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História natural de *Benthana cairensis* (Isopoda: Oniscidea)

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Tese de doutorado aprovada em ________________________

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Prof. Dra. Paula Beatriz de Araujo

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"...como teriam elas por fim se transformado

em espécies distintas, genuínas (...)? Como

teriam surgido esses grupos que constituem

os 'gêneros distintos' (...)? Tudo isso, decorre

inevitavelmente da luta pela sobrevivência..."

Charles Darwin (Origem das Espécies)

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Resumo

A subordem Oniscidea abriga os isópodos terrestres, os quais apresentam uma grande riqueza de espécies em diferentes ambientes com características completamente diversas, desde a zona litorânea até ambientes desérticos. No Brasil há uma diversidade de espécies ainda pouco estudada, sendo que a família Philosciidae representa uma grande parte da fauna de isópodos da América do Sul. O presente estudo tem como objetivos descrever uma nova espécie no gênero *Benthana* para o estado do Rio Grande do Sul, descrever seus estágios de manca e juvenil, assim como caracterizar sua estrutura populacional com respeito aos aspectos reprodutivos e crescimento. *Benthana cairensis* foi descrita como uma nova espécie de Philosciidae e tem como caracteres diagnósticos a presença de 17 estetascos na antênula, exópodo do pleópodo 1 do macho alongado apresentando um lobo na margem lateral interna. Essa espécie é semelhante a três outras do gênero em relação a presença do lobo, no entanto diferencia-se de todas elas devido às diferenças no número de omatídeos, inserção dos ramos do urópodo e dimorfismo sexual nos pereiópodos dos machos. A fase imatura e indiferenciada sexualmente apresenta três estágios, chamados de mancas. O estágio de Manca I é caracterizado pela simplicidade de suas estruturas e pela sua rápida duração, de aproximadamente 4 horas; apresenta 6 pares de pereiópodos, esses ainda glabros e o aparelho bucal fracamente desenvolvido ainda sem a presença dos dentes pectinados na maxilula, característicos de *Benthana*. O estágio seguinte, de Manca II, já apresenta o aparelho bucal mais desenvolvido com os dentes pectinados da maxilula e pereiópodos já com o padrão de setas semelhante ao adulto, inclusive a seta "hand-like" do carpo 1, uma autapomorfia do gênero. O último estágio de manca (Manca III) caracteriza-se pela presença do sétimo par de pereiópodos presente dobrado ventralmente sob o corpo do animal. Os estágios de juvenis caracterizam-se pela diferenciação sexual, mas ainda são imaturos sexualmente. Os três primeiros estágios foram descritos para os machos com destaque para o desenvolvimento da protusão do exópodo do pleópodo 1, outra autapomorfia do gênero, o qual só começa a ficar evidente no segundo estágio (JUII) e somente apresenta-se completamente desenvolvido quando o animal atinge aproximadamente 1.0 mm de largura de cefalotórax (LC). O dimorfismo sexual presente nos quatro primeiros pares de pereiópodos dos machos começa a aparecer após os três primeiros estágios de juvenil e está completamente evidente em machos de aproximadamente 1.2 mm de LC. A população de *Benthana cairensis* do Sítio Cairé caracteriza-se por apresentar uma reprodução contínua durante o ano. As fêmeas investem menos em uma única prole, no entanto são capazes de se reproduzir mais de uma vez ao longo de sua vida. A proporção sexual operacional da população é de 1:1, mostrando que há um equilíbrio no que se refere ao número de machos e fêmeas aptos para reprodução. Os machos vivem menos que as fêmeas e são menores. As fêmeas possuem o corpo maior, o que aumenta a superfície para abrigar a prole o que foi demonstrado pela correlação positiva do tamanho da fêmea com o número de ovos. As características de desenvolvimento de *B. cairensis* são semelhantes a outras espécies de Philosciidae, apresentando três estágios de mancas. Suas características populacionais, no que se referem a estação reprodutiva e investimento reprodutivo, são semelhantes a outras espécies subtropicais e, como esperado, diferentes de espécies de isópodos que vivem em regiões temperadas.

Palavras chave: Isópodo neotropical, estágios de manca, caracteres sexuais secundários, estrutura populacional.

Abstract

Terrestrial isopods are included in the suborder Oniscidea which shows a great species richness living at different places showing diverse environmental conditions, occurring from the littoral zone through desert regions. In Brazil there is a diversity that is still poorly studied in which the family Philosciidae represents a great part of South America's woodlice fauna. The present study aims to describe a new species of the genus *Benthana* to the state of Rio Grande do Sul, to describe its manca and juvenile stages as well as to characterize its population structure concerning reproductive aspects and growth. *Benthana cairensis* was described as a new species of Philosciidae and presents the following diagnostic characters: 17 aesthetascs on the antennula and male pleopod 1 exopod elongated with a lobe on the inner lateral margin. This species resembles other three species of the genus concerning the presence of pleopod lobe; however it is differentiated by the number of omatidia, insertion of uropod endopod and exopod and sexual dimorphism on male's pereiopods. The immature and undifferentiated phase has three stages, called mancas. The Manca I is marked by the simplicity of its appendages and by its queek duration, of about 4 hours; it presents 6 pairs of pereiopods still glabrous and mouth parts weekly developed still without the presence of the pectinate teeth of maxillula, characteristic of *Benthana*. The next stage, Manca II, already shows the mouth parts a little more complete, presenting the pectinate teeth of maxillula and the pereiopods showing the setae pattern of the adult, including the hand-like seta on carpus 1, an autapomorphy of the genus. The last manca stage (Manca III) is characterized by the presence of the 7th pair of pereiopods folded ventrally on the pereion. The juvenile stages are sexually differentiated, but still immature. The first three stages were described for males, enphatizing the development of dentiform protusion of pleopod exopod 1, another autapomorphy of the genus which begins to be evident only at the second stage (JUII) and it is completely formed when the animal reaches 1.0 mm of cephalothorax width (CW). Sexual dimorphism at the first four male pereiopods beggins to develop after the three juvenile stages and it is completely formed on males of approximatly 1.2 mm of CW. The population of *Benthana cairensis* at Sítio Cairé is characterized by showing a continnuous reproduction during the year. Females invest less in a single brood, but are able to reproduce more than once in its lifetime. The operational sex ratio of population is 1:1, which shows that there is an equillibrium concerning the number of males and females that are able to reproduce. Males live less than females and are smaller. Females have a bigger body which increases the surface where the brood develops; this was demonstrated by the positive correlation of female body size with the offspring number. The developmental characteristics of *B. cairensis* are similar to those of the other species of Philosciidae presenting three manca stages. Its population features concerning reproductive season and reproductive effort resembles those of other subtropical species and as expected, is different from species that occur at temperate regions.

Key words: Neotropical isopod, manca stages, secondary sexual characters, population structure.

Prefácio

O estudo de uma espécie recém descrita, que ainda não possui nenhum aspecto de sua biologia estudado abre a possibilidade de um leque de opções para possíveis pesquisas, mas também surgem alguns problemas. A fim de conhecer a estrutura populacional de uma espécie é necessário identificar todas as fases de vida do animal em questão, como por exemplo, diferenças morfológicas entre a fase imatura e o adulto. Por se tratar de uma espécie ainda não estudada, a espécie de isópodo terrestre objeto de estudo da presente tese além de ter sido descrita, foi preciso conhecer sua morfologia e desenvolvimento de suas fases imaturas e juvenis para que o trabalho de estrutura populacional fosse possível.

A tese é composta de quatro capítulos apresentados na forma de artigos científicos, cada um redigido de acordo com as normas dos respectivos periódicos os quais foram ou serão submetidos. Anteriormente, na seção "Introdução Geral", é incluída uma revisão sobre os principais assuntos tratados nos artigos subsequentes, abordando questões e esplanações que não cabiam em artigos científicos. Na seção "Material e Métodos" há uma explicação geral dos métodos de coleta, cultivo e preparação de animais para análises comuns para todos os capítulos.

O primeiro capítulo traz a descrição da espécie *Benthana cairensis*, artigo o qual já foi publicado, o que foi crucial para dar continuidade aos estudos com essa espécie. O capítulo II trata da descrição dos estágios indiferenciados sexualmente, os seja, os estágios de manca. Nesse artigo, o qual também já foi publicado, foram estudadas as principais características morfológicas que diferenciam os estágios e também a constatação de que algumas estruturas que tornam possível o reconhecimento genérico, como os dentes pectinados da maxílula, já estão presentes, assim como algumas estruturas ainda não estão completamente formadas, como o exópodo do pleópodo 1 e dimorfismo sexual nos pereiópodos dos machos. No capítulo III é realizada a descrição e ontogenia dos caracteres sexuais secundários dos machos. Com esse estudo foi possível caracterizar o aparecimento da papila genital no primeiro estágio de juvenil e foi essencial para a diferenciação de machos e fêmeas juvenis no trabalho de estrutura populacional. Nesse artigo também foi identificado o início do aparecimento de outros caracteres sexuais secundários específicos.

O último capítulo aborda a caracterização populacional de *B. cairensis*, trazendo informações sobre período reprodutivo, investimento reprodutivo, crescimento populacional, entre outras características de sua história de vida. Finalmente, comentários e conclusões gerais dos resultados obtidos estão explanados na seção "Considerações finais".

Introdução Geral

Os isópodos terrestres

Dentre as mais de 160 mil espécies de crustáceos há uma imensa diversidade de formas e hábitos de vida, desde os conhecidos camarões e caranguejos até os diminutos membros de classes menos populares como Remipedia e Cephalocarida (CHEN *et al.*, 2001, BUCKUP & BOND-BUCKUP, 1999). A superordem Peracarida abriga os únicos dois grupos de crustáceos que são totalmente independentes do meio aquático, apresentando um modo de vida inteiramente terrestre: os Amphipoda e Isopoda, ambos apresentando também espécies aquáticas (BUCKUP & BOND-BUCKUP, 1999). Os isópodos terrestres, todos incluídos na subordem Oniscidea, apresentam uma gama de características que os habilitam sobreviver no ambiente terrestre e com 3600 espécies descritas (*sensu* SCHMALFUSS 2003), constituem o grupo com a maior riqueza de espécies entre os crustáceos.

As mudanças sofridas nesse grupo em relação aos seus ancentrais aquáticos incluem adaptações morfológicas, fisiológicas e comportamentais. Uma das grandes alterações acontenceu em relação à respiração, uma vez que seus ancestrais aquáticos respiravam através de brânquias. Nos isópodos terrestres essas mudanças vão desde a realização das trocas gasosas por meio da superfície corporal até a presença de órgãos altamente especializados na obtenção de oxigênio, os pulmões pleopodais (EDNEY 1968, ARAUJO 1994, LEISTIKOW & ARAUJO 2001). Apesar de viverem na terra, esses animais são sensíveis à perda de água. Nesse sentido, desenvolveram adaptações que evitam sua perda em excesso, como a excreção em forma de amônia gasosa, presença de um sistema capilar que auxilia a absorção de água do solo ou a eliminação do excesso da mesma. Por outro lado, a água também pode ser bebida, absorvida através do alimento e também obtida através da coprofagia (WARBURB 1989, SUTTON 1980, ARAUJO 1994).

Adaptações comportamentais também fazem parte das estratégias que evitam a perda de água. Os isópodos terrestres possuem a capacidade de se orientar por um estímulo, como em direção contrária à luz ou a procura de locais com maior concentração de umidade, fenômeno conhecido como hidrocinese (WARBURG 1968, SUTTON 1980, BARKER *et al.* 1998). Uma característica marcante desses animais que está relacionada com a retenção de umidade é a agregação; através do estímulo de feromônios os animais formam um grupo e permanecem dessa maneira; sabe-se que esse fenômeno tem grande importância biológica para os isópodos terrestres (TAKEDA 1984).

Todas essas características relativas às adaptações para o meio terrestre são mais ou menos especializadas nas diversas espécies de Oniscidea, sendo que, geralmente, espécies mais litorâneas apresentam tais adaptações com menos intensidade do que espécies que vivem em ambientes desérticos, como *Hemilepistus reaumurii* (Milne-Edwards, 1840) o qual vive em regiões desérticas do leste da Argélia até a Síria ocidental e possui características comportamentais peculiares que lhe permitem viver em tais ambientes (HOFFMANN 1989, SCHMALFUSS 2003).

Uma característica morfológica marcante que está presente em todos os Peracarida é a presença de um marsúpio onde ocorre o desenvolvimento embrionário; nos isópodos terrestres o marsúpio é diferenciado dos outros membros da superordem pelo seu completo isolamento do meio externo e pela presença dos cotilédones, estruturas exclusivas de Oniscidea que possuem um epitélio de transporte que provavelmente atua na nutrição e provê água e oxigênio para os jovens em desenvolvimento (HOESE & JANSSEN 1989, HOESE 1984). Essa estrutura única dos isópodos terrestres lhes permitiu ficar totalmente independentes do meio aquático no que se refere à reprodução (HOESE 1984).

Desenvolvimento

Os isópodos terrestres são dióicos e, com excessão de algumas poucas espécies, como *Trichoniscus pusillus* Brandt, 1833 que é partenogenética (SUTTON 1968, STANDEN 1973), as fêmeas passam por um período de receptividade durante a intermuda e alternam fases de reprodução sazonal com períodos de estase reprodutiva (MOCQUARD *et al.* 1989, ZIMMER 2001). A intermuda é o período no qual acontece a cópula e em seguida acontece a muda parturial, quando há a formação dos oostegitos e as fêmeas ficam ovígeras. As fêmeas podem guardar esperma de diversos machos e/ou ter mais de uma prole através da mesma cópula, pois possuem dois órgãos de reserva espermática, o oviduto e um receptáculo seminal ao final do oviduto (MOREAU *et al.* 2002, SUZUKI & ZIEGLER, 2005). Devido à presença dessa estrutura há registros de fêmeas que após a liberação das mancas e a realização da muda pós parturial ficam ovígeras novamente sem ter contato com machos (MOREAU *et al.* 2002, ARAUJO & BOND-BUCKUP 2005).

O desenvolvimento em Peracarida é direto. Após a fertilização os ovos passam através do oviduto para o marsúpio onde ficam até o final do desenvolvimento e então são liberados na forma de mancas, como acontece em Isopoda e Tanaidacea ou na forma de juvenis nas demais ordens. Nos isópodos terrestres no primeiro estágio os ovos são uma massa globular de vitelo protegidos por uma fina membrana (SUTTON 1980, SURBIDA & WRIGHT 2001). A medida que o desenvolvimento prosegue o ovo passa por uma mudança de forma quando é possível visualizar a segmentação do embrião, apartir dessa fase os embriões já apresentam indícios dos apêndices e dos olhos, mas ainda são involtos na membrana vitelina (SUTTON 1980, OUYANG & WRIGHT 2005). Ao final do período intramarsupial o embrião já se movimenta do interior do marsúpio, o que causa a ruptura da membrana que os involve, então as mancas permanecem ainda por alguns dias no interior do marsúpio antes de serem totalmente exteriorizadas (SUTTON 1980, OUYANG & WRIGHT 2005).

O período de desenvolvimento intramarsupial varia dentre as espécies e até em uma mesma espécie. Há registros de um período de 27-79 dias para *Porcellio dilatatus* Brandt, 1833 (HEELEY 1941, LOYOLA E SILVA & CORAIOLA 1999), entre 16 e 26 dias para *Porcellio scaber* Latreille, 1804 (SUTTON 1980), uma média de 31 dias em *Philoscia muscorum* Scopoli, 1793 (SUDERLAND *et al.* 1976) e em média 23 dias para *Atlantoscia floridana* (van Name, 1940) (ARAUJO & BOND-BUCKUP 2005).

As mancas são caracterizadas pela indiferenciação sexual e ausência do sétimo par de pereiópodos funcionais (HOLDICH *et al.* 1984). Geralmente essa fase possui três estágios, sendo que no terceiro já é possivel a visualização do sétimo par de pereiópodos dobrado ventralmente sob o corpo do indivíduo, no entanto, ainda não funcional (HEELEY 1941; 1942, HADDAD 1982, SOKOLOWICZ & ARAUJO 2008). O estágio seguinte, os juvenis, caracteriza-se pela completa formação dos apêndices locomotores, no entando os indivíduos ainda são imaturos sexualmente, nesse estágio inicia-se a diferenciação sexual e aparecem os caracteres sexuais secundários, evidenciados principalmente por diferenças morfológicas nos dois primeiros pares de pleópodos (TOMESCU & CRACIUN 1987, ARAUJO *et al.* 2004).

O número de estágios compreendendo a fase juvenil é mais variável e possui um período mais longo do que o estágio de manca, o final é caracterizado pelo total desenvolvimento das características sexuais secundárias e pela maturidade sexual. Nos machos os caracteres sexuais secundários compreendem o aparato reprodutor, constituído pela papila genital e endópodos dos pleópodos 1 e 2 modificados (SUTTON 1980), e na maioria das espécies por uma forma diferenciada do exópodo do pleópodo 1, pelo padrão de setas diferencial dos pereiópodos e diferenças no comprimento dos exópodos dos urópodos (TOMESCU & CRACIUN 1987, TOMESCU 1976, LEMOS DE CASTRO 1958, ARAUJO & LOPES 2003, SOKOLOWICZ *et al.* 2008). As fêmeas não possuem estruturas que apresentem uma mudança tão conspícua durante o desenvolvimento como os machos; o aparecimento dos oostegitos do marsúpio está ligado à muda puberal e essas estruturas não estão sempre presentes (HAAHTELA 1978), de forma que o momento exato da primeira muda onde aparecem os oostegitos só pode ser defino em acompanhamento de fêmeas em laboratório

Características de história de vida

Dentre os diversos assuntos tratados na literatura existente sobre os isópodos terrestres, as características de história de vida constituem um tema bastante estudado (WILLOWS 1987, MA *et al.* 1991, QUADROS *et al.* 2009 para revisão). A seleção natural, "fitness", adaptação e os "constrains" moldam o aparecimento dessas características na população e suas plasticidades determinam a dinâmica populacional de espécies (STEARNS 1992). Dentre essas características, as principais são: tamanho ao nascer, padrão de crescimento, idade e tamanho da maturidade, número, tamanho e razão sexual dos juvenis, investimento reprodutivo em idade e tamanhos específicos, idade e tamanho específico ao morrer e tempo de vida (STEARNS 1992).

O benefício adquirido com o melhor uso dessas características é revertido em "fitness", representado pelo número de descendentes deixados (BEGON *et al.* 2006). No entanto, há "trade-offs" que representam um custo quando uma mudança benéfica em uma característica de história de vida acontece em detrimento de outra (STEARNS 1989).

Duas características de história de vida são amplamente estudadas e são um ótimo exemplo de um "trade-off": reprodução x crescimento. O investimento na reprodução pode reduzir a quantidade de energia direcionada ao crescimento e, consequentemente, pode comprometer o sucesso reprodutivo futuro já que o tamanho da prole está intimamente ligado ao tamanho da fêmea (STEARNS 1992). Espécies que se reproduzem repetidamente durante sua vida conseguem distribuir a energia tanto para a reprodução como para atividades somáticas (BRODY *et al.* 1983), no entanto, segundo LAWLOR (1976) fêmeas reprodutivas têm a mesma disponibilidade de energia para investir no crescimento e reprodução que as fêmeas não reprodutivas que investem apenas no crescimento. Com isso, a decisão de qual estratégia reprodutiva é a mais lucrativa deve ser tomada considerando que investir muito na reprodução atual pode compromenter as reproduções futuras através da redução de fecundidade e/ou sobrevivência (LAWLOR 1976).

Há duas estratégias reprodutivas presentes em populações de isópodos terrestres, uma na qual as fêmeas após atingir a maturidade intercalam eventos reprodutivos com crescimento, podendo produzir mais de uma prole em uma estação reprodutiva (fêmeas iteróparas) e outra onde as fêmeas terão apenas um evento reprodutivo por estação (VINK & KURNIAWATI 1996); as vezes somente um durante todo seu tempo de vida, essas últimas chamadas de semélparas (AL-DABBAGH & BLOCK 1981, WARBUG & COHEN 1992). Ambas as estratégias devem permitir a máxima sobreviência da prole nas condições atuais em que a população está inserida (CALOW 1973).

