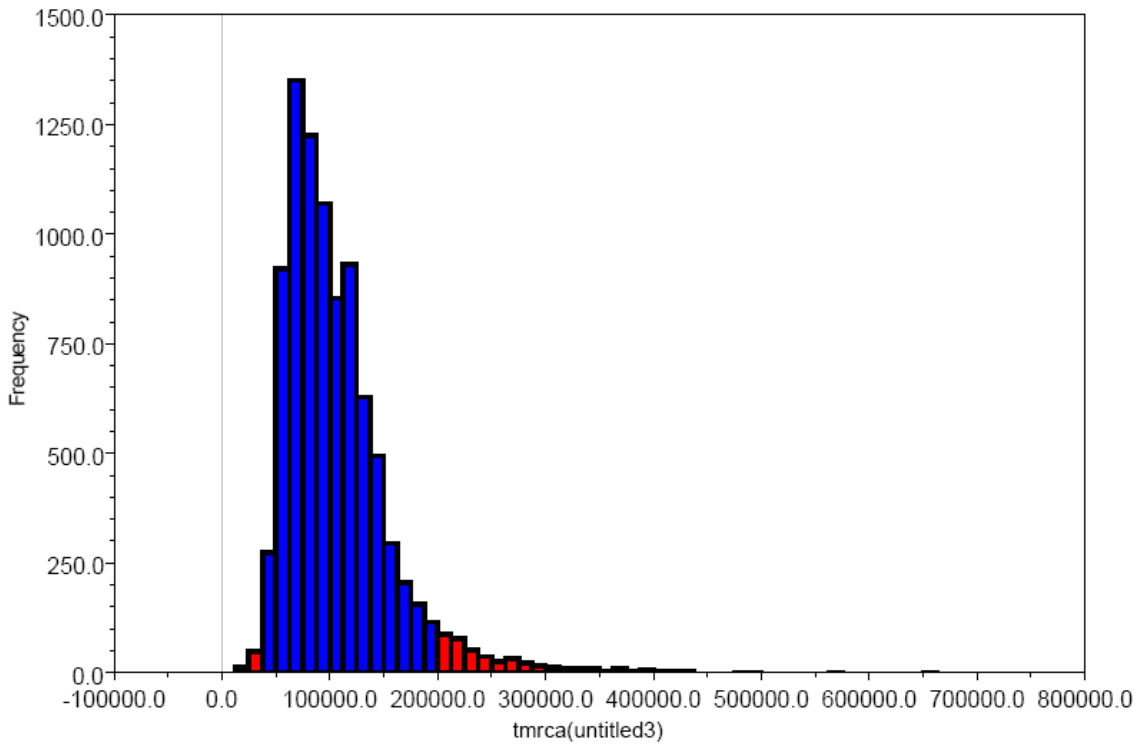
Fig. A5. Distribution density for T_{MRCA} value estimated for BRA population using BEAST.

Fig. A6. Distribution density for T_{MRCA} value estimated for population USA using BEAST.

Table A1. Life-table for *Sterna hierundo*.

Age	Freq.	Survival	Contr.	weight
2	**0.000**	**0.000**	0.000	0.000
3	0.015	**0.400**	0.007	0.021
4	0.031	**0.400**	0.014	0.056
5	0.046	0.645	0.034	0.170
6	0.061	**0.890**	0.063	0.376
7	0.076	**0.890**	0.078	0.548
8	0.092	**0.890**	0.094	0.751
9	**0.107**	**0.890**	0.110	0.986
10	0.096	0.897	0.099	0.994
11	0.086	0.903	0.089	0.979
12	0.075	0.910	0.078	0.941
13	0.064	0.917	0.068	0.880
14	0.053	0.923	0.057	0.796
15	0.043	0.930	0.046	0.687
16	0.032	0.937	0.035	0.554
17	0.021	0.943	0.023	0.395
18	**0.011**	**0.950**	0.012	0.211
19	**0.011**	**0.950**	0.012	0.222
20	**0.011**	**0.950**	0.012	0.234
21	**0.011**	**0.950**	0.012	0.246
22	**0.011**	**0.950**	0.012	0.257
23	**0.011**	**0.950**	0.012	0.269
24	0.009	0.907	0.010	0.234
25	0.008	0.864	0.008	0.199
26	0.007	0.820	0.006	0.164
27	0.005	0.777	0.005	0.129
28	0.004	0.734	0.003	0.095
29	0.003	0.691	0.002	0.062
30	0.001	0.648	0.001	0.030
31	**0.000**	**0.000**	0.000	0.000

Freq. mean relative frequency in the population; Survival, frequency of chick survival until 24 days; Contr., relative contribution to next generation; weight, weight value to use in the weighted mean. Bold values for Freq. and Survival are data compiled form literature, not bold values produced by interpolation. Data from Nisbet (2001, 2002).

CAPÍTULO 5

Evaluation of the status of conservation of the Cabot's Tern in Brazil.

A ser submetido para *Bird Conservation International*.

Evaluation of the status of conservation of the Cabot's Tern in Brazil

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Abstract. The Cabot's Tern, *Thalasseus acuflavidus* is considered to be one of the most vulnerable coastal species in Brazil. Its range is limited to the eastern coast of South America, and it nests on small coastal islands that are susceptible to environmental disturbance. Historically, its colonies have suffered extensive egg collection by fishermen, which has severely decreased its reproductive success. The Brazilian population is mainly confined to the coast of Espírito Santo state. This paper evaluates the population status of *T. acuflavidus* in Brazil and discusses its threat category. Our evaluation of the conservation status of this species follows the criteria and categories adopted by the IUCN. Here, we review several parameters, including taxonomic level, principal threats, area and extent of occurrence, and current population size. Because Cabot's Terns have recently been extirpated from other areas of the Brazilian coast, we recommend that this species should be defined as Vulnerable at the national level. It may also qualify as Endangered at the state level. Finally, we suggest that research and conservation efforts should be increased on Espírito Santo coast, and that conservation actions should be implemented across the whole Brazilian coast.

Keywords: Cabot's Tern, Status, Conservation, Threat, Extent of occurrence**,** Area of occupancy**,** Size population, Brazil.

Introduction

The ornithological literature contains scarce information on Cabot's Tern (Junge and Voous 1955). The species (now *Thalasseus acuflavidus*; Efe et al. in press) breeds on the Caribbean and Atlantic coasts of North and South America. Its range extends from the southern USA, Caribbean along the coasts of Colombia, Venezuela, Surinam, Brazil, and Uruguay south to Argentina as far as Puerto Deseado (Escalante 1973, Shealer 1999).

Antas (1991) identified the Cabot's Tern as the most vulnerable coastal species in Brazil, due to extensive egg collection by fishermen. Since then, this species has been the focus of some studies and conservation initiatives. The Andorinhas do Mar Project curtailed egg collection in Espírito Santo, mainly through inspections and education (Efe et al. 2000); however, the long-term

survival of the Cabot's Tern remains uncertain. This paper evaluates the conservation status of *T. acuflavidus* in Brazil and discusses its current threat level.

Methods and Data Analysis

Our evaluation of the conservation status of the Cabot's Tern follows the criteria and categories established by the IUCN (IUCN 2008). The IUCN categories and criteria are defined for the global evaluation of a taxon. However, these definitions can also be appropriate for regional use (Gärdenfors *et al.* 2001). In this paper we review information on the conservation status, including taxonomic level, main threats, area and extent of occurrence, and current population size in Brazil and we followed the orientations suggested by Gärdenfors *et al.* (2001) for the evaluation at the national and regional level.

In this analysis, we treat *T. acuflavidus* as a valid species, following the proposition presented in Efe et al. (in press) which showed, using a thoroughly molecular phylogenetic analysis, that the European and American *T. sandvicensis* are distinct species and proposed to validate the treatment of the American *acuflavidus/eurygnathus* complex as Cabot's Tern, *Thalasseus acuflavidus*.