Philosciidae

Oniscidea é considerado um grupo monofilético cujas apomorfias reconhecidas encontram-se na musculatura do abdômen, na configuração do aparelho copulatório dos machos, marsúpio, aparelho bucal e estômago (SCHMIDT 2008). Segundo SCHMIDT (2008), a Subordem Oniscidea é dividida em cinco linhagens: Ligiidae, Tylidae, Mesoniscidae, Synocheta e Crinocheta; esta última com cerca de 2500 espécies, representando aproximadamente 80% da diversidade de Oniscidea.

Dentre as famílias de Crinocheta, Philosciidae é considerada a mais basal e o mais importante grupo em ambientes tropicais, constituíndo, na América do Sul, 1/5 das espécies de isópodos terrestres descritas (LEISTIKOW & WAGELE 1999, LEISTIKOW 2001). Atualmente sabe-se que essa família não é um agrupamento monofilético, com suas espécies reunidas principlamente pelo hábito de vida similar e não por caracteres compartilhados (LEISTIKOW 2001). Após uma análise filogenética de vários gêneros da América do Sul, LEISTIKOW (2001) agrupou os membros de Philosciidae dessa região em vários táxons onde o gênero *Benthana* forma um grupo monofilético com *Atlantoscia* e membros de Rhyscotidae. Esse agrupamento em táxons diferentes demonstrou a parafilia de Philosciidae.

Gênero Benthana Budde-Lund, 1908

O gênero *Benthana* compreende um grupo endêmico da América do Sul tropical e subtropical. São conhecidas 24 espécies, 20 dessas no Brasil com 6 no estado do Rio Grande do Sul (LEMOS DE CASTRO 1958, LEISTIKOW & WAGELE 1999, ARAUJO & LOPES 2003)

Os estudos sobre o gênero no Brasil iniciaram-se com o trabalho de LEMOS DE CASTRO (1958), que fez uma revisão do gênero e descreveu sete novas espécies para o país; mais tarde descreveu outras duas (LEMOS DE CASTRO 1985). As autapomorfias do gênero incluem a forma codiforme do exópodo do pleópodo 1 portando uma protusão lateral dentiforme (fig. 1a) e o espinho lateral ornamental no carpo do pereiópodo 1 com um ápice "hand-like", unido à haste cuticular (fig. 1c) (LEISTIKOW 2001). O dente pectinado na maxilulla (fig. 1b) que é uma característica também marcante do gênero não é uma autapomorfia de *Benthana*, é um caracter também compartilhado com *Ctenoscia* Verhoeff, 1928; *Anaphiloscia* Racovitza, 1907 (Sul da Europa); *Benthanops* Barnard, 1932 (África do Sul); *Benthanoides* e *Benthanoscia* (sul da América do Sul) e *Alboscia* Schultz, 1995 (Paraguai e Brasil) e é considerado um caracter plesiomófico de Crinocheta (LEISTIKOW & ARAUJO 2006).

De acordo com uma revisão de *Benthana* realizada por ARAUJO & LEISTIKOW (1999) a diagnose no gênero é a seguinte:

"Cefalotórax com linha supra antenal; linha frontal e lâmina frontal ausentes, lobos laterais pouco proeminentes, omatídeos dos olhos compostos arranjados em quatro fileiras. Antênula com artículo distal coniforme com grupos de estetascos em diferentes níveis de inserção, antena com flagelo tri articulado, órgão apical muito menor do que o artículo distal, sensilas livres tão longas quanto as lamelas cuticulares. Penicílio molar dicotomizado, maxilula com endito mediano sem a ponta apical, endito lateral com 4+6 dentes mais uma aste adicional, cinco dentes internos pectinados, maxila com lobo lateral duas vezes mais largo do que o endito mediano, base do maxilípodo com sulco lateral, endito sem penicílio "knob-like", tufo da seta proximal do palpo com duas setas. Pereiópodos alongados, carpo 1 com pincel de limpeza da antena transverso, dáctilo com o gancho interno longo, seta do dáctilo simples e pequena, placas coxais com nódulos laterais e sulco marginal, sem poros glandulares. Pleópodos com área respiratória no exópodo 1, exópodo do macho triangular com uma protusão subapical lateralmente direcionada na maioria das espécies. Urópodo com endópodo inserido proximalmente ao exópodo.”

Benthana cairensis Sokolowicz, Araujo & Boelter, 2008

A espécie que é objeto de estudo dessa tese foi a sexta descrita para o estado do Rio Grande do Sul e assemelha-se a três outras espécies do Brasil: *Benthana serrana* Araujo & Lopes, 2003, *Benthana taeniata* Araujo & Buckup, 1994 e *Benthana olfersii* (Brandt, 1833). Além de diferenças morfológicas como número de omatídeos, inserção dos ramos do urópodo e padrão de setas do pereiópodo 7 (SOKOLOWICZ *et al.* 2008), essas espécies diferem também geograficamente; *B. serrana* ocorre na depressão da Serra Geral no Rio Grande do Sul (ARAUJO & LOPES 2003). *B. taeniata*, descrita originalmente para o Rio Grande do Sul e Santa Catarina, foi recentemente registrada no Rio Janeiro, o que aumenta a área de distribuição espacial e ecológica já que é dessa forma encontrada nas regiões tropical e subtropical (LEISTIKOW & ARAUJO 2006). A espécie mais semelhante é *B. olfersii*, com ocorrência em São Paulo e Rio de Janeiro e que recentemente foi incluída no subgênero *Benthanoscia* por apresentar urópodos sexualmente dimórficos (SOKOLOWICZ *et al.* 2008).

Apartir da descrição da espécie alguns trabalhos já foram realizados sobre sua biologia, como a descrição de números de cotilédones do marsúpio e sua preferência alimentar (APPEL 2008, BOELTER *et al.* 2009).

Objetivos

A presente tese teve como objetivo geral conhecer aspectos populacionais e o desenvolvimento pós marsupial de *Benthana cairensis*, mais especificamente:

- Descrever uma espécie nova do gênero *Benthana*.

- Acompanhar e descrever o desenvolvimento de mancas e juvenis;

- Caracterizar e acompanhar a ontogenia dos caracteres sexuais secundários de machos de *B. cairensis*.

- Descrever alguns padrões de história de vida, como tamanho ao nascer, idade e tamanho da primeira reprodução, padrão de crescimento, investimento reprodutivo e tempo de vida;

- Caracterizar a estrutura da população de *B. cairensis* do Sítio Cairé, em Taquara (RS).

Baseado em tais objetivos foram traçadas as seguintes hipóteses:

1) As características dos estágios de mancas ainda não evidenciarão caracteres sexuais secundários característicos da espécie;
2) A descrição do desenvolvimento do dimorfismo sexual presente do exópodo do pleópodo 1 e pereiópodos dos machos tornará possível identificar o início da maturidade sexual nos mesmos;
3) A analise da estrutura populacional e reprodução de *B. cairensis* fará possível a confirmação do padrão de reprodução característico dos isópodos subtropicais, reprodução contínua, não restrita á uma única estação do ano.

Material e Métodos

A espécie

Além da localidade tipo em Taquara (RS), *Benthana cairensis* (fig. 2) também foi registrada para o município de Sapiranga (RS); sua distribuição pode ser aumentada, o que depende de coletas na região de ocorrência da espécie para obter-se mais registros (fig. 3).

Umas das características peculiares dessa espécie é uma preferência apresentada por locais específicos utilizados como abrigo, principalmente fêmeas reprodutivas e mancas que concentram-se em grandes quantidades sob troncos de árvores, inclusive em seus orifícios (fig. 4).

Área de Estudo

As coletas foram realizadas no Sítio Cairé, no município de Taquara, RS (29 46'S - 50 53' O), em uma área de aproximadamente 1/2 hec. Inicialmente, o local era uma área de campo que, há aproximadamente 20 anos, sofreu o plantio de diversas espécies vegetais nativas do estado do Rio Grande do Sul, constituindo uma área de mata com espécies arbustivas e arbóreas (fig. 5).

Nessa localidade foi encontrado o espécime tipo de *B. cairensis.* Em uma coleta preliminar verificou-se os animais encontravam-se distribuídos por toda extensão da mata, tanto nos ambientes com maior acúmulo de serapilheira e madeira como em locais onde a mata é mais fechada e não há tanto acúmulo de serapilheira no solo.

Amostragens

Anteriormente ao início das coletas mensais foi realizado um projeto piloto a fim de definir a metodologia adequada para a captura dos animais. Foi feita uma avaliação do tamanho e acesso à área de coleta e disponibilidade de animais.

Os espécimes de Isopoda foram amostrados em uma área de 18 m de comprimento e 6 m de largura, dividida em 12 quadrantes de 3 x 3 m cada (fig. 6). Mensalmente, de março/2006 até agosto/2007, uma unidade amostral foi sorteada aleatoriamente em cada um dos 12 quadrantes para a coleta dos animais. O sorteio foi feito com um dado, cujo primeiro lance indicava o número de metros à frente e o segundo o número de metros para o lado (fig. 6). Um amostrador circular de ferro com diâmetro de 30 cm foi utilizado para demarcar a área de coleta (Fig. 7). Todo material contido na área do amostrador foi retirado e acondicionado em sacos plásticos, previamente identificados com a data da coleta e número do ponto.

Em cada uma das 12 unidades amostrais mensais foram também registradas a temperatura do solo (3 cm de profundidade) e do folhiço. Para a obtenção da quantidade de água, amostras do solo e folhiço foram coletadas e levadas para o laboratório. Para obter a quantidade de água foi utilizado o método de Allen (ALLEN 1984), onde as amostras do solo e do folhiço de cada ponto, separadamente, foram pesadas para obtenção do seu peso úmido e após 72 horas em estufa a 60°C obteve-se seu peso seco. O peso úmido dividido pelo peso seco e multiplicado por 100 dá o valor, em porcentagem, da quantidade de água no solo e no folhiço.

Análises em laboratório

Para a descrição de mancas e juvenis fêmeas ovígeras de *B. cairensis* foram coletadas e levadas ao laboratório onde foram mantidas em condições de cultivo (fotoperíodo 12:12 h; temperatura 20ºC ± 1ºC) até a exteriorização das mancas. Após o nascimento, as mancas foram individualizadas em placas de Petri com um fundo de gesso umedecido. Os animais eram alimentados com pedaços de folhas provenientes do local de coleta diariamente. Para o acompanhamento das mudas os animais foram monitorados diariamente; quando houve ecdise alguns exemplares foram fixados no alcóol 70% para a descrição do estágio. O mesmo procedimento foi realizado para a descrição dos juvenis.

Para o experimento de tempo de desenvolvimento intramarsupial, fêmeas e machos adultos foram mantidos em placas de Petri nas mesmas condições descritas acima. Cada casal foi observado diariamente quanto a presença de ovos no marsúpio das fêmeas; aquelas que se tornaram ovígeras foram separadas dos machos e mantidas individualmente até a eclosão das mancas.

Preparação para Microscopia Eletrônica de Varredura

Com a finalidade de uma melhor descrição morfológica, alguns exemplares foram submetidos à Microscopia Eletrônica de Varredura (MEV); a metodologia descrita a seguir foi utilizada para a descrição da espécie, mancas e juvenis.

Para o processo de desidratação do material os animais passaram for diferentes concentrações de etanol, 70%, 80%, 90% e 100%, permanecendo 30 minutos em cada concentração; então foram fixados em uma solução de etanol 50% e cetona 50% até o dia da secagem no Ponto Crítico.

No Centro de Microscopia Eletrônica (CEM) da UFRGS, os indivíduos passaram por um processo de secagem conhecido como Ponto Crítico, no qual todo o líquido é substituído por gás carbônico. Após esse processo os animais, ou apêndices, foram banhados em ouro e/ou carbono para a observação no microscópio eletrônico de varredura, onde as imagens foram realizadas.

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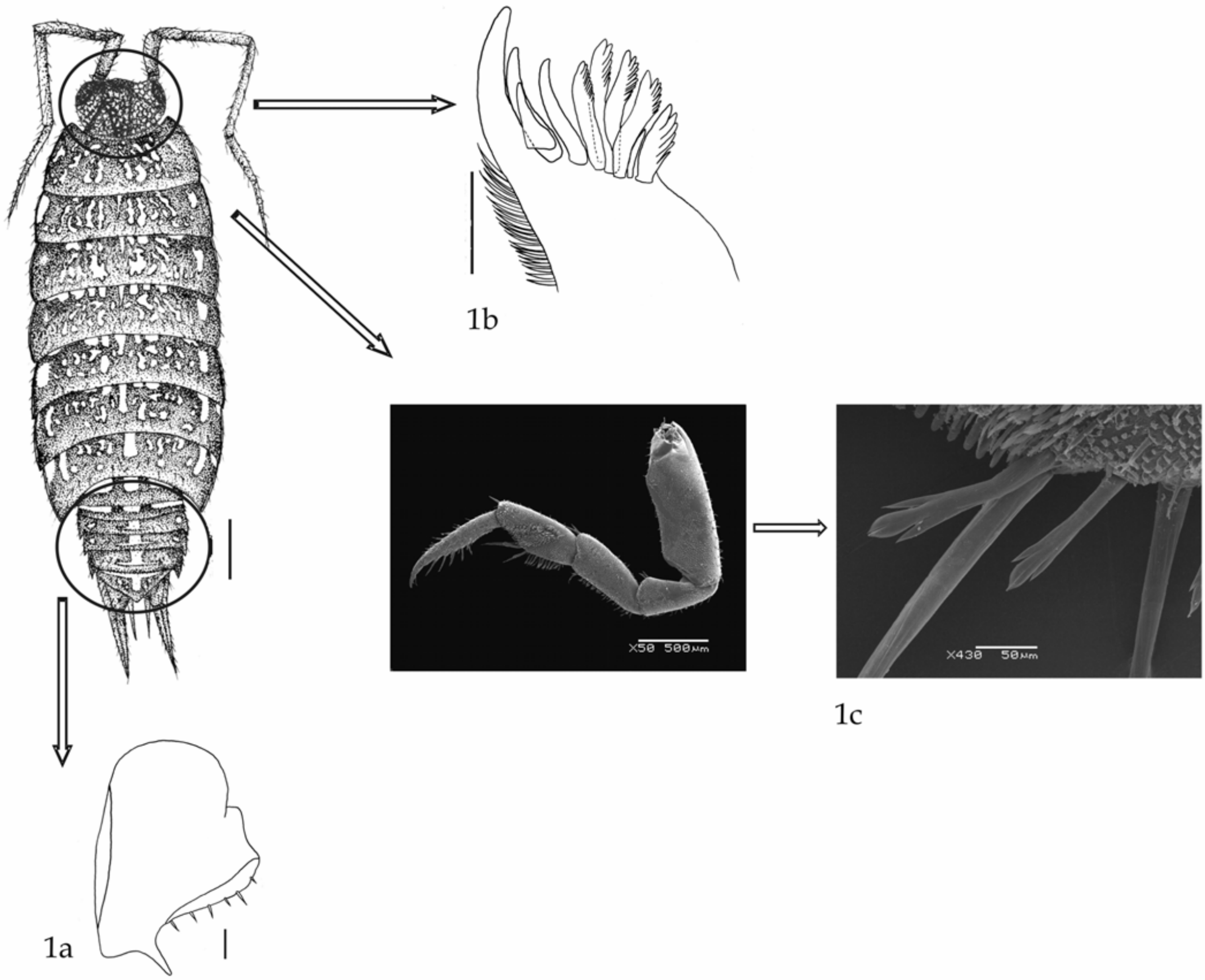

Figura 1: *Benthana cairensis*, macho, vista dorsal; escala 1.0 mm. (1a) exópodo do pleópodo 1, escala 0.1 mm; (1b) dente pectinado da maxilula, escala 0.1 mm; (1c) seta "hand-like" no carpo do pereiópodo 1, escala 50 µm.

Figura 2: *Benthana cairensis*. Escala 1.0 mm.

Figura 3: Mapa do Brasil e do estado do Rio Grande do Sul em detalhe mostrando a localização dos municípios de Taquara e Sapiranga, onde há registro de *Benthana cairensis*.

Figura 4: Tronco de madeira com orifícios onde abrigam-se os espécimes de *Benthana cairensis* (em detalhe).

Figura 5: Área de coleta no Sítio Cairé, Taquara (RS).

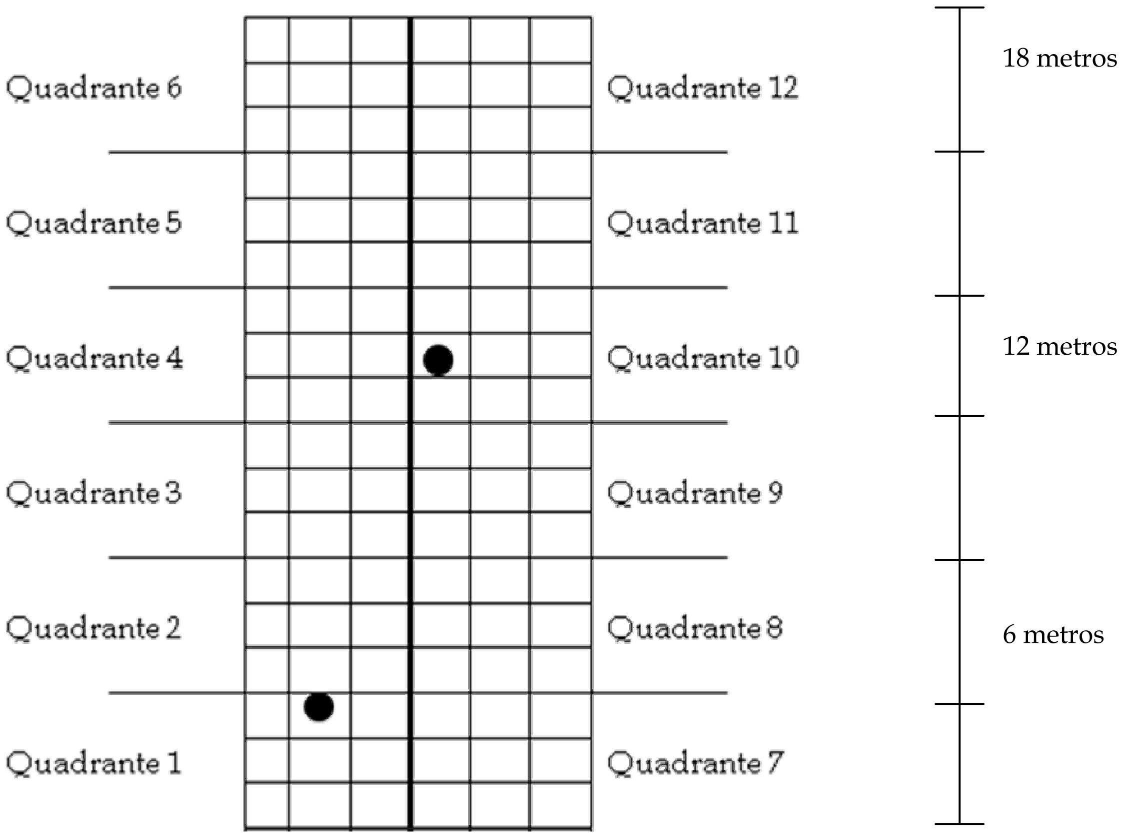

Figura 6: Desenho esquemático da área de coleta mostrando as 12 unidades amostrais e exemplo de pontos sorteados.

Figura 7: Amostrador circular utilizado para delimitar a área de coleta.

Capítulo I

"A new species of *Benthana* Budde-Lund (Crustacea: Isopoda: Philosciidae) from Brazil"

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Sokolowicz, C.C.; P.B. Araujo & J.F. Boelter. 2008. A new species of *Benthana* (Crustacea: Isopoda: Philosciidae) from southern Brazil. **Revista Brasileira de Zoologia 25**(2): 314-318.

A new species of *Benthana* Budde-Lund (Crustacea: Isopoda: Philosciidae) from Brazil.

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ABSTRACT. A new species of *Benthana* Budde-Lund, 1908 is described from southern Brazil. *Benthana cairensis* **sp. nov** is distinguished from the other species in the genus by the presence of the 17 aesthetascs on the antennula and the elongated male pleopod 1 exopod with a lobe on inner lateral margin and up to six setae on outer lateral margin. The new species most resembles *B. olfersii* (Brandt, 1833), but may be distinguished by lacking the lobe with six setae on the internal margin of the proximal extremity of the merus of pereiopod 7. Furthermore, we include *B. olfersii* in the subgenus *Benthanoscia* based on the examined material as described herein.

Key Words. Neotropical; Oniscidea; terrestrial isopod.