According to Birdlife International (2001), the extent of occurrence is defined as the area contained within the shortest continuous imaginary boundary which can be drawn to encompass all the known, inferred or projected sites of present occurrence of a taxon, excluding cases of vagrancy. For the estimate of the extent of occurrence in this analysis we used the following reasoning: coastal breeders usually forage from tidal creeks and estuaries to ocean waters, usually close inshore, but occasionally ranging across the continental shelf (Gochfeld and Burger 1996). Since studies shown that other terns can be found until 25 km offshore (Pearson 1968, Veen 1977, Bugoni and Vooren 2004), we considered this distance for the estimate of the extent of occurrence of the Cabot's Tern in Brazil.

Area of occupancy is the area inside the extent of occurrence which is occupied by a taxon, excluding cases of vagrancy. This category reflects the fact that a taxon will not usually occur throughout its extent of occurrence, which may contain unsuitable or unoccupied habitats. In some cases the area of occupancy is the smallest area essential at any stage to the survival of existing populations of a taxon (BirdLife International 2001). For the estimate of the area of occupancy in this analysis we used the following reasoning: according to Shealer (1999) most breeding birds forage at a maximum distance of ~15-25 km from their breeding sites (mean value of 20 km). Bugoni et al. (2005) showed that Common Tern on their wintering ground in southern Brazil fed over waters 10-20 m depth, corresponding to 8 km from the coast. Therefore, we considered for the estimate of the area of occupancy of the Cabot's Tern in Brazil a circular area around the breeding sites with 20 km of ray (i.e., area of 1,256 km^2) and as feeding area the distance of 8 km of shore.

Results and Discussion

Using the above definition, the Cabot's Tern is distributed all along the Brazilian coast (~6.100 km). Therefore, in this analysis, we estimate the extent of occurrence as the area contained within the imaginary boundary of 6,100 km of extension for 25 km of width, i.e., 125,500 km^2. However, this area is not completely occupied for the Cabot's Tern. Breeding colonies have recently been located in south and southeast of Brazil. A review of historical records of nesting distribution indicates that Cabot's Terns have bred in at least 16 breeding sites in Brazil (Table 1) and was registered in more four feeding areas considered important for the species (Figure 1) during the non-breeding period. Therefore, in this analysis, the area of occupancy in the appropriate scale of the 20 suitable habitats with relevant biological aspects of the taxon was estimate for 16 breeding sites in 20,096 km^2. For the feeding sites (Mangue Seco beach with 240 km^2; Coroa Vermelha island with 201 km^2; Coast of Paraná with 856 km^2 and Rio Grande do Sul's coast 4,960 km^2), the total area is 6,257 km^2.

Therefore, both the estimated extent of occurrence and area of occupancy are larger than the necessary limits for inclusion in the criteria for Critically Endangered, Endangered or Vulnerable.

Table 1. Location and size (in breeding pairs) of Cabot's Tern colonies in coastal Brazil. Nests correspond to the last available census. All locations where terns have bred in the past are listed.

Number in Map (Figure 1)	State	Site	Location	Size (nº nests)	Year	Source
3	Espírito Santo	Pacotes Is.	20º21'S,40º16'W	NC	1994	1
4		Escalvada Is.	20º42'S,40º24'W	6500	1996	1
5		Itatiaia Is.	20º21'S,40º17'W	1500	1996	1
6		Branca Is.	21º00'S,40º47'W	5000	1990	1
7	Rio de Janeiro	Papagaios Is.	22º24' S,41º48' W	NC	1981	2
8		Rio-Niteroi Bridge	22º52'S,43º10'W	66	2001	3
9		Casa da Pedra Is.	22º47'S,43º08'W	NC		3
10	São Paulo	Prainha Is.	23º51'S,45º25'W	75	U	4
11		Apara Is.	23º50'S,45º33'W	25	U	4
12		Laje de Santos Is.	24º19'S,46º11'W	142	U	4
13		Castilho Is.	25º17'S,47º57'W	40		5
14		Figueira Is.	23º55'S,45º18'W	NC	1985	2
15	Paraná	Itacolomis Is.	25º50'S,48º24'W	100	1995	6
17	Santa Catarina	Deserta Is.	27º16'S,48º20'W	65	1999	7
18		Moleques do Sul Is.	27º51'S,48º26'W	200	2000	7
19		Cardos Is.	27°48'S, 48°34'W	76	2002	7

Notes: NC: not assessed; 1 - Efe et al. (2000); 2 - Antas (1991); 3 - Alves et al. (2004); 4 - Campos et al. (2004); 5 - Olmos et al. (1995); 6 - Krull (2004); 7 - Branco (2003).
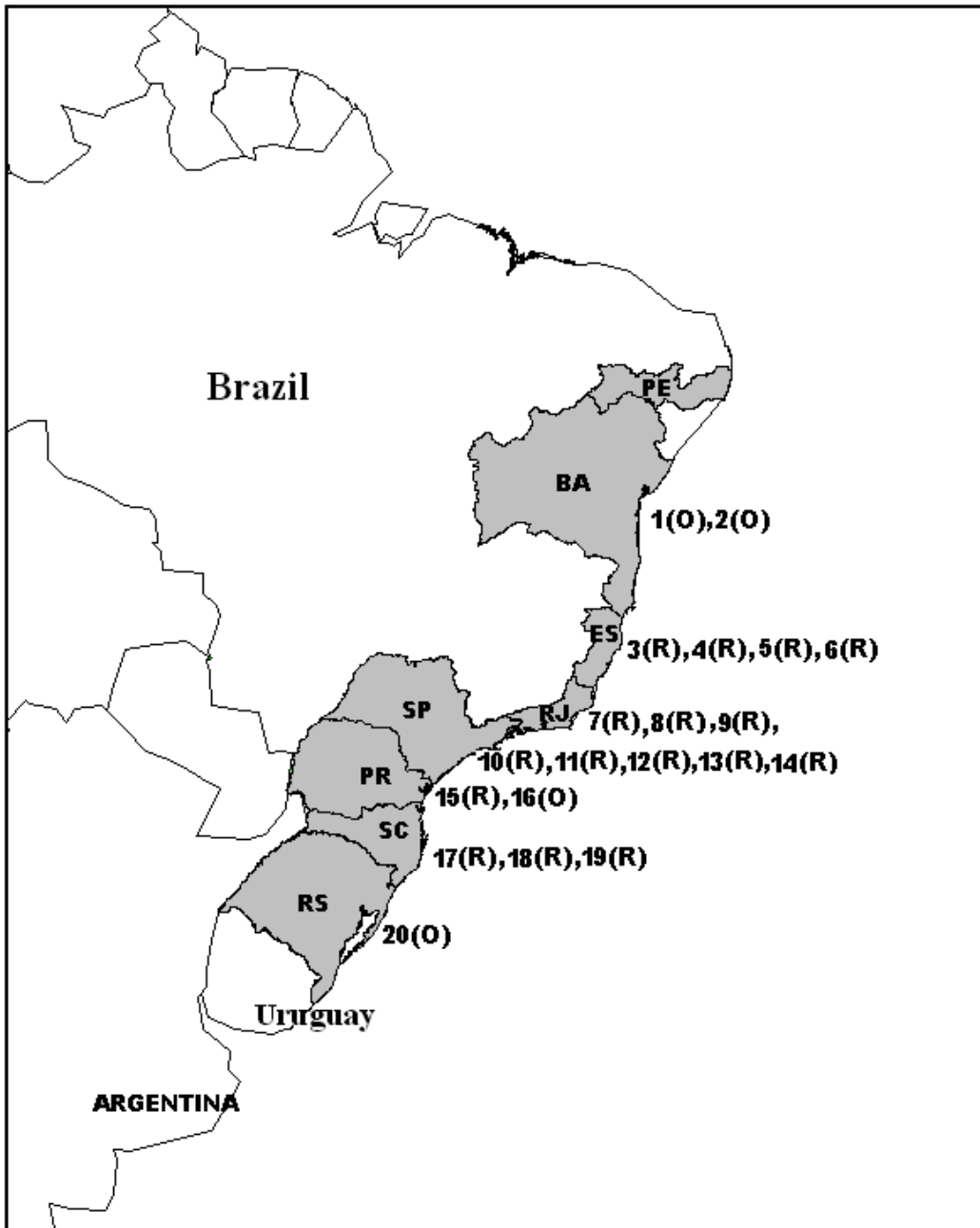
Figure 1. Records of *T. acuflavidus* in South American Atlantic coast. Adapted from Efe *et al.* (2000). Sources in Efe *et al.* (2000) and Table 1. Status: O = occurrence and important feeding areas, R = breeding areas. Localities: 1- Mangue Seco beach; 2- Coroa Vermelha island; 3- Pacotes island; 4- Itatiaia Archipelago; 5- Escalvada island; 6- Branca island; 7- Papagaios island; 8- Rio-Niteroi Bridge, 9- Casa da Pedra island; 10- Prainha island; 11- Apara island; 12- Laje de Santos island; 13- Castilho island; 14- Figueira island; 15- Itacolomis island; 16- Coast of Paraná; 17- Deserta island; 18- Moleques do Sul island; 19- Cardos island; 20- Coast of Rio Grande do Sul (Bugoni e Vooren, 2005).