RESUMO. Uma nova espécie de *Benthana* Budde-Lund (Crustacea, Isopoda, Philosciidae) do Brasil. Uma nova espécie de *Benthana* Budde-Lund, 1908, é descrita de material coletado no sul do Brasil. *B. cairensis* **sp. nov.** distingue-se das outras espécies do gênero por apresentar 17 estetascos na antênula e o exópodo do pleópodo 1 do macho alongado, com um lobo e portando seis setas na margem lateral externa. A nova espécie assemelha-se a *B. olfersii* (Brandt, 1833) em várias características, mas pode ser diferenciada pela ausência do lobo com seis setas na margem externa da extremidade proximal do mero do pereiópodo 7. Além disso, *B. olfersii* é incluída, nesse trabalho, no subgênero *Benthanoscia*, com base em material examinado.

Palavras-Chave. Isópodo terrestre; neotropical; Oniscidea.

Nineteen species of *Benthana* Budde-Lund, 1908 (LEISTIKOW & WÄGELE 1999, ARAUJO & LOPES 2003) are registred to Brazil, the genus is distribuited in eastern tropical and subtropical South America (LEISTIKOW 2001). In the state of Rio Grande do Sul (RS), five species were recorded, *B. picta* Araujo and Buckup, 1994, *B. taeniata* Araujo and Buckup, 1994, *B. serrana* Araujo and Lopes, 2003, *B. trinodulata* Araujo and Lopes, 2003, and *B. araucariana* Araujo and Lopes, 2003. This genus is characterized by two autapomorphies, which are the cordiform shape of the male pleopod 1 exopod with a lateral dentiform protusion, and the robust distally dentate seta on the carpus of pereiopod 1, slightly merged with cuticular stalk (LEISTIKOW 2001, LEISTIKOW & ARAUJO 2006).

LEISTIKOW (2001) proposed a phylogeny of South American Philosciidae that he considered paraphyletic. *Benthana* is included as a sister group of *Atlantoscia* and Rhyscotidae, and these three taxa together form the new *Benthana*-group with the autapomorphy being the maxillipedal endite without setae, distal margin transverse.

LEMOS DE CASTRO (1958a) described *Benthanoscia longicaudata*, a new species and genus of philosciid from eastern Brazil that he did not include in *Benthana*. LEISTIKOW & ARAUJO (2006), however, proposed that *Benthanoscia* should be interpreted as a subgenus of *Benthana* with sexually dimorphic uropods as an autapomorphy.

Here, we describe a new species of *Benthana* based on material collected in the municipality of Taquara and Sapiranga, in eastern Rio Grande do Sul, southern Brazil.

MATERIAL AND METHODS

Pleopod 1 exopod shape in males (z:y ratio) was defined following the morphometric method of ARAUJO & LOPES (2003). Measurements of noduli lateralis were obtained as in VANDEL (1962). Drawings were prepared using a camera lucida; pereiopods were prepared for analyses on Scanning Electron Microscopy (SEM) following LEISTIKOW & ARAUJO (2001). The material examined of *Benthana olfersii* (Brandt, 1833) is MNRJ 8145 and MNRJ 8152.

The holotype and paratypes were deposited in the Museu Nacional (MNRJ), Universidade Federal do Rio de Janeiro, Rio de Janeiro; Museu de Zoologia (MZUSP), Universidade de São Paulo, São Paulo; and in the Crustacean Collection of the Department of Zoology, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre.

Benthana **Budde-Lund, 1908**

Type species: *Philoscia picta* Brandt, 1833

A diagnosis for the genus and a key to distinguish *Benthana* from the other Neotropical genera of Philosciidae are found in ARAUJO & LEISTIKOW (1999) and LEISTIKOW (2001), respectively.

Benthana cairensis **sp. nov.**

Figs 1- 27

Material: Holotype: male, BRAZIL, Rio Grande do Sul: Taquara (29°46'S, 50°50' W, Sítio Cairé), in leaf litter, 25.IV.2004 (MNRJ 20574). Paratypes: same locality as holotype, 1 male and 1 female, 26.II.07 (MZUSP 17362) and 5 males and 5 females on 26.II.07 (UFRGS 4399), 5 males and 5 females on 19.III.07

(UFRGS 4421) and 5 males and 5 females on 23.IV.07 (UFRGS 4422). Sapiranga, (29°37′52″S, 51°00′34″W), 8 males, 7 females, 29.XII.2001 (UFRGS 4471).

Diagnosis: Eye with 22-25 ommatidia, antennula with 15 + 2 aesthetascs; antenna, when extended posteriorly, reaches anterior margin of the fifth pereionite; lateral endite of maxillula with 4+6 setae, 5 of inner set pectinate, proximal teeth with 5+1 denticles. Male pleopod 1 exopod elongated (*sensu* ARAUJO & LOPES 2003), with lobe on inner lateral margin; bearing up to 6 setae on outer lateral margin. Exopods and endopods of uropods inserting at different levels.

Description: Maximum cephalothorax width: male 1.70 mm, female 2.60 mm. Maximum length: male 9.7 mm, female 11.5 mm. Body color chestnut, with unpigmented regions along pereion; unpigmented spots on anterior region of each coxal plate; elongated unpigmented lines on median longitudinal part of the pereionites (Fig. 1). Antenna articles 1-5 with few small areas without pigmentation; flagellum uniformly chestnut colored. Pleonites 1-2 with 3 unpigmented spots, pleonites 3-5 with elongated spots with fine lateral projections without pigmentation on median longitudinal line. Pleotelson with same pattern of pleonites 3-5. Uropods uniformly pigmented. Pereiopods partially pigmented.

Pereion with smooth and bright tegument bearing setae on all pereionites. *Noduli laterales* (Fig. 15a and 15b) with d/c coordinates showing a maximum on pereionite 4. Pleon narrows abruptly in relation to pereion; neopleura well-developed on segments 3-5. Pleotelson triangular with slightly concave lateral margins, and with rounded apex, reaching more than halfway down the protopodite of uropods.

Cephatothorax with eyes with 22-25 ommatidia. Antennula with coniform distal article bearing 5 rounds of 3 aesthetascs each and 2 apical aesthetascs (Fig.2). Antenna, when extended posteriorly, reaches anterior margin of fifth pereionite. Antenna article 5 and flagellum approximately same length. Second flagellar article smallest, apical organ about one-third of size of distal article (Fig. 3). Mandibles: right mandible with three penicils on incisor process and tuft of at least 10 plumose setae on molar (Fig. 4); left mandible with two penicils on incisor process and tuft of at least 10 plumose setae on molar (Fig. 5). Maxillula: lateral endite with 4+6 setae, 5 of inner set pectinate, proximal teeth with 5+1 denticles (Fig. 6), medial endite with 2 apical penicils; (Fig. 7). Maxilla: external lobe with sinuous posterior margin and internal margin with 3 long and fine setae (Fig. 8). Maxilliped: endite with long and short seta; 2 short teeth on the distal external margin, with 3 small protuberances. Apex of palp with tuft of short fine setae (Fig. 9). Pereiopods: with tricorns on all articles, antenna-groming brush of the carpus and dentate carpal seta present on pereiopod 1 (Fig. 26). Uropod: insertion of endopod and exopod at different levels, endopod extending to half of exopod (Fig. 1).

Sexual differentiation: pereiopods 1-4 of male with rows of bifid setae, particularly on merus and carpus, missing in female; ischium and merus of all pereiopods of male of sub-equal length. Ischium of pereiopod 7 of male robust with setae approximately in the middle part; female setae placed on distal third of ischium (Fig. 16-26). Pleopods: pleopod 1 exopod of male elongated (z:y ratio = 3.1, excluding the lobe) with subapical dentiform expansion and lobe on inner lateral margin and up to six setae on outer lateral margin (Fig. 10), endopod with 5 fine apical spines (Fig. 11). Pleopod 2 with exopod distally elongated carrying setae on external margin, endopod tapered on distal third (Fig. 12, 13). Pleopod 5 exopod with up to approximately 9 setae on external lateral margin (Fig. 14).

Etymology: The specific name refers to a location, Sítio Cairé, at which the species was found, in Taquara, Rio Grande do Sul, Brazil.

Remarks: *Benthana cairensis* **sp. nov.** has 5+1 denticles in the proximal teeth of the lateral endite in the maxillula, while the other 19 species have at least 6+1denticles. The new species does have characteristics found in other congeneric species, such as: the lobe on the inner lateral margin on the male pleopod 1 exopod and setae on the opposite margin, five pectinate teeth on lateral endite of the maxillula, insertion of the endopod and exopod at different levels, and male sexual dimorphism on pereiopods 1-3(4).

Three species of the genus have a lobe on male pleopod 1 exopod: (1) *B. serrana*, lacks setae on the pleopod 1 exopod and has 20 ommatidia (22-25 on the **sp. nov.**); (2) *B. taeniata*, also lacks setae on pleopod 1 exopod, has endopod and exopod insertions of the uropod at the same level, sexual dimorphism of males only in the first pereiopod and head with 17 to 19 ommatidia; (3) *B. olfersii* has all the above mentioned characteristics for *B. cairensis* **sp.nov.**, except that the sexual dimorphism of males is restricted to the pereiopods 1-3, and, unlikely, a lobe with six setae on the internal margin of the proximal extremity of the merus on pereiopod 7 (Fig. 27).

In a review of *Benthana*, sexual dimorphism was described on the exopods of the uropods in *B. olfersii*, which are longer in males than in females (LEMOS DE CASTRO 1958b). In the examined material of *B. olfersii*, one male and one female of similar size were examined and had this same dimorphism and so this species should be included in the subgenus *Benthanoscia*, in which dimorphism on male uropods exopods is an autapomorphy (LEISTIKOW & ARAUJO 2006). Therefore, by lacking this dimorphism (autopomorphy), the new species is not included in the subgenus *Benthanoscia*.

Benthana cairensis **sp. nov.** is commonly abundant in leaf litter and under fallen tree branches of *Araucaria angustifolia* (Bert.) O. Ktze., the only native conifer in Brazil. While classified as runners following SCHMALFUSS (1984) this species is also capable of performing thanatosis.

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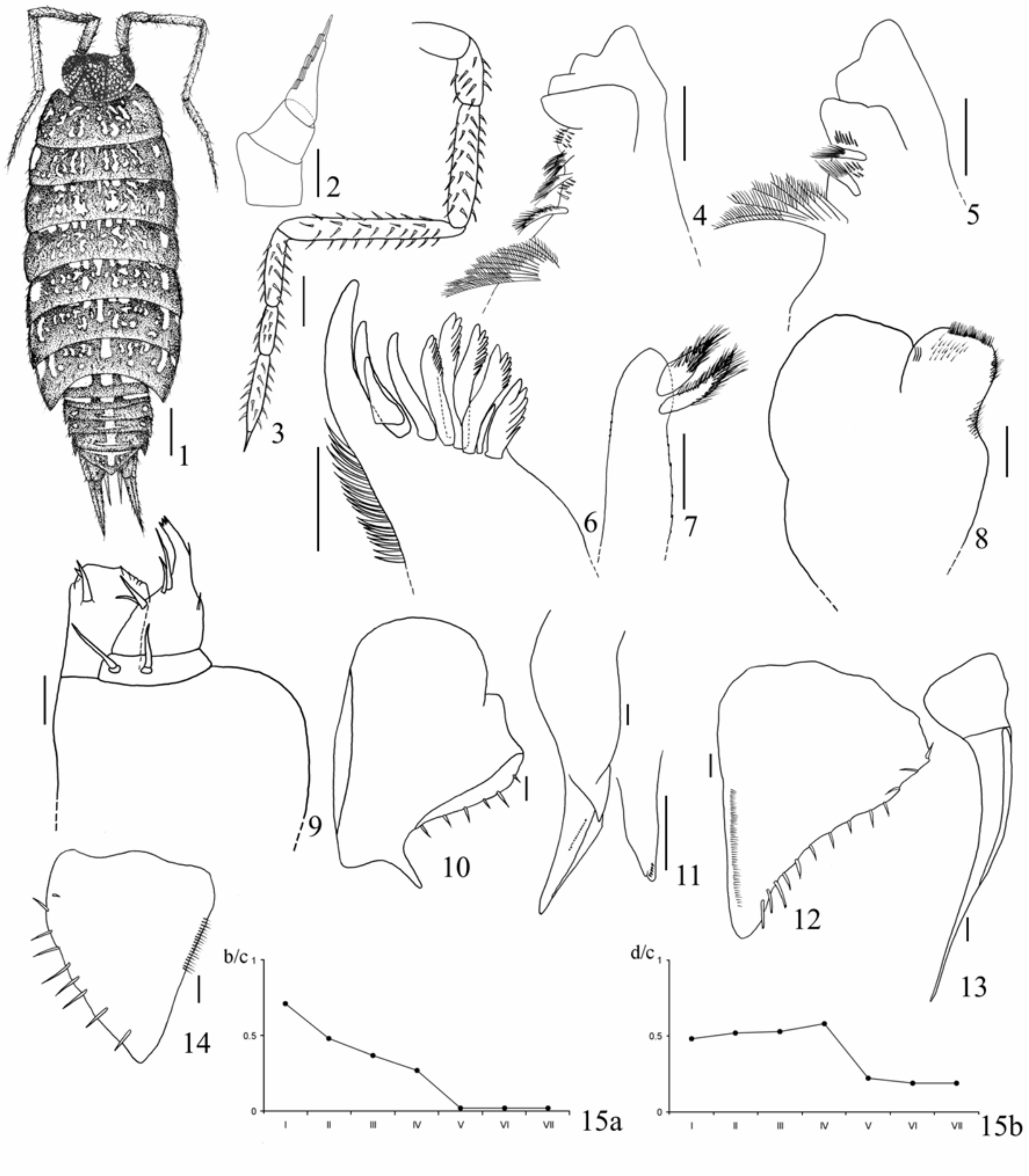
Legends of figures

Figures 1-15. *Benthana cairensis* **sp. nov.** Male: (1) dorsal view; (2) antennula; (3) antenna; (4) right mandible; (5) left mandible; (6) lateral endite of maxillula; (7) medial endite of maxillula; (8) maxilla; (9) maxilliped; (10) pleopod 1 exopod, (11) pleopod 1 endopod, with detail of five small spines; (12) pleopod 2 exopod; (13) pleopod 2 endopod; (14) pleopod 5 exopod; (15a-15b) co-ordinates b/c and d/c of the noduli laterales. Scales: 0.1 mm.

Figures 16-27: *Benthana cairensis* **sp. nov.:** (16) male pereiopod 1; (17) male pereiopod 2; (18) male pereiopod 3; (19) male pereiopod 4; (20) male pereiopod 7; (21) female pereiopod 1; (22) female pereiopod 2; (23) female pereiopod 3; (24) female pereiopod 4; (25) female pereiopod 7; (26) male pereiopod 1 carpus; (27) *B. olfersii*: male pereiopod 7 merus; arrow indicate unique lobe with 6 setae. Scales: (16-25) 500 µm; (26) 100 µm; (27) 200 µm.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Capítulo II

"Postmarsupial mancas of *Benthana cairensis* Sokolowicz, Araujo & Boelter (Isopoda, Philosciidae)"

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Postmarsupial mancas of *Benthana cairensis* Sokolowicz, Araujo & Boelter (Isopoda, Philosciidae)

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Running head: Manca stages of *Benthana cairensis*.

Abstract

The aim of this study is to present a description of manca stages of the terrestrial isopod *Benthana cairensis* Sokolowicz, Araujo & Boelter, 2008, and to elucidate whether there is a sexual dimorphism on pereiopods already in the early stages. The species presents three manca stages. Manca I shows an embryonic appearance, with very fragile body and lasts approximately 4 hours. Manca II already has many characteristics of adult, including the pectinate teeth of maxillula, antenna grooming brush and ornamental setae with hand-like apex of carpus 1; rudimentary seventh pereiopod. Manca III shows the seventh pair of pereiopods ventrally folded on the pereon; although completely segmented it is not functional. No sexual dimorphism was found on setae distribution on pereiopods 1-4. The simplicity of MI demonstrate that this phase is very incipient and seems to be a preparatory stage for the following ones, MII and MIII already show several characteristics of adult although have not sexual differentiation.

Key-words: *Benthana*, morphology, manca stages, Oniscidea.

Introduction

Being independent of the aquatic environment, terrestrial isopods (Oniscidea) exhibit several characteristics that allow them to live on land. One adaptation of crucial importance for reproduction was the development of a closed marsupium with cotyledons, typical of terrestrial isopods, which serves as an aquarium, providing protection, nutrition, and oxygen to the developing offspring (Hoese, 1984). The marsupium allows development to occur independently of a water source, which makes terrestrial isopods fully emancipated from external liquid water, a unique feature among Crustacea (Surbida & Wright, 2001).

Similarly to other Peracarida, terrestrial isopods undergo direct embryonic development. The stage that hatches from the marsupium is active and woodlouse-like, but lacks the last pereional segment and its associated appendages. The phases before complete development of the seventh pair of pereiopods are called manca stages (Holdich *et al.*, 1984).

Early studies on this subject used terms such as larva, immature, and juvenile for describing the developmental stages of terrestrial isopods, or the stages were designated by the number of completed moults. These first studies provide a brief description of immature individuals, with information on pigmentation, size, and general body structure, in addition to the period between moults (Verhoeff, 1920; Heeley, 1941, 1942; Vandel, 1943; Matsakis, 1955; Haddad, 1982; Katakura, 1984; Tomescu, 1976; Kacem-Lachkar, 1997).

Holdich *et al.* (1984) proposed a terminology to define terrestrial isopod developmental stages. Thus postmarsupial mancas correspond to individuals that leave the marsupium, and are characterized by the absence of a functional seventh pair of pereiopods. Juvenile stages comprise the individuals with the beginning of sexual differentiation until the complete formation of adult characteristics. This definition has been already used in the most recent works on developmental stages of *Porcellio scaber* Latreille, 1804 (Tomescu & Carciun, 1987); and recently in *Atlantoscia floridana* (van Name, 1940), and *Porcellio dilatatus* Brandt, 1833 by Araujo *et al*. (2004a) and Brum & Araujo (2007), respectively; who described in detail the manca stages of these species, including body morphology and appendages.

Vandel (1957) stressed the importance of understanding the evolution of morphological characteristics during isopod ontogenesis in order to define species and genera, or to establish phylogenetic relations.

The genus *Benthana* Budde-Lund, 1908 is distributed in eastern tropical and subtropical South America (Leistikow, 2001). There are 24 known species of this genus, including six in the state of Rio Grande do Sul (RS) (Leistikow & Wägele, 1999; Araujo & Lopes, 2003), of which *Benthana cairensis* Sokolowicz, Araujo & Boelter, 2008 is the most recently described one.

Most species of this genus exhibit sexually dimorphic pereiopods, with densely setose merus and carpus of the first three pairs in males (Lemos de Castro, 1958a,b; Lemos de Castro, 1985; Lima & Serejo, 1993; Araujo & Buckup, 1994; Araujo, *et al.*, 1996; Araujo & Lopes, 2003). However, the time when this dimorphism becomes evident is unknown.

This study presents a description of the postmarsupial manca stages of *B. cairensis*, aiming at contributing to the knowledge on this species, and also to elucidate whether there is a sexual dimorphism of pereiopods already in the early stages. The species is found in eastern RS, and one of its characteristics is the sexual dimorphism of male pereiopods 1-4, which show rows of bifid setae, particularly on the merus and carpus.

Material & Methods

Ovigerous females, already bearing intramarsupial mancas, were collected in Taquara, Rio Grande do Sul, Brazil (29°46′S, 50°50′W). They were kept with food until postmarsupial mancas were released under controlled temperature (20±1°C) and photoperiod (12:12h) on wet Petri dishes with the bottoms of which were wetted whenever necessary to maintain humidity.

When mancas started hatching from the marsupium, they were separated from their mothers and kept individualized on closed Petri dishes with a bottom of plaster to maintain humidity. Some pieces of decaying leaves from the sample site were provided as food. Water and food supply were monitored daily.

Animals were checked for moulting events daily. Representative examples of each stage were collected for description, illustration, and cephalothorax width measurement (Sunderland *et al.*, 1976, Araujo *et al.*, 2004a; Brum & Araujo, 2007). The stages were described using general appendage characteristics. Manca stages were designated as manca I (MI), manca II (MII), and manca III (MIII).

Several individuals of each stage were analyzed under a stereomicroscope for the presence of sexual dimorphism of pereiopods. The preparation of animals for Scanning Electron Microscopy (SEM) was carried out according to Leistikow & Araujo (2001). Animals were coated with gold at the Electronic Microscopy Center, UFRGS (CME), except for MI, due to its fragility – at this stage the body structure did not resist the preparation processes.