Following the orientations suggested by Gärdenfors *et al*. (2001) for the evaluation in the national and regional level we analyzed questions such as: contact of the national population with the neighboring populations; capacity of dispersion of the species; abundance and threats in the neighboring populations; differences in local adaptation between the national and foreign populations; the environmental situation within each country/region; environmental conditions in the country for immigrants' establishment and probabilities for recolonization within 100 years in case the species is extinguished at the country.

Contact with the neighboring populations

The extent of possible hybridization between North and South American Cabot's Tern specimens has been documented quantitatively (see Hayes 2004) and the migration between nearby colonies has been demonstrated (Shealer 1999, Efe et al., 2000). Efe et al. (unpublished results) show a high gene flow between a Brazilian and a North American population, supporting the contact of the Brazilian population with Northern populations. On the other hand, notwithstanding the South American populations are in close contact in some localities in Brazil (e.g., Lagoa do Peixe, RS), which is constantly used for both populations as a feeding site (Efe et al., 2000, Bugoni e Vooren, 2005) and individuals banded in Brazil have been captured in feeding sites at Argentine and Uruguay and vice-versa (Efe et al., 2000), Efe et al. (unpublished results) show a lower gene flow between the Brazilian and Argentinean populations they studied. They suggested that difference in breeding season could explain this low level of gene flow between these two populations.

Capacity of dispersion of the species

Most terns are migratory and some tropical species, including some Cabot's Tern populations, move great distances during the non-breeding period (Gochfeld and Burger 1996).

Cabot's Tern in Brazil show a post-breeding dispersal moving along the northeastern coast of Brazil and southern coast of South America, including Argentina (Efe et al., 2000), showing an excellent capacity for long-distance dispersion.

Abundance and threats in the neighboring populations

The total census population of Cabot's Tern worldwide may be less than 80,000 pairs. The North American population is estimated in about 47,000 pairs (Shealer 1999); the Caribbean about 8,000 pairs (Norton 1984); 1,700-3,470 pairs from Aruba; 1,200 pairs for Guiana (data compiled by Shealer, 1999); and the Argentinean population is about 10,000 pairs (Yorio and Efe 2009).

Egg collection and disturbance at breeding sites are among the main factors limiting reproductive success of terns (Gochfeld and Burger 1996, Shealer 1999) and main threats to the species include also predation by Gulls, fisheries, egging and introduced predators (Gochfeld and Burger 1996). On Punta León (Argentina), Kelp Gulls were the main predators of eggs of the Royal Tern (*Thalasseus maximus*) and the Cabot's Tern, decreasing reproductive success in all colonies studied (Quintana and Yorio 1997).