Results

General remarks

Manca stages can be distinguished through the morphology of some appendages, especially the antenna, which increases its length as the development proceeds, and also through number of ommatidia (Tab. 1). The cephalothorax width in the different stages varies from 0.59 to 0.66 mm (MI and MIII, respectively) (Tab. 2); the three manca stages show overlapping size ranges.

Manca I

The first manca stage shows mild pigmentation concentrated on the lateral margins of the pereionites. It has an embryonic appearance; the head shows lateral projections and a frontal u-shaped indentation (Fig. 7G). The body, including pereiopods, has a soft appearance; calcium deposits on the ventral pereon are visible. Eyes with four ommatidia. Pereionite VII not visible.

Antennula tri-articulated with two apical aesthetascs (Fig. 1A). Antenna with bi-articulated flagellum with the same size of the fifth peduncular article; proximal article four times longer than the distal one, apical organ less than a half the length of distal flagellar article (Fig. 1B). Mouthparts weakly developed, showing few setae and a pattern much simpler than the adult mouthparts, especially the maxillula, which lacks pectinate teeth (Fig. 1C); maxilla and maxilliped missing setae (Fig. 1D, 1E). Pereiopods 1-6 practically glabrous, showing reduced number of setae (Fig. 2A-F), carpus 1 lacking antenna-grooming brush and ornamental setae with hand-like apex *sensu* Leistikow (2001). Pleopod 1 lacking; one seta on exopods 2 and 3 and two setae on exopods 4 and 5 (Fig. 2G-J).

Manca II

The time interval between MI and MII is very short; four hours after hatching the animals undergo the first moult to MII. It was noticed that right after birth the newly hatched individuals stay very close to their mother, the majority staying under her body. When individuals enter MII, they start to explore the environment. The intermoult stage of MII lasts from 5 to 8 days.

Pigmentation more intense on lateral margins of pereionites. Shape of the head similar to that of adults. Eyes with 6 ommatidia (Fig. 7A). Pereionite VII weakly developed. (Fig. 7C).

Antennula similar to MI, with two additional aesthetascs in the middle part of the distal segment (Fig. 3A). Antenna with distal flagellum article almost twice as long as the proximal one; apical organ about a third the length of distal flagellum article (Fig. 3B). Mouthparts similar to those of adults, except the mandible which is not completely formed, lacking plumose setae and fully formed penicils on incisor and molar process; maxillula with lateral endite with 4+6 teeth, five of inner set pectinate, the internal one with 5 denticles (Fig. 3C), maxillary external lobe with a sinuous posterior margin (Fig. 3D); maxilliped endite with a long seta and a smaller one; one short teeth on the distal external margin; apex of the palp with a tuft of short fine setae (Fig. 3E). Pereiopods 1-6 more setose than in MI, but still not like the adult (Fig. 4A-F); carpal antenna grooming brush and ornamental setae with hand-like apex on carpus 1 present (Fig. 7F); pereiopods 1-4 showing no characteristics of sexual dimorphism (Fig. 4 A-D); pereiopod 7 rudimentary with no distinguishable articles. Pleopods 2-3, 4 and 5 present one, two and three setae, respectively (Fig. 4G-J).

Manca III

Intermoult stage lasts from 7 to 13 days.

Pigmentation almost like in the adult, eyes with 7 ommatidia (Fig. 7B); pereionite VII half of the length of pereionite VI (Fig. 7D).

Antennula with three aesthetascs on the middle part of distal segment (Fig. 5A). Antenna distal flagellum article almost the same length as the other two articles, apical organ about half the length of distal flagellar article (Fig. 5B). Mouthparts similar to those of MII, except for an additional denticle on the inner pectinate teeth of maxillula (Fig. 5C) and the presence of three fine setae on internal margin of maxilla (Fig. 5D). Maxilliped like in MII (Fig. 5E). Mandible like that of the adult. Pereiopods like those of MII, with an additional seventh pair which is ventrally folded (Fig. 6A-G, 7E); pereiopods show no sexual dimorphism. Pleopods 2, 3 like those of MII, 4 and 5 exopods with three setae (Fig. 6H-L).

Discussion

After hatching, individuals go through a series of moults until they reach the adult stage. Except for *Hemilepistus reaumurii* (Milne-Edwards, 1840) and *Trichoniscus pusillus* Brandt, 1833, studied by Kacem-Lachkar (1997) and Helley (1942), respectively, all species which development was followed present three manca stages (Verhoeff, 1920; Heeley, 1941, 1942; Matsakis, 1955; Haddad, 1982; Araujo *et al.*, 2004a; Brum & Araujo, 2007).

Heeley (1941, 1942) described intramarsupial mancas in several isopod species as being individuals with incomplete segmentation, body with soft, pearly-white appearance without pigmentation, and with almost transparent pereion side-plates. This pattern seems very similar to the embryonic appearance of the *B. cairensis* Manca I. The entire body is very fragile, and the presence of calcium deposits in all observed animals lead to the assumption that the MI leaves the marsupium in an already premoult stage. This is also supported by the fact the first moult is rapidly completed, and by the poorly developed appendages. This was also mentioned by Araujo *et al*. (2004a) in *A. floridana*, where MI represents the final stage of the marsupial manca, as they are very close to moulting and do not feed.

The first moult was completed in less than 4 hours, and it is the shortest intermoult period among the already studied species. In other species, the duration of this stage ranges from approximately 12 hours for *A. floridana* (Araujo *et al.*, 2004a) to 24 to 48 hours for *H. reaumurii* (Kacem-Lachkar, 1997).

Several newly-hatched individuals already resembled the appearance of MII with respect to the posterior part of the body, as the moult is biphasic in isopods. First, the posterior part of the body is eliminated, and then the anterior part; the division line between the two stages is the junction between the fourth and fifth thoracic segments (Helley, 1942).

The first moult does not seem to be a critical development step for *B. cairensis*, as practically all individuals studied herein completed this stage. According to Ouyang & Wright (2005), the marsupial development of mancas is critical with respect to calcium uptake. Marsupial mancas apparently ingest marsupial fluid, which may be important to store calcium (Ouyang & Wright, 2005). This may have been the reason why mortality was low during this phase. After the first moult, exuviae are ingested by the individuals, which was also observed in several other isopod species (Helley, 1941, Araujo *et al.*, 2004a; Brum & Araujo, 2007). Calcium lost during moulting is replaced by an uptake of calcium from the environment after moulting, and/or by use of calcium reservoirs formed before the moult (Luquet & Marin, 2004). The biphasic moult permits the storage of calcium in the integument, including sternal deposits. These are resorbed in the time between the posterior and anterior moults, before the deposits would be lost when the anterior cuticle is shed (Ziegler *et al.*, 2007).

After the second moult, individuals showed a more complete appearance even the tegument seemed to be harder. Pereiopods were stronger, and the animals fed normally. This stage is identified by the presence of a more developed pereionite VII, including the epimera, and also by the inversion of the proportions between the articles of the flagellum of the antenna. In MI, the proximal article is longer than the distal one, whereas this proportion changes in MII, with the proximal article becoming smaller than the distal one. This pattern is common to the developmental stages of several species of Oniscidea belonging to different families (Verhoeff, 1918; Heeley, 1941; Matsakis, 1955; Tomescu & Craciun, 1987; Araujo *et al*., 2004a; Brum & Araujo, 2007).

The transition from MII to MIII can be predicted by the presence of calcium plates, which is a clear indication of the premoult stage (Zidar *et al*., 1998). These plates appeared approximately 1 or 2 days before moulting.

MIII is very similar to MII, except for the seventh pereionite, which is larger, and for the presence of a completely segmented seventh pair of pereiopods. However, this pair is not functional, and it is ventrally folded against the animal body. These two stages present several similarities with the adult like the presence of the ornamental seta with hand-like apex of carpus 1 which is an autapomorphy of the genus *Benthana*, and the maxillula serrate teeth, which is also present in other Oniscidea genera (Leistikow & Araujo, 2006; Leistikow, 2001). The presence of these two characteristics is particularly important to taxonomy of the genus, since it allows its identification already in early developmental stages. While this is not possible to MI, the very brief period that this phase is free in the environment, the probability of finding MI individuals in the field is very low. In a parallel work on the life history of this species, not a single MI individual was found during two years of field sample collection (C. Sokolowicz, personal observation).

The overlap of the size ranges of all three stages was also observed in *Porcellio laevis* Latreille, 1804, *A. floridana,* and *P. dilatatus* (Matsakis, 1955; Araujo *et al.*, 2004a; Brum & Araujo, 2007), and seems to be a common feature during the first developmental stages.

The pleopod 1 exopod is sexually dimorphic, with a lateral dentiform protusion in males, an autapomorphy of the genus. Male merus and carpus of pereiopods 1-4 carry rows of bifid setae and the ischium of pereiopod 7 is different with respect to shape and position of setae. As manca stages lack sexual genital dimorphism, and fully developed pereiopod 7, setae dimorphism in the first pereiopods of males could be used to differentiate sexes. Electron microscopy and SEM analyses did not present any evidence of differentiation in setae pereiopod patterns on among individuals. These would emerge during the subsequent stage (juvenile), when external sexual differentiation of pleopods begins (Heeley, 1941; Matsakis, 1955; Tomescu, 1976; Katakura, 1984; Tomescu & Craciun, 1987; Kacem-Lachkar, 1997; Araujo *et al.*, 2004b).

The study of developmental stages of terrestrial isopods are still in its infancy. Comparisons of immature forms from different families in searching for characters to be use in phylogenic analysis are still not possible. Possibly, this is due to different characters that have been used in different species.

Further, early developmental stages have thus far been described in only few species. Thus, within the "Philosciidae", besides the present study, only the manca stages of *A. floridana* have been studied in detail. Since the "Philosciidae" are considered paraphyletic (Leistikow, 2001), even a comparison of these two species is of limit value. On the other hand, we hold that with the detailed description of early developmental stages of species from different genera that have previously been included in this family, it will be possible to provide new characters from immature forms for studies on the phylogeny of this group.

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Table 1: Main characters on manca stages of *Benthana cairensis*.

Stage	Number of ommatidia	Antennula	Antenna: flagellum	Seventh pereiopod	Mouth parts
MI	4	two apical aesthetascs	bi-articulate; proximal article the largest	Absent	weakly developed, maxillula lacking pectinate teeth
MII	6	two aditional aesthetascs on middle part of distal segment	bi-articulate; distal article the largest	rudimentary, without segmentation.	almost like adult, maxillula with pectinate teeth
MIII	7	three aestetascs on middle part of distal segment	tri-articulate; distal article the largest	folded in ventral pereion, all segments distinct	like the adult

Table 2: Cephalothorax width and duration of each manca stage of *Benthana cairensis*, M ± SD (N).

Stage	Cephalothorax Width (mm)	Stage duration
MI	0.582 ± 0.017 (17)	ca 4 hours
MII	0.617 ± 0.033 (35)	6.6 ± 0.79 days (39)
MIII	0.655 ± 0.029 (44)	10.4 ± 2.62 days (35)

Figure captions.

Figure 1. *Benthana cairensis*. Manca I. (A) antennula; (B) antenna; (C) maxillula; (D) maxilla; (E) maxilliped. Scales 0.1 mm.

Figure 2. *Benthana cairensis*. Manca I. (A) pereiopod 1; (B) pereiopod 2; (C) pereiopod 3; (D) pereiopod 4; (E) pereiopod 5; (F) pereiopod 6; (G) pleopod 2; (H) pleopod 3; (I) pleopod 4; (J) pleopod 5. Scale 0.1 mm.

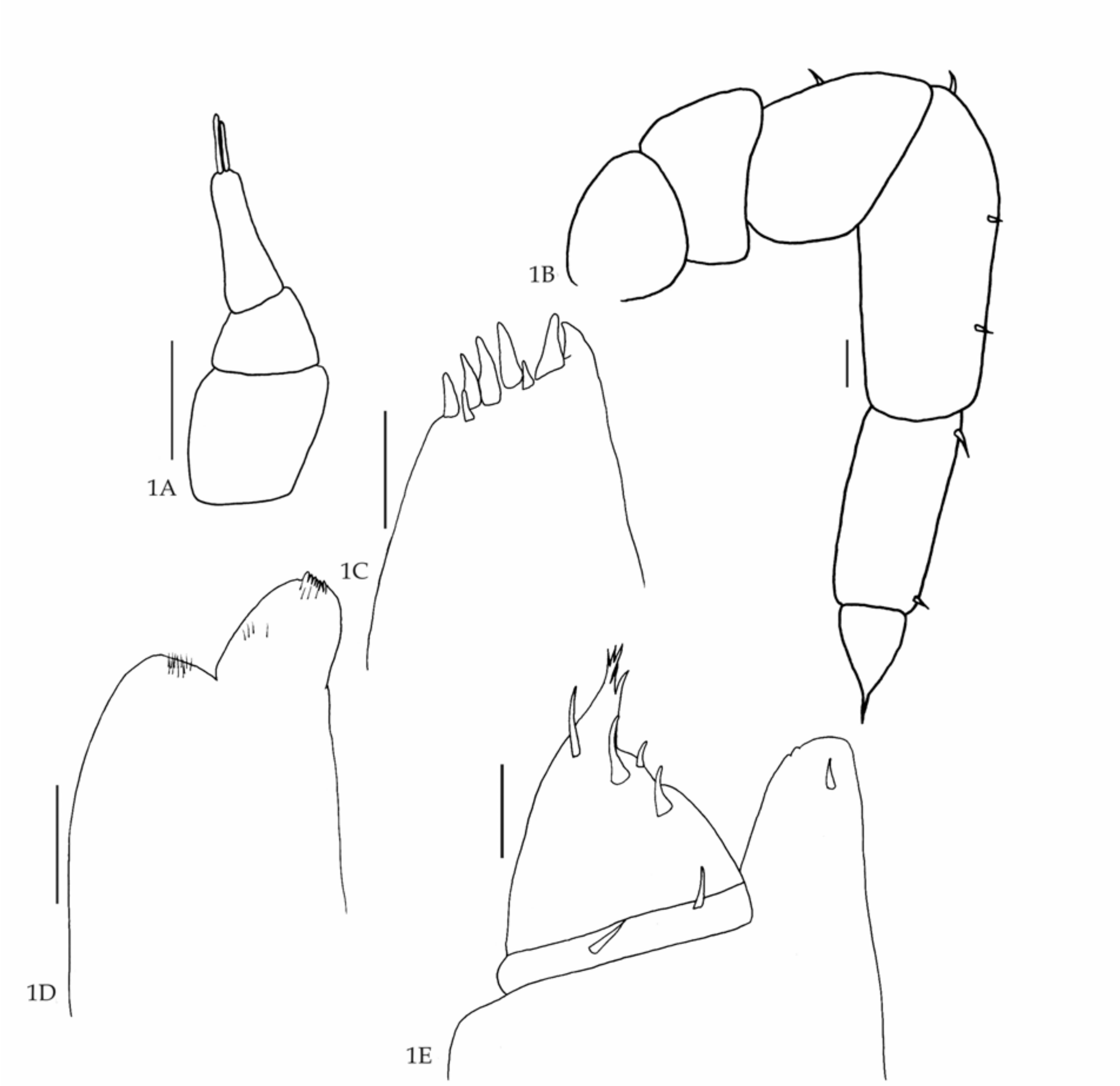
Figure 3. *Benthana cairensis*. Manca II. (A) antennula; (B) antenna; (C) maxillula; (D) maxilla; (E) maxilliped. Scales 0.1 mm.

Figure 4. *Benthana cairensis*. Manca II. (A) pereiopod 1; (B) pereiopod 2; (C) pereiopod 3; (D) pereiopod 4; (E) pereiopod 5; (F) pereiopod 6; (G) pleopod 2; (H) pleopod 3; (I) pleopod 4; (J) pleopod 5. Scale 0.1 mm.

Figure 5. *Benthana cairensis*. Manca III. (A) antennula; (B) antenna; (C) maxillula; (D) maxilla; (E) maxilliped. Scales 0.1 mm.

Figure 6. *Benthana cairensis*. Manca III. (A) pereiopod 1; (B) pereiopod 2; (C) pereiopod 3; (D) pereiopod 4; (E) pereiopod 5; (F) pereiopod 6; (G) pereiopod 7; (H) pleopod 2; (I) pleopod 3; (J) pleopod 4; (L) pleopod 5. Scale 0.1 mm.