Differences in local adaptation between the national and foreign populations

Ecological variability is common in Cabot's Tern, revealing important differences necessary to the adaptation in the different geographic areas. In the U.S. and Gulf coasts, they typically nests on low, sandy, flat islands close to shore (Oberholser, 1974, Visser and Peterson, 1994). In the Caribean region the breeding sites are flat islands situated in an extensive saline lagoon of shallow water or on bare coral rocks and patches of coral debris and sand and a few elevated rocks locally covered with thorny scrub and opuntias. The breeding grounds in Brazil have low shrub vegetation with cactaceans predominating (Efe et al., 2000). In Argentina colonies are characterized by extensive cliffs 30-100 m high and gravel beaches along the shoreline (Yorio et al., 1998). In the

U.S., Cabot's Tern usually nests in dense groups among Royal Tern, *T. maximus*, Laughing Gulls, *Larus atricilla*, and sometimes Black Skimmers, *Rynchops niger* (Shealer, 1999). In North Carolina and Caribe it breeds with the Royal Tern (McGinnis and Emslie, 2001, Hayes, 2004) and Roseate Tern, *S. dougallii*. In Brazilian coast breeding occur in mixed colonies with the South American Tern, *S. hirundinacea* (Efe et al., 2004). The colonies of Argentina are located within the Kelp Gull colony in mixed colonies with the Royal Tern (Quintana and Yorio, 1997). Breeding season also differs between colonies, in most U.S. and Caribbean colonies first adults arrive in late April or early May (Shealer, 1999.) The terns in Brazilian colonies also began to arrive in mid-April and the settlement at the colony site occurs from May onwards. In mid-September birds begin to leave the colonies and after the end of October they are rarely found on the coast of the State of Espírito Santo (Efe, 2004). However, in Argentina, Cabot's terns started to arrive in mid-September (Quintana and Yorio, 1997) and the breeding activity has been registered until January (Escalante, 1970).

Environmental situation within Brazil

Several breeding colonies on islands on the Rio de Janeiro coast have been abandoned due to disturbances caused by fishermen, and the Espírito Santo colonies have been depopulated by constant egg collection by local fishermen (Antas 1991). The Brazilian coast has also suffered severe environmental degradation in recent decades. Coastal islands are particularly vulnerable to degradation since they are used by both fishermen and tourists visiting from the mainland. In some areas, however, weather and predation also limit the reproductive success of Cabot's Terns. Here, we summarize the primary threats affecting each of the main nesting areas along the Brazilian coast.

A recent analysis found that on the islands of Espírito Santo, storms are the most common cause of mortality (Efe *et al*. 2005). The Rio de Janeiro islands colonies are also disturbed by humans and, the eggs and chicks being predaded by both native (*Coragyps atratus* and *Larus*

dominicanus) and introduced predators (cats and mice). Cabot's Terns have also used the pillars of the Rio-Niterói Bridge for breeding (Alves *et al*. 2004). On the São Paulo coast, Cabot's and other terns species are threatened due to disturbance in nesting colonies and roosting sites. Egg collection, fire and intense human presence in the beaches and in the sea increase the susceptibility of these colonies (Campos *et al*. 2004). On the Paraná coast, Cabot's Terns feed on fish discarded by fishermen, but their nesting colonies are often disturbed by fishermen and tourists (Krul 2004). On Deserta island (27°16'23" S 48°19'53" W - part of the Federal Biological Reserve), on the Santa Catarina coast, the main threat to Cabot's Terns is predation by Kelp Gulls (Branco 2003). Kelp Gull predation on eggs and nestlings is well-known in June and July on Deserta island, and has forced birds to abandon the colony (Branco 2004).

Kelp Gulls are widely distributed in the Southern Hemisphere, breeding in South America, southern Africa, Australia, New Zealand, on sub-Antarctic islands, and on the Antarctic Peninsula (Burger and Gochfeld 1996). In Brazil, Kelp Gulls breed from the coast of Santa Catarina north to the coast of Rio de Janeiro, rarely reaching the Espírito Santo coast. In fact, the absence of Kelp Gulls, in addition to conservation activities and abundant food, is considered to be responsible for the reproductive success of colonies on the Espírito Santo coast.