Figure 7: *Benthana cairensis*. (A) Manca II: compound eye; (B) Manca III: compound eye; (C) Manca II: pereionite VII; (D) Manca III: pereionite VII; (E) Manca II: pleon in ventral view, with seventh pair of pereiopods (arrow); (F) Manca II: carpus pereiopod 1; (G) Manca I individuals inside the marsupium. Scales (A, B) 50 µm; (C) 200 µm; (D) 500 µm; (E) 100 µm; (F) 20 µm; (G) 0.5 mm.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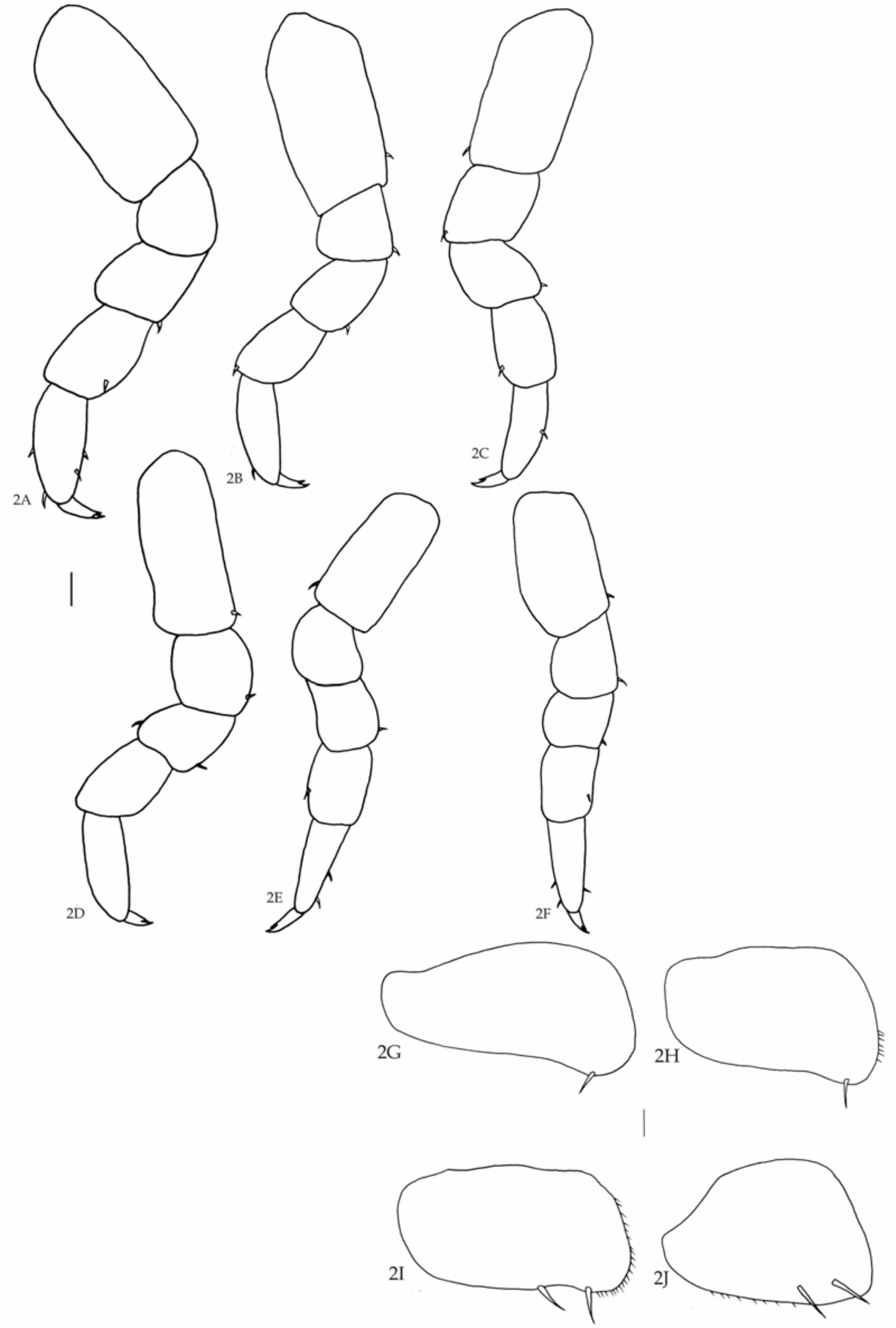
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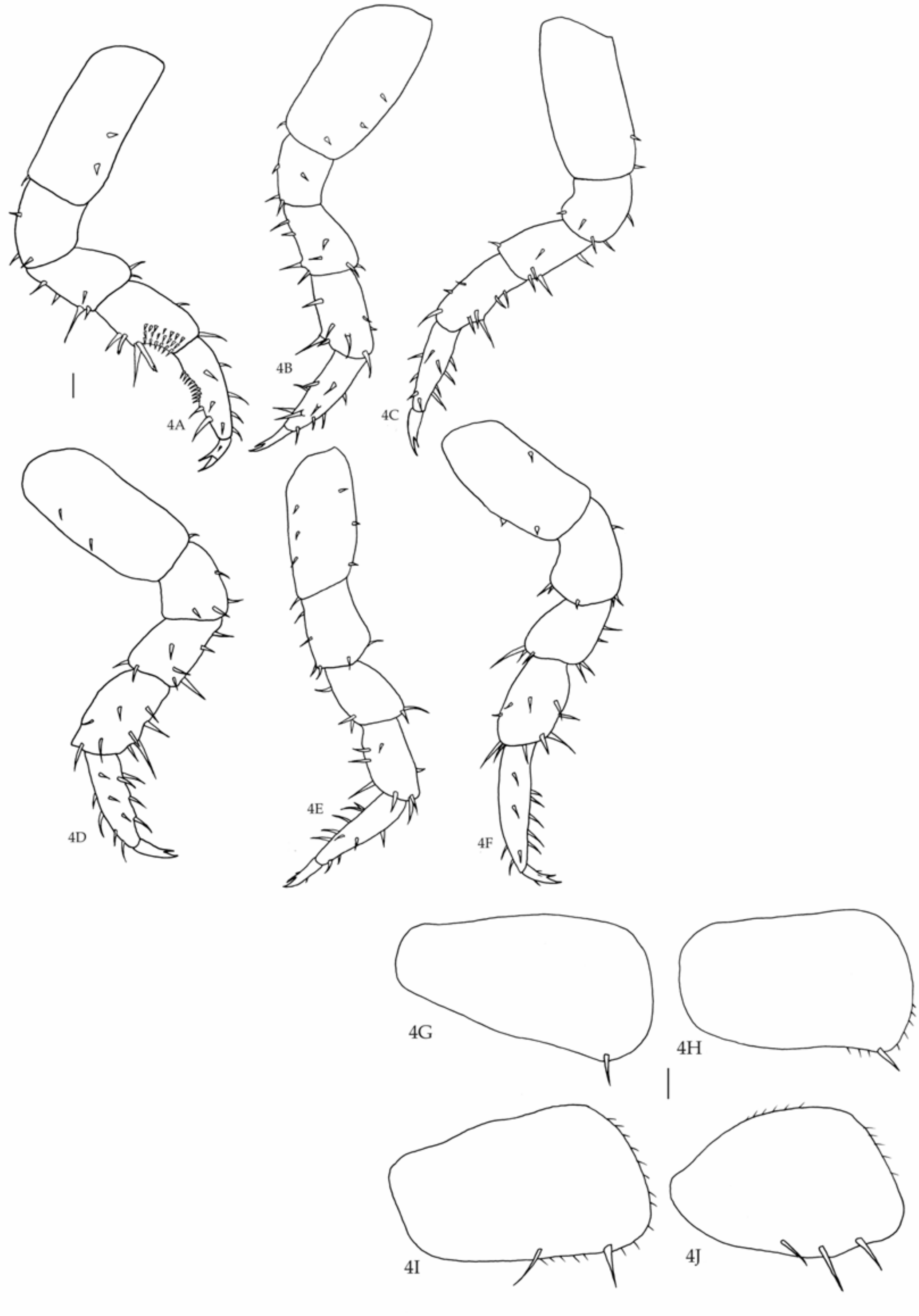
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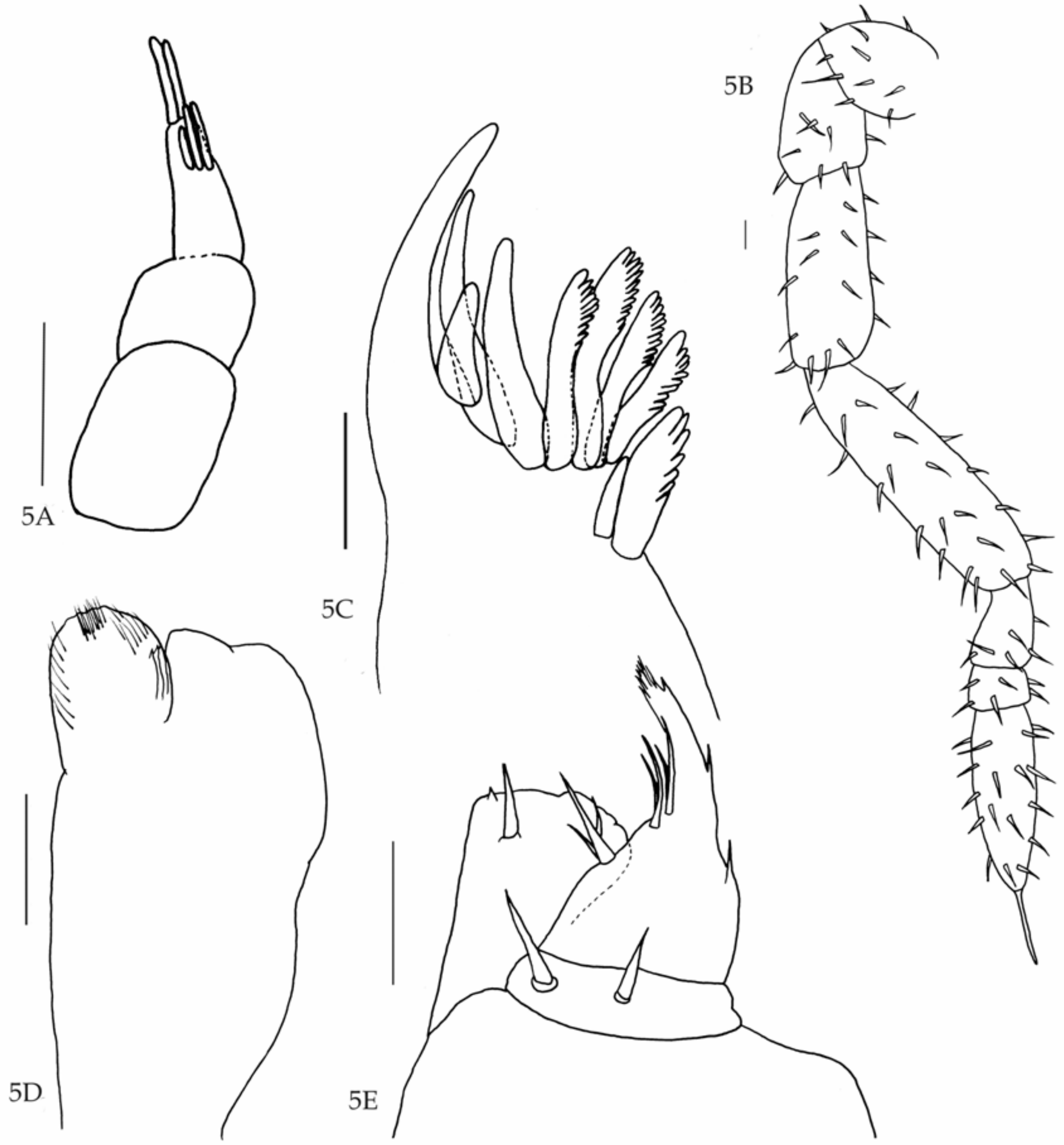
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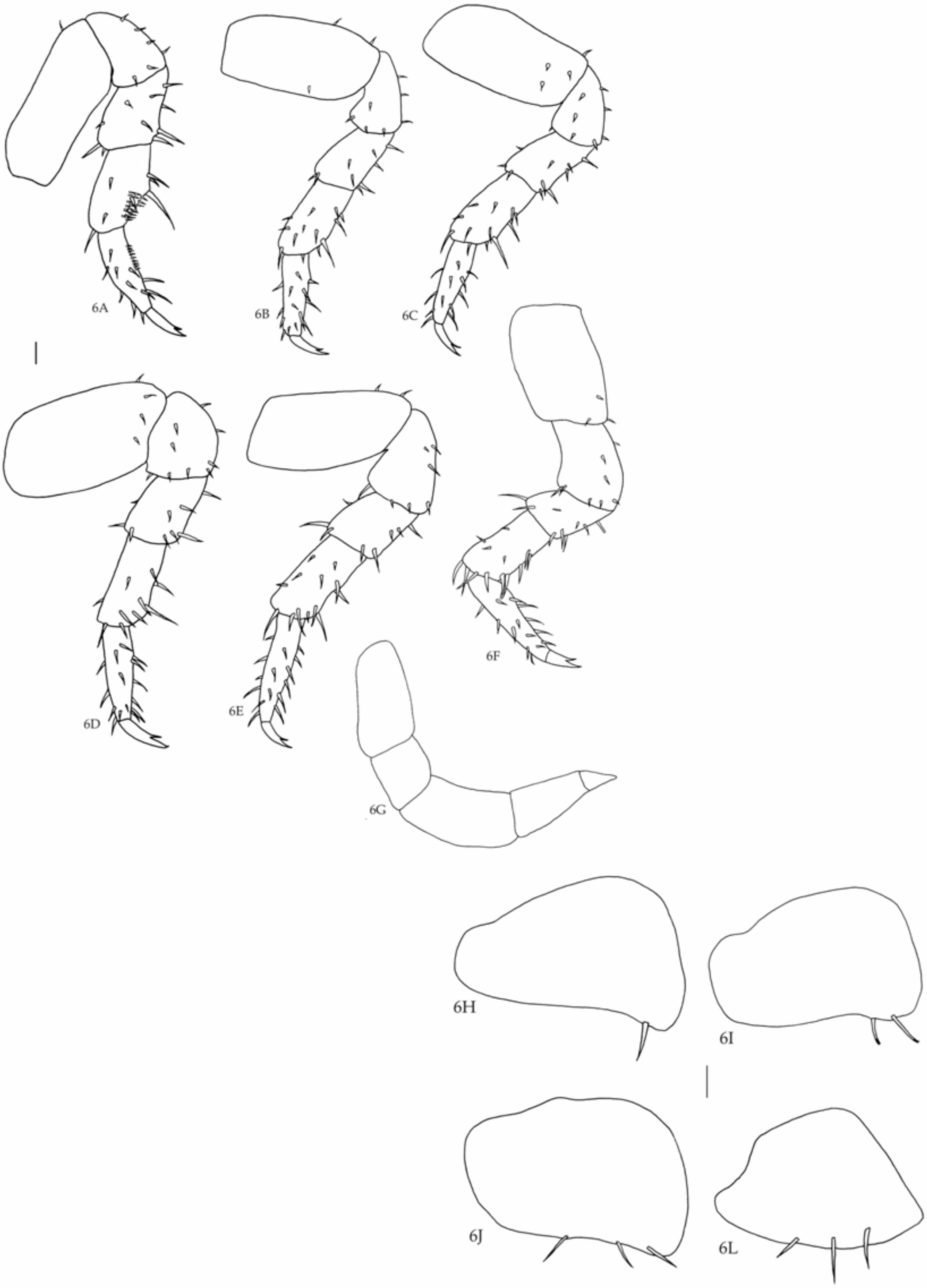
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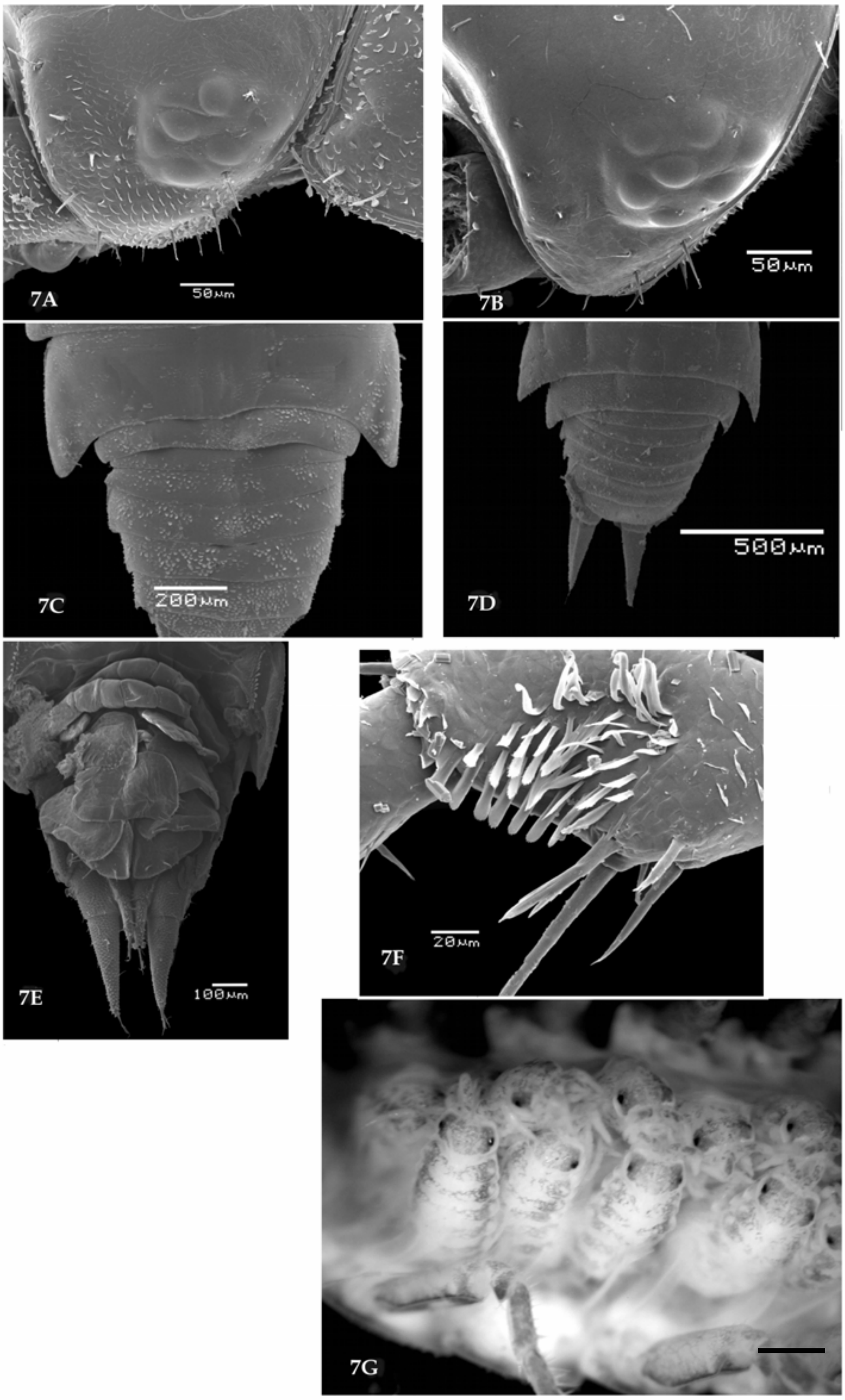
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Capítulo III

"Development of sexual secondary characteres on juvenile males of *Benthana cairensis* (Crustacea, Oniscidae, Philosciidae)"

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Sokolowicz C.C. & P.B. Araujo. Development of sexual secondary characters on juvenile males of *Benthana cairensis* (Crustacea, Oniscidae, Philosciidae). **Acta Zoologica (Stockholm)**.

Development of sexual secondary characters on juvenile males of *Benthana cairensis* (Crustacea, Oniscidae, Philosciidae).

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Abstract

Sokolowicz C.C. and Araujo P.B. 2009. Development of sexual secondary characters on juvenile males of *Benthana cairensis* (Crustacea, Oniscidae, Philosciidae). Acta Zoologica (Stockholm)

Terrestrial isopods undergo direct development, after the undifferentiated stage of mancas the animals moult to juvenile stages and the sexes can be recognized. The first three juvenile stages of *Benthana cairensis* were described with enphasis on the male's sexual secondary characters, the ontogeny of genital papilla, pleopods 1 and 2 and pereiopods were followed. On juvenile I stage, males can be characterized by a slightly developed papilla although dimorphism of pleopod 1 exopod and pereiopods is still not evident at this stage. On juvenile II the subapical protrusion of pleopod 1 exopod begins to appear and on JUIII it is more evident. The completely formation of such protrusion is only seen when males reach about 1.0 mm of cephalothorax width (CW); the dimorphism on setation of the four pairs of pereiopods begins later and is evident when males are about 1.04 mm of CW.

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Introduction

In gonochoric animals, males and females are differentiated by morphologic characteristics that appear when they reach maturity, which is defined as sexual dimorphism (Fairbairn 1997). In malacostracan crustaceans the differentiation of sex depends on the stimuli of the androgenic hormone, whose presence determines the male individual and absence, the female (Juchault 1967; Azzouna *et al*. 2004). In isopods the secondary morphological characters that define sexes appear progressively, and not at once, as the animal develops (Tomescu 1976; Tomescu and Craciun 1987), in a way that it is possible to verify the sex even before sexual maturity begins.

The sex can be recognized in adult males of terrestrial isopods by the modification of the first two pairs of pleopods into copulatory organs. The exopod of the first pair of pleopod is little modified but the endopodite has a projection that points backwards and is distinctively shaped; the endopodites of the second pleopods are more elongated and form a second pair of stylets (Sutton 1980). These structures, together with the genital papilla, form the sexual apparatus of male isopods. Except by the genital aperture on the fifth thoracic segment, females do not present distinct morphological features; the presence of the marsupium on reproductive females represents its reproductive status, while those not bearing one might already had reached sexual maturity but are not on reproductive state (Haahtela 1978; Sutton 1980).

Besides the genital papilla, other morphological characteristics are used to identify males, usually appearing as secondary sexual characters, such as the form of pleopod 1 exopod and some segments of pereiopod VII; the shape of pereiopod meropodite; differences in length of uropod exopods and endopodites, and differences on setation of pereiopods (Lemos de Castro 1958; Tomescu 1976; Tomescu and Craciun 1987; Araujo and Lopes 2003; Sokolowicz *et al.* 2008).

Woodlice show direct development, an immature and undifferentiated form concerning sex leaves the marsupium, this phase is often comprised by three stages in which the body morphology is not complete, specially the seventh thoracic segment and its appendages; these stages are called mancas (Araujo *et al.* 2004a; Brum and Araujo 2007; Sokolowicz and Araujo 2008). After the manca stages, the development continue throughout juvenile stages, where the sexes can already be distinguished and body morphology is quite complete except for the sexual secondary characters (Tomescu and Craciun 1987; Araujo *et al.* 2004b).

Some previous studies were done to describe immature forms of terrestrial isopods, including the period when sexes can be recognized (Heeley 1941; Legrand 1941; Heeley 1942; Matsakis 1955; Tomescu 1976, Tomescu and Craciun 1987; Kacem-Lachkar 1997); more recently, Araujo *et al.* (2004b), described the juvenile stages of *Atlantoscia floridana* (van Name, 1940), using morphological measurements of the genital papilla to determine the reproductive status of males.

Males of *Benthana* species are characterized, besides the genital papilla, by the presence of a subapical dentiform expansion of pleopod 1 exopod, which is an autapomorphy of the genus; some species show sexual dimorphism on the first pairs of pereiopods, that in this case are provided with a set of setae on merus and carpus (Leistikow 2001; Sokolowicz *et al*. 2008); none of these features appear on manca stages (Sokolowicz and Araujo 2008).

This study presents the ontogeny of sexual secondary characters of *B. cairensis* males, precisely the development of the subapical dentiform expansion on the pleopod 1 exopod and the sexual dimorphism on the pereiopods 1-4, already described for the adults (Sokolowicz *et al*., 2008). The general morphology of the first three juvenile stages is also provided.

Material and Methods

Juvenile individuals were obtained from ovigerous females kept under laboratory conditions. After postmarsupial mancas were released they were kept individually on Petri dishes with a bottom of plaster to maintain humidity under controlled temperature (20 ± 1 ºC) and photoperiod (12:12h). Pieces of decaying leaves from the sample site were provided as food. Water and food supply were monitored daily.