It is important to evaluate the potential of the national population to be self-sustaining or if it dependents on immigration for its long-term survival. However, during the past 20 years, only a few studies have been conducted on important topics such as basic reproductive biology and population dynamics of this species (Shealer 1999). For example, a study from 1993 to 1997 showed an annual population growth of 1.051% and an intrinsic rate of population growth, **r**, of 0.199 in Escalvada island, in Brazil (Efe *et al*. 2005), showing a low capacity of self-sustaining and some immigration dependence.

Environmental conditions in the country for establishment of immigrants and probabilities for recolonization

The distribution of Cabot's Tern in Brazil was poorly known until 1963, when breeding colonies were first identified (Sick and Leão 1965). Cabot's Terns have a limited breeding distribution restricted to small coastal islands that are vulnerable to unpredictable environmental conditions. The breeding distribution of the Cabot's Tern is highly fragmented in some areas, and the species is known to breed at no more than ten truly representative locations (p.e. Espírito Santo colonies). Continuing declines have been documented in the quality of habitats, the total area of occupancy, and the total number of locations or subpopulations.

The majority of the Brazilian population is restricted to the Espírito Santo coast, on the islands of Escalvada, Itatiaia, and Branca (Table 1). In addition, several smaller colonies exist that are used by different subpopulations; population estimates of these colonies rarely surpass a few hundred individuals. From the six islands that Cabot's Terns nest on the Espírito Santo coast (500 km), four are protected by the Andorinhas do Mar Project; however, reproductive success has been low on two of them for several years. On the Rio de Janeiro (635 km), São Paulo (390 km), Paraná (107 km), and Santa Catarina coasts (670 km), small breeding colonies are threatened by human intervention and by Kelp Gull predation. They feed in areas that are heavily used by fishermen and contaminated by marine pollutants. All these factors turn more and more difficult to maintain the necessary prerequisites for the immigrants' establishment in the available areas in the country.

Evaluation and suggestion of status

As a comparison, the estimated population of the Elegant Tern (*T. elegans*) is between 51,000 and 90,000 individuals; 95% of these breed on Isla Rasa in the Gulf of California, and small populations breed on other islands. IUCN classifies this species as Near Threatened, and population fluctuations are considerably less than one order of magnitude (BirdLife International 2008). The

total population of *Sterna balaenarum* (Near Threatened) was estimated at 14,000 birds, and its breeding colonies suffer considerable human disturbance (BirdLife International 2008).

The population of Sandwich Tern (*T. sandvicensis*) in Great Britain is 14,000 pairs with an additional 4,400 pairs in Ireland (Ratcliffe et al. 2000). The continental European breeding population is between 82,000 and 130,000 pairs, and underwent a moderate decline between 1970–1990 (BirdLife International 2004). The UK Government's wildlife adviser (JNCC) considers the conservation status of *T. sandvicensis* to be precarious, and recommends general protection of breeding grounds. The IUCN currently classifies this species as Least Concern; however it includes the data of the American populations, which now must be deducted and transferred to *T. acuflavidus*.

Although for one side the estimated extent of occurrence and area of occupancy in Brazil are larger than the necessary limits for inclusion in the criteria for Critically Endangered, Endangered or Vulnerable, for the other the serious trends that have been quantified, the estimated population around 16,000 individuals, and, the alarming and recent extirpation of the Cabot's Tern in several areas of the Brazilian coast, all suggest that the Brazilian population may be in greater danger in the foreseeable future. For these reasons we suggested that Cabot's Tern does merit classification in category VULNERABLE at the national level and may qualify as ENDANGERED at the state level.

Regional lists of conservation status directly reflect the status of local populations, and can be used to suggest necessary conservation measures for specific situations (Lins *et al.* 1997). Therefore, we suggest that conservation efforts be increased in the research and conservation programs in Espírito Santo coast, and that conservation actions should be implemented across the entire Brazilian coast.