When individuals molted to manca III they were monitored daily to follow the exuviations to juvenile phase. The time interval between molts, size and sex were recorded. Only males were used for the morphology description; all individuals (including females) were used to determine intermolt duration and size increment.

In order to follow the ontogeny of male's sexual morphologic characters individuals of juvenile I, II and III (henceforth JUI, JUII and III) were used. The description was based on slide preparations of the genital papilla, pleopods 1 and 2 endopods and exopods of individuals of different sizes; pleopods of adult males were also analyzed for comparison. The pereiopods of those same individuals were taken to be evaluated on Scanning Electron Microscopy (SEM), in order verify when sexual dimorphism on pereiopod 1-4 begins. The size of the animals from which the pleopod 1 exopod and pereiopods were analyzed varied from 0.71 to 1.19 mm of cephalothorax width (CW).

The illustrations were made through a camera lucida attached to a stereomicroscope and the SEM preparation followed Leistikow and Araujo (2001). Entire bodies of JUI individuals were also observed in SEM to verify the beginning of development of genital papilla.

Results

Juvenile individuals can be recognized by the complete development of the seventh pereionite and its functional pair of pereiopods. At this stage the animals already present the pigmentation and general appearance of the species. The indivisual from the first three juvenile stages that were followed measured from 0.70 to 0.81 mm of CW, showing overlapping size ranges and intermolt period (table I).

Juvenile I

At this stage individuals ranged from 0.61 to 0.78 mm CW. The sexes can be distinguished by the presence of a slightly developed genital papilla on males that outgrowths from the separation of the seventh thoracic sternite and the abdominal segment and reaches the beginning of the insertion of the endopod 1 on the first abdominal segment; the structure is short and round at the appex (Fig. 1A and 2A). On females this structure is absent (Fig. 1B).

The pleopod 1 endopod is short with a round apex and it doesn't even reach the inner end of exopod 1 (Fig. 2A). The pleopod 1 exopod is triangular and still do not show the subapical expansion (Fig. 2A and 3A). The pleopod 2 endopod is elongated but shorter than the exopod 2 and it doesn't have the lobular expansion on proximal part (Fig. 2F); the pleopod 2 exopod is slightly distally elongated (Fig. 2F).

Concerning the pereiopods, the distinct setation on carpus and merus which characterize the male is still not developed, only the common setae that appear on both sexes are present (Fig. 1C).

Juvenile II

The CW of the animals at this stage ranged from 0.70 to 0.82 mm. The genital papilla is still not fully developed (Fig. 2D), but the ejaculatory ducts can already be seen by transparency on the stereomicroscope. The pleopod 1 endopod is longer than the exopod 1 and has a styliform apex (Fig. 2B). The pleopod 1 exopod begins to have the form of an adult pleopod, and the beginning of the formation of the subapical expansion is already seen, although is far from being complete (Fig. 2B and 3B). The pleopod 2 endopod is still shorter than the exopod 2 but already shows a slightly lobe on proximal part; the exopod is more elongated than the previous stage (Fig. 2G).

Juvenile III

The individuals varied from 0.76 to 0.87 mm of CW. At this stage the sexual apparatus resembles the one of the adult. The genital papilla is elongated, reaching the middle part of pleopod 1 endopod and presents a rectangular spatula below the ventral shield, wider than the shield (Fig. 2C). The pleopod 1 endopod is almost twice longer than the exopod and already shows the pointed apex, as the adult (Fig. 2C); the exopod 1 is elongated and presents a slightly developed subapical expansion (Fig. 2C and 3C). The pleopod 2 endopod presents the form seen on the adult, with a lobe on proximal part and extending until it passes the distal apex of exopod 2, which is elongated like in the adult (Fig. 2H).

Adult male of Benthana cairensis

The genital papilla is elongated, reaching the middle part of pleopod 1 endopod and presents a shield on the appex, which is wider than the papilla; the ejaculatory ducts can be seen by transparency. The pleopod 1 endopod is rounded on the external margin of its first half and then elongated with a styliform apex. The pleopod 1 exopod is elongated showing a subapical dentiform expansion on its outer margin (Fig. 2E). Pleopod 2 endopod presents a lobular form on the proximal part and is elongated with a fine apex that overpasses the distal end of the exopod 2; the latter is distally elongated (Fig. 2I).

Male pereiopods 1-4 show rows of bifid setae, particularly on merus and carpus that are shorter than the common setae shown on males and females (Fig. 1G and 1H).

Several male individuals were analyzed concerning the formation of the expansion of pleopod 1 and the setae of pereiopods. The subapical expansion shows gradual development as the animals grow; males at about 0.90 mm of CW have an evident expansion (Fig. 3 D), which grows until it reaches its typical form at 1.0 mm of CW (Fig. 3E).

The setation of pereiopods also appears to be a gradual characteristic and it begins to be visualized in males of about 1.04 mm of CW (Fig. 1E) and gradually develops until it is completely developed (Fig. 1F).

Discussion

The beginning of the stage in which sexes can be recognized in oniscideans is marked by the appearance of the genital papilla on males (Sutton 1980; Araujo and Leistikow 1999). Concerning the period when this structure appears the available information in literature is not precise, some authors show this period in days, others in number of molts and stages. *Porcellio scaber* Latreille, 1904 becomes differentiate within 20 to 25 days of development (Tomescu and Crauciun 1987); Heeley (1942) followed the development of several genera of Oniscidea and concluded that in the end of a period of three months the sexes can be distinguished by small abdominal limbs and that individuals bear slight sexual distinctions. In *Ligia italica* Fabricius, 1798 after the first molt the male papilla can already be seen (Berreur-Bonnenfant and Inagaki 1973); *Hemilepistus* *reaumurii* (Milne-Edwards, 1840) can be recognized as males after the fourth molt (Kacem-Lachkar 1997); at this period males of *Armadillidium vulgare* (Latreille, 1804) can also be differentiated (Katakura 1984). Heeley (1941) stated that only after the fifth molt individuals of *Porcellio dilatatus* (Brandt, 1833), *P. scaber*, *Oniscus asellus* Linnaeus, 1758, *Philoscia muscorum* (Scopoli, 1763) and *A. vulgare* have their sexes differentiated through special characteristics on pleopods.

In the present study males in the first juvenile stage can already be distinguished by the presence of the genital papilla although slightly developed. This structure is of difficult perception and its presence could only be confirmed through SEM analysis. Araujo *et al.* (2004b) also verified that males of *A. floridana* can be recognized by slightly developed genitalia in the first juvenile stage. Even though information on literature is not clear, it is more likely that in the first juvenile stage the majority of oniscideans can already have their sexes differentiated, since although sexually immature, the juvenile stage comprises the period when sexual differentiation begins.

In *B. cairensis* it was noticed a continuous growth of the papilla as the development of the three juvenile stages proceeded. Even though not all stages leading to adult were followed, considering the size of adult genital papilla, this structure probably continues to grow till it reaches a certain specific size. In *L. italica* the growth of genital papilla has two critical moments, when the appendix emerges and in the puberal phase when it acquires the adult form (Berreur-Bonnenfant and Inagaki 1973). The morphometric growth of *A.*

floridana was followed considering genital papilla size to verify individual sexual status and the authors considered that after the third juvenile stage juvenile males undergo their puberal molt and become adults (Araujo *et al.* 2004b).

Besides the genital papilla, dimorphism on the first two pairs of pleopods is probably the most conspicuous one on terrestrial isopods. The differences of these structures on males and females begin to be noticed in the first juvenile stage and continue to be more conspicuous throughout ontogeny. The most pronounced difference in *B. cairensis* is the shape of the male pleopod 1 exopod, which begins as a triangular appendage with no lateral projections and culminates in an elongate pleopod bearing a subapical protrusion, typical of genus *Benthana* (Leistikow 2001) Even though there is no record in literature about the ontogeny of pleopods on members of this genus, several other species have been studied considering this subject. On *P. scaber* the pleopod 1 exopod extends progressively throughout the development (Tomescu and Craciun 1987); in *H. reaumurii* it presents an extension of its posterior apex as the animal develops (Kacem-Lachkar 1997) and in *A. floridana* the pleopod 1 exopod do not show the complete adult morphology until the end of the juvenile stage (Araujo *et al.* 2004b). The dimorphism of pleopod 1 exopod is a well know feature on isopods (Legrand 1941; Heeley 1942; Tomescu and Craciun 1987; Kacem-Lachkar 1997) and it is said that the differential ornamentations, sizes and structures help to protect the copulatory system and they develop together with it (Legrand 1941), a fact that seems to be confirmed in the present study, since the dimorphism on pleopod 1 begins to develop together with the formation of genital papilla.

The same seems to happen with the dimorphism of pereiopods, but this is not as gradual as the dimorphism of pleopods, since the appearance of the first differential setae is noticed when male reaches 1.04 mm of CW, by this time the animal is probably already adult. More than half of the species of *Benthana* are known to have sexual dimorphism on male pereiopods, most of them represented by the presence of dense setae on the first three or four pereiopods (Lemos de Castro 1958; Lima and Serejo, 1993; Araujo *et al*. 1996) but there is no information about when this character begins to appear on the animals.

Based on the present results it can be said that sexual dimorphism on *B. cairensis* is recognizable in the first juvenile stage by observation of a little genital papilla. The secondary sexual characters, such as the lateral expansion on first pleopod exopod and setae on the first four pairs of pereiopods begin later on development; the first feature is slightly perceptive when the animal is still juvenile and the latter is completely formed until the male reaches a greater size, probably related with sexual maturity.

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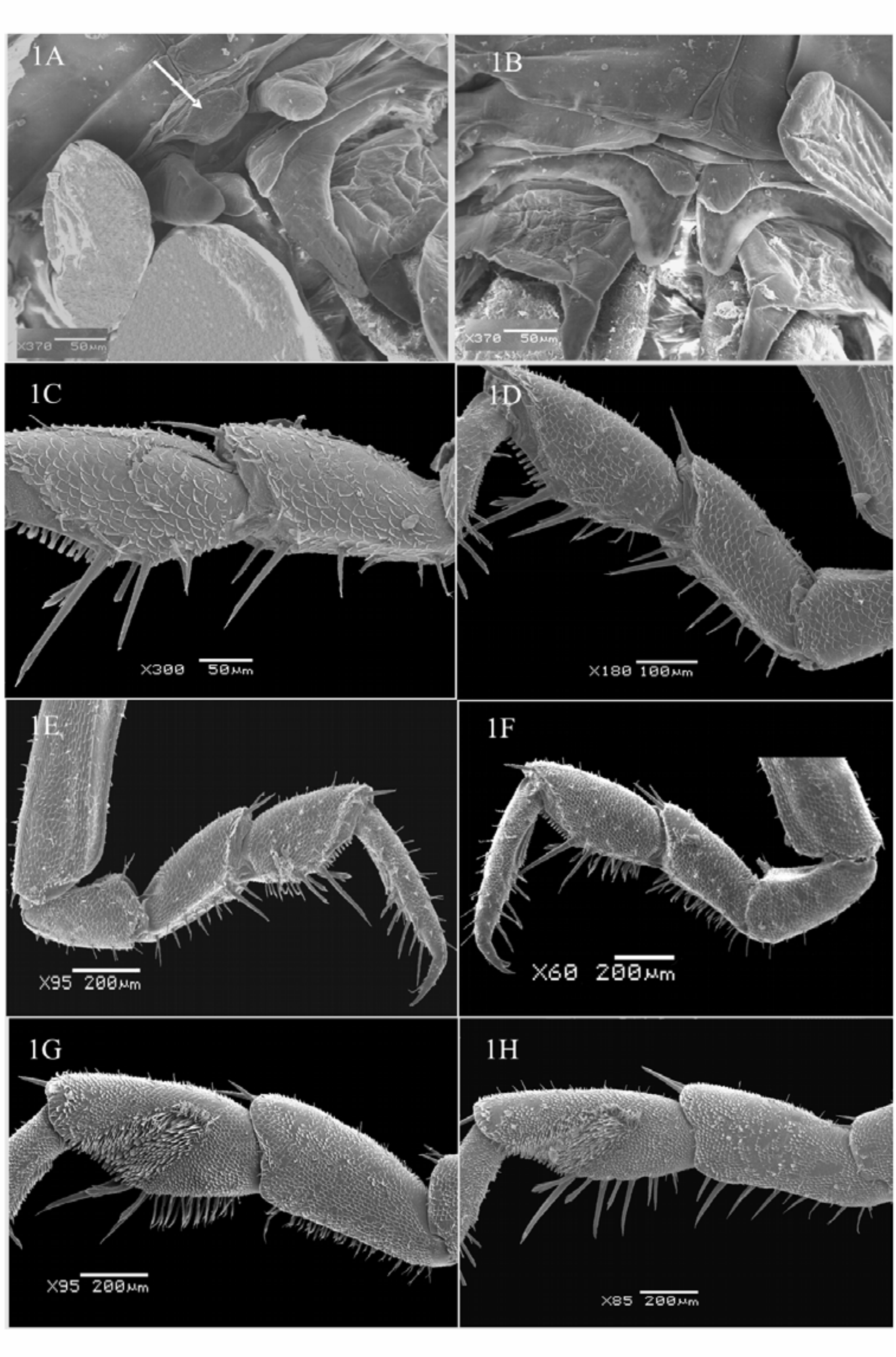
Table I: Cephalothorax width and duration of the first three juvenile stage of *Benthana cairensis* $\bar{x}$ ± S.D. (Number of Individuals).

Stage	**Cephalothorax Width (mm)**	**Stage duration (days)**
Juvenile I	0.70±0.03 (46)	9.74±2.71 (46)
Juvenile II	0.74±0.03 (20)	12.85±3.06(20)
Juvenile III	0.81±0.06 (3)	12.67±1.53(3)

Figure 1: Scanning Eletronic Microscopy of *Benthana cairensis*: A – Juvenile I, ventral view of male pleon, arrow indicates genital papilla, scale: 50 µm. B – Juvenile I, ventral view of female pleon, scale: 50 µm. C – male pereiopod 1 of Juvenile I, scale 50 µm. D – pereiopod 1 of a 0.9 mm CW male, scale 100 µm. E – pereiopod 1 of a 1.04 mm CW male, scale 200 µm. F – pereiopod 1 of a 1.27 mm of CW male, scale 200 µm. G – pereiopod 1 of an adult male, scale: 200µm. H – pereiopod 1 of an adult female, scale 200 µm.

Figure 2: Illustration of *Benthana cairensis* male reproductive apparatus. A – Juvenile I: genital papilla and pleopod 1 endopod and exopod. B – Juvenile II: genital papilla and pleopod 1 endopod and exopod. C – Juvenile III: genital papilla and pleopod 1 endopod and exopod. D – Juvenile II: genital papilla. E – Adult: genital papilla and pleopod 1 endopod and exopod. F – Juvenile I: Pleopod 2 endopod and exopod. G – Juvenile II: Pleopod 2 endopod and exopod. H – Juvenile III: Pleopod 2 endopod and exopod. I – Adulto: Pleopod 2 endopod and exopod. Scales: 0.1 mm.

Figure 3: Male´s pleopod 1 exopod of *Benthana cairensis*. A – Juvenile I. B – Juvenile II. C – Juvenile III. D – male with CW of 0.9 mm. E – male with CW of 1.0 mm of CW. Scales: 0.1 mm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1D
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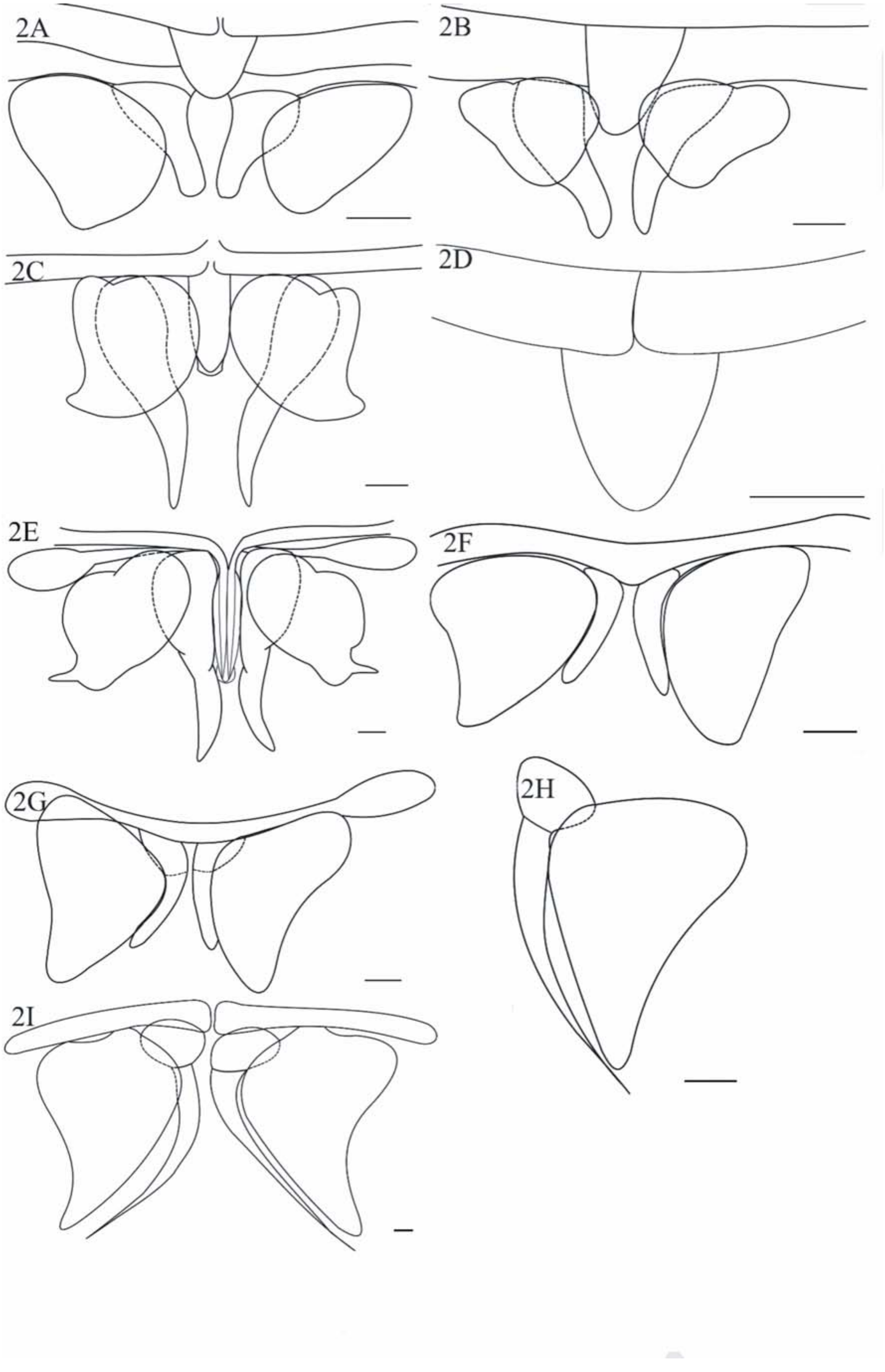
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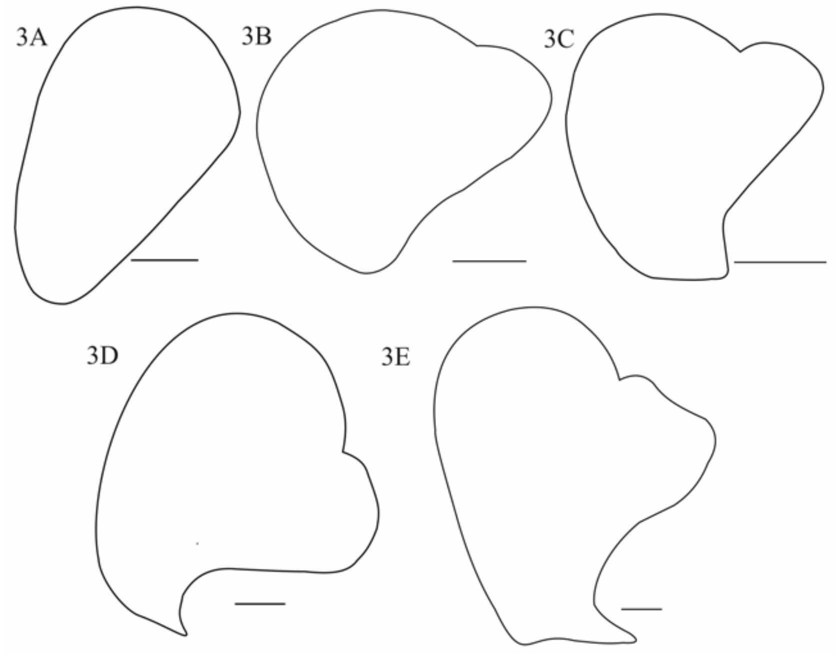
3A
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Capítulo IV

"Population characteristics and reproduction of a neotropical terrestrial isopod from Brazil (Isopoda, Philosciidae)"

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Sokolowicz, CC. & P.B. Araujo. Population characteristics and reproduction of a neotropical species of terrestrial isopod from southern Brazil (Isopoda, Philosciidae). **Acta Oecologica.**

Population characteristics and reproduction of a neotropical terrestrial isopod from Brazil (Isopoda, Philosciidae).