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CONSIDERAÇÕES FINAIS

Todos os anos milhares de aves de *Thalasseus sandvicensis eurygnathus* e de *Sterna hirundinacea* usam as ilhas costeiras do litoral sul do Espírito Santo para reproduzirem, entre os meses de maio e setembro. Além da compilação das informações já publicadas pelo autor sobre a ecologia reprodutiva do trinta-réis-de-bando, bem como das outras espécies de aves marinhas que ocorrem no litoral do Espírito Santo, dados inéditos apresentados aqui possibilitaram a identificação e descrição dos principais eventos comportamentais relacionadas à côrte do trinta-réis-de-bando. As ilhas costeiras do litoral sul do Espírito Santo, ao longo dos anos vinham sofrendo uma enorme degradação de seus ecossistemas por estarem próximas ao continente urbanizado e receberem visitas periódicas de pescadores e turistas que ateavam fogo à vegetação insular. As atividades visando à proteção dos sítios de reprodução foram importantes como iniciativa de conservação dessas espécies e funcionam como catalisador de uma postura conservacionista, que vem contribuindo para a melhoria da qualidade de vida das populações litorâneas, pois a presença de colônias de reprodução de aves migratórias em ilhas próximas a costa, é um evidente indicador biológico das condições de conservação dos ecossistemas costeiros no estado do Espírito Santo.

Em 13 localidades, a maioria localizada na Argentina, o trinta-réis-de-bando e o trinta-réis-real nidificam em associação, muitas vezes com os seus ninhos entremeados. A população total do trinta-réis-real foi estimada em 750 pares no Brasil e menos de 5.000 na Argentina, enquanto que a população total do trinta-réis-de-bando foi estimada em 8.000 pares no Brasil e menos de 10.000, na Argentina. As principais ameaças enfrentadas pelas respectivas populações, em ambos os países são a perturbação por humanos, a predação por parte do gaivotão, *Larus dominicanus*, a pesca predatória, a coleta de ovos e a introdução de predadores.

Nossos dados corroboram a monofilia do gênero *Thalasseus* e indicam que a população européia e as americanas de *Thalasseus sandvicensis* são altamente divergentes e se agrupam em clados filogenéticamente distintos dentro do gênero *Thalasseus*. Estes resultados sugerem fortemente que o atual arranjo taxonômico do complexo *T. sandvicensis / acuflavidus / eurygnathus* como uma única espécie ou como uma espécie no hemisfério norte (*T. sandvicensis*) e outra no hemisfério sul (*T. eurygnathus*) são inapropriados, demandando um novo arranjo no qual as populações européias e as americanas sejam consideradas como duas espécies diferentes. Nós propomos, portanto a validação do tratamento do complexo *acuflavidus / eurygnathus* como um táxon nominado *Thalasseus acuflavidus*.

No estudo da variabilidade genética do trinta-réis-de-bando nas Américas a partir de seqüências nucleotídicas de DNA mitocondrial e nuclear, além de microsatélites verificou-se que a diversidade do MtDNA é baixa na espécie como um todo, com a população dos EUA sendo divergente das demais populações da Argentina e Brasil, as quais não são diferenciadas. Todas as três populações apresentam sinais de efeito gargalo e expansão populacional, com as populações sulamericanas apresentando uma expansão a cerca de 30.000 anos atrás. Por outro lado, dados de microsatélites sugerem um forte e recente fluxo gênico entre as populações do Brasil e EUA e, um baixo fluxo entre as populações do Brasil e Argentina. Estes resultados sugerem a ocorrência de uma zona de hibridização entre o Brasil e a América do Norte. A diferença entre os períodos

reprodutivos no Brasil e Argentina pode ser importante no recente isolamento destas aves costeiras, sugerindo que a alocronia pode ser um processo negligenciado na formação da biodiversidade Neotropical.

Na avaliação do estado de conservação da espécie após a revisão de vários parâmetros incluindo o nível taxonômico, as principais ameaças, a área e a extensão de ocorrência e o atual tamanho populacional, recomendamos que a espécie seja categorizada como Vulnerável no nível nacional e como Ameaçada no nível regional. Finalmente sugerimos que esforços de pesquisa e conservação sejam ampliados na costa do Espírito Santo e que ações semelhantes de conservação sejam implementadas ao longo da costa brasileira.

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