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Abstract

Population studies on terrestrial isopods are often carried out taking into account life history traits, such as size at sexual maturity, fecundity and life span, especially of temperate species and some tropical ones. In this study we focused on population structure and growth and reproductive biology of the neotropical species *Benthana cairensis*. Samples were taken during eighteen months at a small area in southern Brazil. *Benthana cairensis* showed a continuous reproduction throughout the year with a peak of reproductive females on spring and its mean fecundity was 10.4 eggs while mean reproductive allocation circa 17%. Females attain sexual maturity early in life and live over one and a half year. Males grow faster; attain smaller sizes than females, and live approximately thirteen months. The reproduction characteristics of *B. cairensis* is similar to those isopods in tropical climate, where females allocate relatively small amount of energy in one progeny are able to continue to grow and reproduce more than once at its life time.

Keywords: *Benthana cairensis*, reproductive biology, population growth.

Introduction

Terrestrial isopods are one of the most important invertebrate soil groups among the detritivore community, being closely linked to the nutrient cycle. It has been demonstrated that these isopods have great consumption ability which might represent the consumption of 16% of the annual input of leaves as demonstrated for *Atlantoscia floridana* (van Name, 1940) and *Balloniscus glaber* Araujo & Zardo, 1995 in a semi-deciduos forest in southern Brazil (Quadros and Araujo, 2008).

Studies on population and reproduction of terrestrial isopods include species from temperate, xeric, and in less number from subtropical and tropical regions. It is known that environmental conditions such as temperature, humidity, and rainfall periodicity and amount influence the population dynamics of isopods, mainly the reproduction (AlJetlawi & Nair, 1994; Warburg, 1994). Temperate species concentrate their reproduction period often on spring and summer (Warburg and Cohen, 1992; Sutton et al., 1984; Zimmer, 2003).

Among the species studied the worldwide *Armadillidium vulgare* (Latreille, 1804) has been widely studied in different regions (Lawlor, 1976; Hassall and Dangerfield, 1997; Al-Dabbagh and Block, 1981; Sorensen and Burkett, 1977; Souty-Grosset et al., 1998) and its reproductive pattern might change according to world region of occurence, females from low latitudes breed earlier than those from high latitudes (Souty-Grosset et al., 1998), but often the breed period occurs in hottest months (Lawlor, 1976; Al-Dabbagh and

Block, 1981). Other species had also been studied as *Trichoniscus pusillus* Brandt, 1833 (Standen, 1973; Sutton, 1968) and *Philoscia muscorum* Scopoli, 1793 (Suderland et al., 1976).

It has been demonstrated that tropical and subtropical species have an extented reproductive period (Dangerfield and Telford, 1995; Araujo and Bond-Buckup, 2005; Quadros et al., 2009) and show similarities in some other aspects of reproduction as low reproductive investment and higher number of broods per life time (Ma et al., 1991; Vink and Purwanti, 1994; Dangerfield and Telford, 1995; Quadros et al., 2008). On the other hand, species from xeric and arid environments often have their reproductive activity restricted to the wet season, which is the case of *Hemilepistus reaumurii* (Milne-Edwards, 1840) (Shachak and Yair, 1984; Warburg, 1992), *Porcellio laevis* Latreille, 1804 (Nair, 1984) and *Porcellio scaber* Latreille, 1804 (Nair, 1998).

In Brazil among the 120 species of terrestrial isopods recorded, only a few species had been studied concerning patterns of population dynamics, reproduction and life histories traits. Araujo and Bond-Buckup (2005) studied the population of the most common species inhabiting a Restinga forest in southern Brazil, *A. floridana*, which later had its life history traits described along with *B. glaber*, both living at the same site in a subtropical forest (Quadros et al., 2009). The widespread *Ligia exotica* Roux, 1828, a littoral species, also had aspects of its reproduction studied, in southern Brazil (Lopes et al., 2006). For these three species the growth patterns are also known (Araujo and Buckup, 2004; Lopes-Leitske et al., 2009; Meinhardt et al., 2007).

Description of population characteristics brings important information on how individuals are structured in the population. It is also a tool to understand life history traits that have direct effect on reproduction and survival, such as the growth pattern, age at maturity, size, number and sex ratio of offspring and reproductive output (Stearns, 1992).

Reproduction is probably one of the most important features of the life history of a species, being closely linked to fitness and bringing consequences to future growth. For some organisms that present indeterminate growth the energy allocated on reproduction could detract from growth and then decrease fitness at later stage (Brody et al., 1983) which constitutes a trade-off since female body size is positively related with the brood size (Lawlor, 1976; Brody et al., 1983; Quadros et al., 2009).

Although southern Brazil is not inserted in the tropical region it is considered at a subtropical climate, which is temperate with hot summers and no defined wet season. Based on this climate characteristics we hypothesize that *B. cairensis* has a pattern of reproduction similar to those of tropical species in which the population is able to reproduce during all year since environmental conditions are not so harsh in winter when compared with temperate regions.

Material and Methods

Study area and samplings

Individuals of *B. cairensis* were collected from March/2006 to August/2007, once in a month, outside the urban area in Taquara, southern Brazil (29° 46′S 50° 53′W) in a site of approximately ½ hectare. This place was initially a grassland field and at about 20 years ago it was planted with several vegetal species both introduced an endemic from the state of Rio Grande do Sul. Nowadays it is small woodland covered with brushy and arboreal species.

The animals were taken randomically within an area 18 m long and 6 m wide divided in 12 quadrants. In each quadrant a circular bottomless recipient of 30 cm diameter was used to delimit the sampling area; the leaf litter and a soil sample of about 2 cm deep was taken and stored in plastic bags. In the laboratory the extracted material was hand-searched to collect the isopods and then the content was left in a Berleze Funnel for 72h to ensure that all individuals were taken.

Extra samples were taken in sites near to the quadrants, to represent microhabitats that were supposed to be suitable to isopods, such as under tree branches and woods. The animals collected at these places were used to calculate the correlation between female body size and fecundity and also to calculate the growth curve.

The following microclimate variables were taken in each one of the 12 sites/month (quadrants): soil (3cm deep) and leaf litter temperature, and water content of leaf litter and soil, determined through the gravimetric method (Allen, 1984).

To verify if there was differential distribution of animals according to environmental parameters a multiple regression was performed, using the animal abundance as the dependent variable and the environmental variables as the independent one. Litter and soil temperature weren't used since showed high colinearity with air temperature ($r \geq 0.90$). All data were logaritimized and analyzed using Bio Estat 5.0 (Ayres et al., 2007). Cluster Analyses was performed using soil and litter water content, air temperature and humidity to verify possible grouping patterns within these abiotic factors during the months.

Population characteristics

In laboratory isopods were sorted out by sex, sex-undifferentiated state individuals (mancas) and reproductive status of females (ovigerous and post-ovigerous). All animals had the cephalothorax width (CW) measured under stereomicroscope (0.001 mm accuracy) (adapted from Araujo and Bond-Buckup, 2004).

To quantify population aggregation in each month the Morisita's Index of Dispersion (MID) was applied: $MID=[n(\sum i^2 - \sum i)/((\sum i)^2-\sum i)]$, where $n=12$ (samples per month) and i=number of individuals per sample (Elliot, 1983); this index is independent of the number of individuals in each sample (Araujo and Bond –Buckup, 2004; Quadros and Araujo, 2008).

Ovigerous females were separated into three categories according to the stage of development of the embryos: with eggs (EG), with embryos (EM) and with intra-marsupial mancas (MA) (Araujo and Bond-Buckup, 2005).

A relationship between number of ovigerous females and their mean fecundity at each month was made to attain the potential and the actual recruitment. The potential recruitment was obtained multiplying the average fecundity of the month by the number of ovigerous females of the month; the actual recruitment was obtained with the number of mancas in the population on that month.

The sex ratio was estimated through the operational sex ratio (OSR) (Araujo and Bond-Buckup, 2005) considering only females apt to reproduce, being excluded ovigerous and post ovigerous ones. To identify differences between the proportion of each sex, a χ^2 test was employed (α=0.05) for all samples.

Reproduction

Females were considered sexually mature by the presence of the marsupium, with or without eggs. We considered adult females based on the size of the smallest ovigerous females found in the population. We differentiated Potential Reproductive Females (PRF) and Actual Reproductive Females (ARF), which represented the total number of adult females of a given month and the total number of reproductive females of a month, respectively (Quadros and Araujo, 2007). This differentiation was made in order to calculate the Index of Reproductive Mobilization (IRM) of the population. According to

Quadros and Araujo (2007), if we consider that the maximum mobilization would be if 100% of females were adults (PRF=1) and if 100% of these females were reproductive (ARF=1), and that they were at its mean fecundity capacity these parameters multiplied would give the IRM_{max} of the species population. The observed IRM for each month was calculated as [(PRF x ARF x mean fecundity) x 100/IRM_{max}.

The relationship between the number of EG, EM and MA and females size (CL) was compared through Pearson´s correlation. Ovigerous females in the beginning of egg development were kept under 20°C ± 1 at a photoperiod of 12h dark and 12h light to follow development until hatching. To calculate egg production in the months in which there were ovigerous females, the following formula was used (Sutton 1968):

Embryo production = embryo standing crop x sampling interval/development time. The embryo standing crop refers to the total number of eggs found in a month and the sampling period the time interval between the months in which occurred ovigerous females.

The reproductive allocation was estimated through a gravimetric index proposed by Sutton et al. (1984), which is based on females and mancas dry weight to measure the proportion between the females' body mass and offspring body mass, being independent of female size (Dangerfield and Telford, 1995). The Reproductive Allocation (RA) is calculated as follows: RA = BM/FM x 100; where BM is the brood body mass (mg) and FM is the female body mass (mg). For that matter ovigerous females already in the late stage of development (bearing intramarsupial mancas) were dried for 72 h at 60 ˚C. Afterwards the mancas were taken from the marsupium and the female and brood were separately weighted.

Growth curve

Growth was analyzed through von Bertalanffy's model: $W_t=W_\infty[1-e^{-k(t+t0)}]$, where W_t is the cephalothorax width for a given age t (in days); W_∞ is the maximum mean cephalothorax width (in mm); k is the growth rate, and t_0 is a parameter related to age at length zero (in days) (von Bertalanffy, 1938).

The frequency distribution of class interval (0.08 mm) was used to follow the displacement of CW; this interval was defined based on one-fourth of the standard deviation of the mean size of males and females pooled together (Araujo and Buckup, 2004; Meinhardt et al., 2007). The first class interval included all mancas and represented the monthly recruitment for both males and females since mancas are not yet sexually dimorphic. The modes were chosen using the PeakFit 4.12 – SeaSolve Software Inc.®. After the modes were chosen they were transformed by the method of Ford-Walford (Walford, 1946) to obtain the parameters "a" and "b" through a linear regression.

The growth curve obtained for males and females separately was linearized (Allen, 1976): $W_t= a-b.r^t$, where $a= W_\infty$; $b= W_\infty.e^{kt0}$; and $r = (e^{-k}$ male + e^{-k} female$)/2$. In the transformation the dependent variable was calculated using the data obtained from the growth curve. The linear regressions were compared through ANCOVA, with a confidence interval of 95% (Snedecor and Cochran, 1967). Based on the growth curve it is possible to obtain approximately the life span of individuals. This is possible taking the size of the biggest animal found in the field and checking in the curve which is the corresponding age.

Results

Microclimate variables

Air temperature varied from 6.7 to 27.6°C ($\bar{x}$ =19.8°C) and litter and soil temperature varied from 7.10 to 25.4 ($\bar{x}$=17.8°C) and 9.4 to 23.3 ($\bar{x}$ =16.7°C), respectively. Litter water content ranged from 16.3 to 64 ($\bar{x}$ =35%) and soil water content varied from 6.4 to 22.4 ($\bar{x}$ =12.3%). The results of multiple regression showed no correlation between environmental variables and the animal abundance (Multiple R^2= 0.22, F=2.23, p=0.12).

The cluster analysis did not show a clear grouping regarding months abiotic variables although there was a clear distintiction of May/2007 as a completely separated group showing the lowest temperature (6.4°C) and a reduced abundance of individuals was found (60). The cluster also put together July and August/2007, the months which had the lowest abundance (52 and 42, respectively) (Fig. 1).

Population description

In total 1834 individuals were found. Of these 91 were mancas (4.96%), 830 were males (45.26%) and 792 were females (43.18%), of which 121 were reproductive (ovigerous and post-ovigerous) (6.6%) The cephalothorax width of males ranged from 0.64 to 1.74 mm (1.17±0.21), females from 0.62 to 2.00 mm

(1.18 ± 0.30), reproductive females from 1.32 to 2.10 mm (1.68 ± 0.18) and mancas from 0.54 to 0.70 (0.64 ± 0.03) (Fig. 2).

The month with the highest abundance was October/2006 with 79 males and 131 females, the winter months of July and August/2007 were the months with the lowest number of individuals with a total of 48 and 39 individuals, respectively. Reproductive females were present in almost all months with a peak of ovigerous females occurring on spring (Oct/Nov/Dec-2007). Mancas were often present in the population, with highest abundance on March/2006 and December/2006 (Fig. 3).

In general, the population presents animals of all sizes through the year, with no defined period of recruitment and reproductive females were often present. Males show a unimodal distribution and females a bimodal one. In general, individuals in the highest size classes are less abundant and females reach bigger size than males (Fig. 2).

Considering the OSR there were no statiscally differences between the proportion of males and females in population ($p>0.05$), accounting for a 1:1 sex ratio. When post-ovigerous females were included, females outnumbered males (1.02-1.00) ($p<0.05$) as well as when all males and females were considered (1.12-1) ($p<0.05$). Females were also significantly bigger than males ($F=40.36$, $p=0.0001$).

The population of *B. cairensis* showed an aggregate distribution (MID>1) which varied in intensity during the 18 months with the highest in April/2007 (2.5) and the lowest in May/2006 (1.1).

Reproduction and recruitment

The reproductive pattern of *B. cairensis* seems to be continuous through the year, since the lack of reproductive females in June/06 and January, June and August/2007 doesn't represent a pattern following a specific season. The mean fecundity was 10.4 eggs but varied, according to the stage of development of the brood mass: EG females = 10.9, EM females= 9.9 and MA = 9.3, the fecundity was only statistically different between EG and EM females ($F=4.42$, $p<0.05$).

Concerning the relationship of female body size and offspring there was a positive correlation between EG and EM females size and fecundity: 0.62 and 0.65 ($p>0.01$) respectively. On the other hand, there was no correlation between MA female size and fecundity ($p<0.01$). Concernig all females found during the sample months the highest fecundity was 18 eggs in a females of 2.05 CW.

The table I shows a description of the fecundity in the samples months related with the recruitment of population.

The highest IRM occurred on October (21%), November (15%) and December (23%) and PRF were present in all months (Table II). Females at onset of reproduction occur approximately at the size of 1.32 mm of CW, which corresponds to the smallest ovigerous female found in the population. From this size on there were always reproductive females at this size class and from the size class of 1.75 mm, 50% were ARF (Fig. 5).

The incubation period, based on 5 ovigerous females is 21 days. The estimated egg production was highest on spring (324 in October) (Table III).

Females use *circa* 17 % of its body mass on the progeny; the biggest female (11.1 mg) allocated 14% on brood mass and the smallest (5.5 mg) showed the highest allocation percentage, 23%. In average, individual manca mass represent 1.9 % of the total weight of females.

Population growth

The chosen modes (Table IV), obtained from the histograms of frequency distribution of CW of both males and females (fig. 4), were those which the values, after the linear regression, showed the best approximation with the maximum CW obtained in the population, since the $W\infty$ is obtained with the linear regression parameters. The resulted equation for male is W_t=1.98 [1-$e^{0.0555\ (t+59.46)}$], and for females W_t=2.39 [1-$e^{0.0378\ (t+69.10)}$] (figs. 6a,b).

From the growth curve it was possible to estimate the age of animals. Males reach biggest size at about 420 days old (1.15 years) and females at 660 days (1.8 years); this lifespan was estimated based on the size of animals found in field and corresponds to the biggest male (1.83 mm of CW) and female (2.23 mm of CW) found. Based on the size of the smallest ovigerous female, they reach sexual maturity with 160 days old and the population of females have their peak reproductive activity by the age of 280 days old.

The linearized curves obtained to compare the growth between males and females show that they show different growth curves (F= 579.86; $p>0.001$), the intercepts and slope are also different (F=4005.94; $p>0.001$) (Fig. 7 and Table V).

Discussion

Terrestrial isopods is a intersting group to perform studies of life histories traits, since they have a common pattern of morphology, physiology and behavior. That makes it easier also to compare these traits with woodlice from different habitats and regions. The population characteristics of *B. cairensis* are similar in several aspects with those isopods in tropical and other subtropical regions, like the extended reproductive period but show some features common of mostly isopods, for example the big and long living females.

Although there are four marked seasons on subtropical climate, the winter of 2006 did not show typical winter temperatures, the mean temperature on these months was 19.2 °C, especially on the samples of August in which the mean temperature was 23.2 °C. Low temperatures seem to affect the population abundance, only in the following year, when the months in which there were a fewest number of animals were those when the temperature was the lowest. The influence of environmental conditions on isopod population is well known and it can affect breeding length, incubation time and population structure throughout the year (Sunderland et al., 1976; Brody et al., 1983; Dangerfield and Telford, 1995).

Although there were no correlation of the environmental parameters and the abundance of animals, the cluster analysis showed some patterns that can be discussed. The separation of May/2007 from the rest of the months is related with the low number of animals found in this month; it is known than low temperature may affect the individuals in a way that they should find more suitable places, like sheltered places or lower layer in the soil (Sunderland et al., 1976; Brody et al., 1983).

Other aspect that is well known about woodlice population is its aggregate pattern of distribution (Takeda, 1984; Hassall et al., 2002; Tuck and Hassall, 2005), often gathered in microhabitat in which humidity, temperature e food availability are suitable. *Benthana cairensis* showed this aggregation pattern common to woodlice populations; Quadros and Araujo (2008) also found an aggregation pattern for four species of woodlice that also varied along the year.

Although there were some months when neither ovigerous nor post-ovigerous females were found this species probably reproduces during the whole year, since some extra samples were always taken during the period of field work and reproductive females were always found. Reproductive females are recognized either with brood mass or when still bearing only the marsupium. The marsupium is formed during the parturial molt and lasts until the following molt after the release of mancas (Sutton, 1968; Hoese, 1984), so females with the marsupium can be considered reproductive ones (Sutton, 1968). Continuous reproduction is a common feature of other subtropical species, as *A. floridana* studied in southern Brazil (Araujo and Bond-Buckup, 2005, Quadros et al., 2008). Other species living at tropical regions, as *P. pruinosos* rose in laboratory in Indonesia (Vink and Kurniawati, 1996) and also in Zimbabwe (Dangerfield and Telford, 1990) also show a continuous reproduction. An extended brooding period for *Burmoniscus ocellatus*, *Spherillo* *raffaelei* (cited as *Formosillo raffaelei*) and *Dryadillo maculatus* (cited as *Orodillo maculates*) was found in Hong Kong (Ma et al., 1991).

It is worth to mention that there were individuals of varied sizes through the year which is probably related to the continuous reproduction and consequently constant input of young. Mancas were not found in several months and it can be explained by a possible habitat preference show by the species. During the study period it was noticed that a great amount of animals were aggregated under three branches and woods, both providing moist and shelter in small crevices. Those microhabitats were highly used for females, ovigerous females and specially mancas (CCS, pers. obs.). This can explain the fact that both ovigerous females and mancas weren't so abundant on the random sampling but could be found when certain microhabitats, not included in the experinmental design, were explored.

Other fact that can corroborate the continuous reproduction was the relatively low IRM in population during the peak of reproduction (21% and 23%), this can result from a constant recruitment, as already mentioned to other populations (Quadros and Araujo, 2007; Quadros et al., 2008). The presence of animals in different size classes during a year has been observed for other species, as *A. floridana* and *Philoscia muscorum* (Araujo and Bond-Buckup, 2005; Sunderland et al., 1976). For the latter the authors stated that the cohort-splitting was an explanation, in that case some juveniles members of a single cohort could show differential growth, therefore reaching maturity and reproducing at distinct sizes. Although this cohort-splitting is possible to occur in the population herein studied, the existence of this different structure concerning size and reproductive status probably occur due to the continuous reproduction, especially due to the presence of ovigerous females all year round.

Concerning the sex ratio of males and females the great majority of studies take into account all females to calculate the proportion between sexes, most of them favoring females (Ma et al., 1991; AlJetlawii and Nair, 1994, Warburg et al., 2001**)** and sometimes showing different sex ratios depending on age (Moreau and Rigaud, 2000). The use of the OSR is one of the best tools to evaluate the proportion of males and females that are actually on reproductive status, since females with eggs do not reproduce and there's no evidence that post-ovigerous can do so (Moreau and Rigaud, 2000). These latter authors explain male-biased sex ratio with the fact that males are ready to reproduction faster than females and also that females are asynchronous concerning the onset of reproduction which leads to a higher proportion of males receptive to mating than females. That fact was also brought up by Araujo and Bond-Buckup (2005) that found a sex ratio favoring males in a population of *A. floridana*. The OSR on the present study demonstrated equilibrium on sex proportion when only receptive animals were considered, this condition can lead to the assumption that there is no competition among males for receptive females which can occur on population where males are in higher number. The presence of the feminizing bacteria *Wolbachia* which could distort this equilibrium on woodlice populations (Rigaud et al., 1999) has been discarded since there is no evidence of the bacteria on this population of *B. cairensis* (Almerão, pers. comm.).

Several studies have demonstrated the positive relation between female body size and number of offspring on isopods species from different world regions (Brody et al., 1983, Sutton, 1984; Quadros et al., 2009) since the brood development takes place in a limited space on female body; this positive relationship was also seen in the present study.

The pattern of an extended period of reproduction, lower reproductive investment and higher number of broods per life time found in *B. cairensis* is similar to those of species living in tropical and other subtropical regions. Often temperate species live longer, begin to reproduce later, allocate a great energy on a small brood and present a limited reproductive season (Sutton et al., 1984; Willows, 1987; Warburg, 1994). Reproductive allocation is predicted to be lower in tropical species probably due to a higher degree of iteroparity (Quadros et al. 2008). The mean value of 17% of reproductive allocation found in the present study is similar to those found in tropical species (Ma et al., 1991; Dangerfield and Telford, 2005) and for two neotropical species (Quadros et al., 2009).

According to Vink and Kurniawati (1996) a good strategy of iteroparous population is that after reaching maturity the female alternate periods of growth with periods of growth plus reproduction which implies that they can reproduce more than once in a season. The fact that females of *B. cairensis* reach maturity early in life, at about 6 months old and from this time on a great amount of females on population were always carrying eggs and also that the biggest females found in population had a mean fecundity similar to the small ones can demonstrate that this species allocate a small quantity of energy in one progeny although they might be capable of reproducing successfully during a longer period of time.

Although it is fully known that reproduction and growth constitute a major trade off on animal populations, species that reproduce repeatedly over their lifetime typically allocate energy to both reproductive and somatic growth (Brody et al., 1983). The low reproductive investment of *B. cairensis*, the small fecundity comparing to other Philosciidae, specially *A. floridana* which is smaller and bear similar number of eggs (the ovigerous females of this species have in average 1.3 mm of CW (Araujo and Bond-Buckup, 2005; Quadros et al., 2009)) and the rapid maturity, which also occurs in other neotropical and tropical species (Ma et al., 1991; Araujo et al. 2004) accomplishes for an itereoparous mode of reproduction. The positive relation between female size and number of offspring also corroborates the fact that biggest (oldest) females might not invest less on progeny than youngest ones and therefore, big size females have an advantage of a greater fitness.

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Table I: Number of ovigerous females, mean fecundity, potential and actual recruitment of *Benthana cairensis* during the sampling period.

	Ovigerous females	**Mean fecundity**	**Potential recruitment**	**Actual recruitment**
Mar/06	0	-	-	31
Apr/06	4	9.5	38	10
May/06	1	10	10	2
Jun/06	0	-	-	-
Jul/06	4	11	44	-
Aug/06	8	9.7	59	-
Sept/06	6	8.6	62	3
Oct/06	31	10.9	337	-
Nov/06	5	10.8	55	3
Dec/06	16	10.4	166	26
Jan/07	0	-	-	4
Feb/07	2	10	20	1
Mar/07	6	8.7	52	14
Apr/07	5	7.2	36	1
May/07	0	-	-	-
Jun/07	0	-	-	-
Jul/07	1	13	13	-
Aug/07	0	-	-	-

Table II: Potential reproductive females (PRF), Actual reproductive females (ARF) and index of reproductive mobilization (IRM) of female population of *Benthana cairensis* in each sample month.

	PRF	**ARF**	**IRM**
Mar/06	0.20	0.00	0%
Apr/06	0.32	0.23	7%
May/06	0.39	0.05	2%
Jun/06	0.40	0.00	0%
Jul/06	0.42	0.14	6%
Aug/06	0.44	0.16	6%
Sep/06	0.44	0.19	8%
Oct/06	0.74	0.27	21%
Nov/06	0.50	0.28	15%
Dec/06	0.50	0.46	23%
Jan/07	0.14	0.00	0%
Feb/07	0.31	0.17	5%
Mar/07	0.25	0.46	10%
Apr/07	0.23	0.50	8%
May/07	0.30	0.00	0%
Jun/07	0.46	0.00	0%
Jul/07	0.45	0.11	6%
Aug/07	0.28	0.00	0%

Table III: Number of ovigerous females, number of eggs, sample interval (in days) and estimated egg production of female population of *Benthana cairensis*.

	Number of ovigerous females	**Number of eggs**	**Sample interval**	**Egg production**
28/07/06	2	24	28	32
25/08/06	4	32	29	44
26/09/06	2	9	32	14
24/10/06	21	243	28	324
23/11/06	2	20	30	28
21/12/06	9	87	28	116
22/03/07	3	21	22	22
24/04/07	1	9	33	14

Table IV: Mode values of cephalothorax width and its respective correspondent days used to calculate de growth curves of of males and females of *Benthana cairensis*.

Males		**Females**	
Day	**Mode value**	**Day**	**Mode value**
1	0.55	1	0.55
36	0.77	36	0.79
64	0.96	64	0.95
127	1.34	99	1.11
155	1.43	127	1.27
187	1.49	155	1.35
-	-	306	1.83
-	-	342	1.91

Table V: Values of cephalothorax width (mm) and r^t used to linearize the growth curve of males and females of *Benthana cairensis* according to Allen's (1976) method.

		Cephalothorax Width	
Age (days)	r^t	**Males**	**Females**
20	0.9109	0.70	0.68
120	0.5714	1.25	1.22
220	0.3584	1.56	1.59
320	0.2249	1.74	1.84
420	0.1410	1.84	2.02
520	0.0885	1.90	2.14
620	0.0555	1.93	2.22
640	0.0506	1.94	2.23
660	0.0461	1.94	2.24

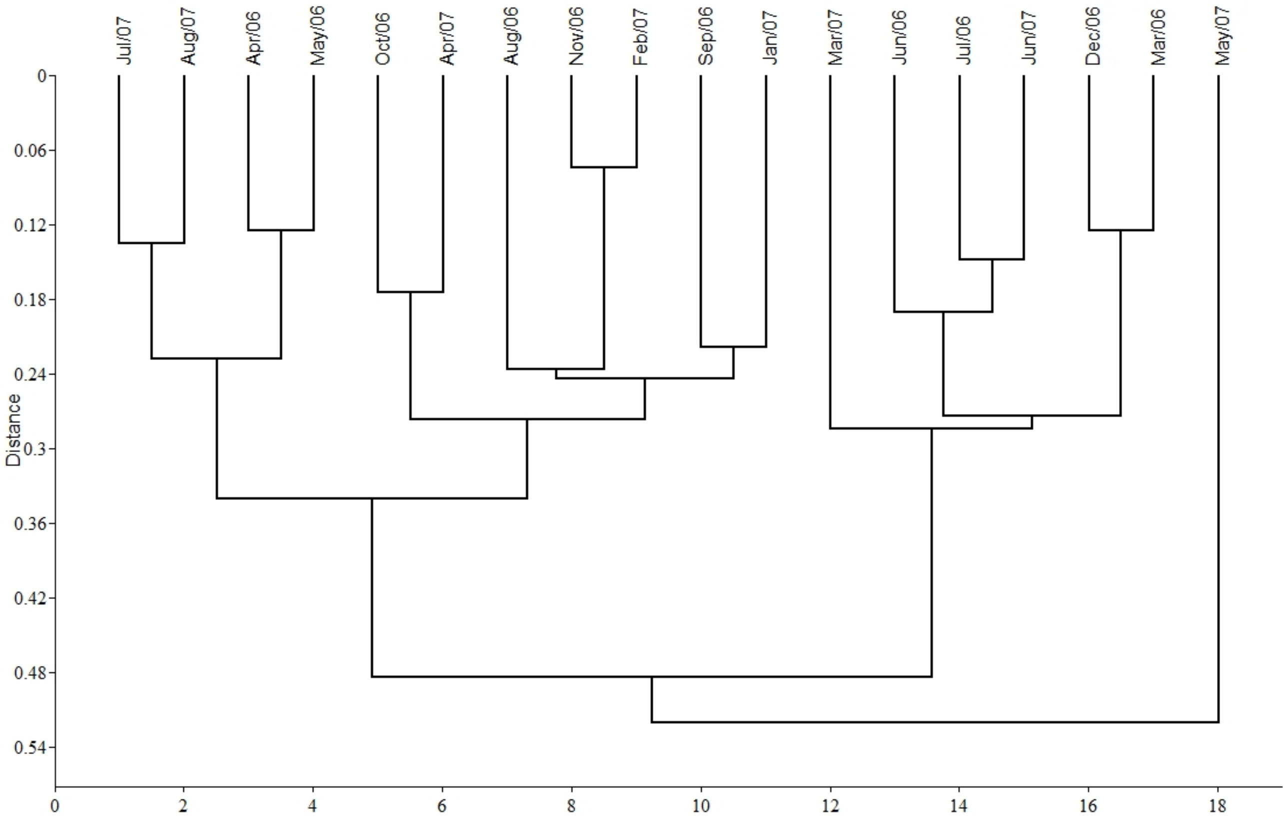

Jul/07
Aug/07
Apr/06
May/06
Oct/06
Apr/07
Aug/06
Nov/06
Feb/07
Sep/06
Jan/07
Mar/07
Jun/06
Jul/06
Jun/07
Dec/06
Mar/06
May/07
Distance
0
0.06
0.12
0.18
0.24
0.3
0.36
0.42
0.48
0.54
0
2
4
6
8
10
12
14
16
18

Figure 1

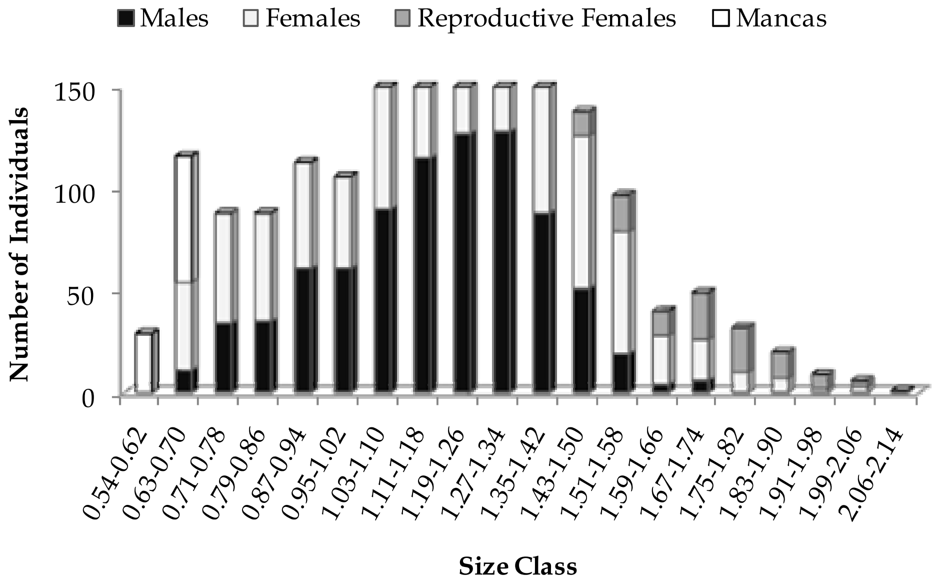

■ Males
□ Females
▨ Reproductive Females
□ Mancas
Number of Individuals
150
100
50
0
0.54-0.62
0.63-0.70
0.71-0.78
0.79-0.86
0.87-0.94
0.95-1.02
1.03-1.10
1.11-1.18
1.19-1.26
1.27-1.34
1.35-1.42
1.43-1.50
1.51-1.58
1.59-1.66
1.67-1.74
1.75-1.82
1.83-1.90
1.91-1.98
1.99-2.06
2.06-2.14
Size Class

Figure 2

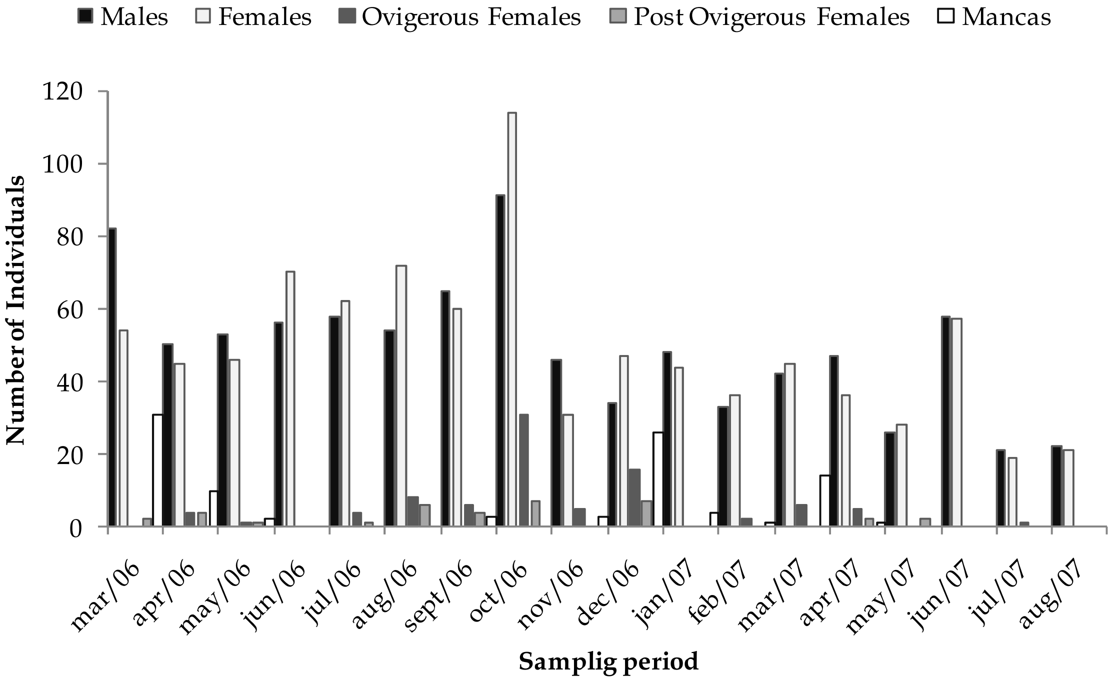
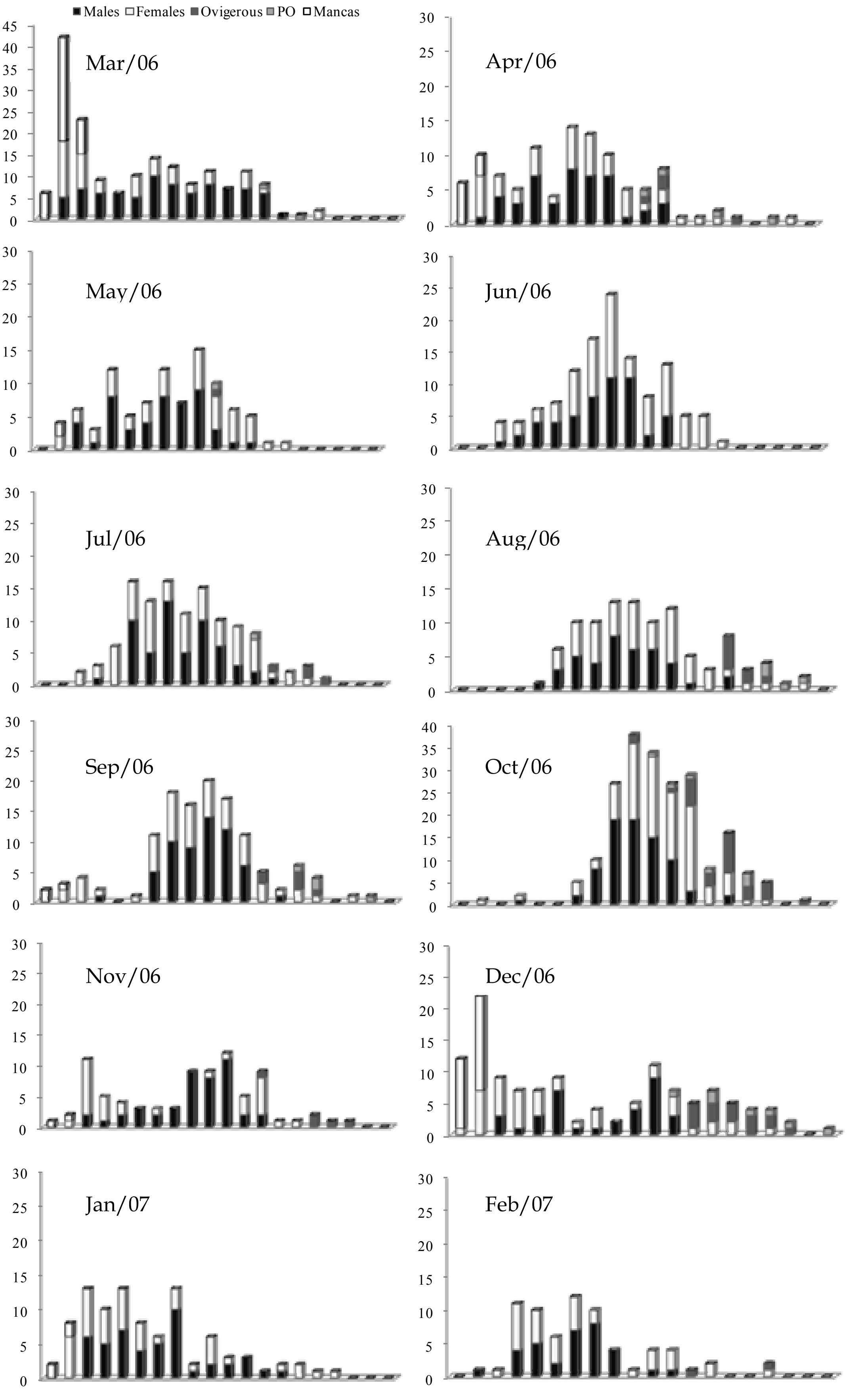

■ Males
□ Females
■ Ovigerous Females
■ Post Ovigerous Females
□ Mancas
Number of Individuals
120
100
80
60
40
20
0
mar/06
apr/06
may/06
jun/06
jul/06
aug/06
sept/06
oct/06
nov/06
dec/06
jan/07
feb/07
mar/07
apr/07
may/07
jun/07
jul/07
aug/07
Samplig period

Figure 3


■ Males □ Females ■ Ovigerous ■ PO □ Mancas
Mar/06
Apr/06
May/06
Jun/06
Jul/06
Aug/06
Sep/06
Oct/06
Nov/06
Dec/06
Jan/07
Feb/07
0
5
10
15
20
25
30
35
40
45

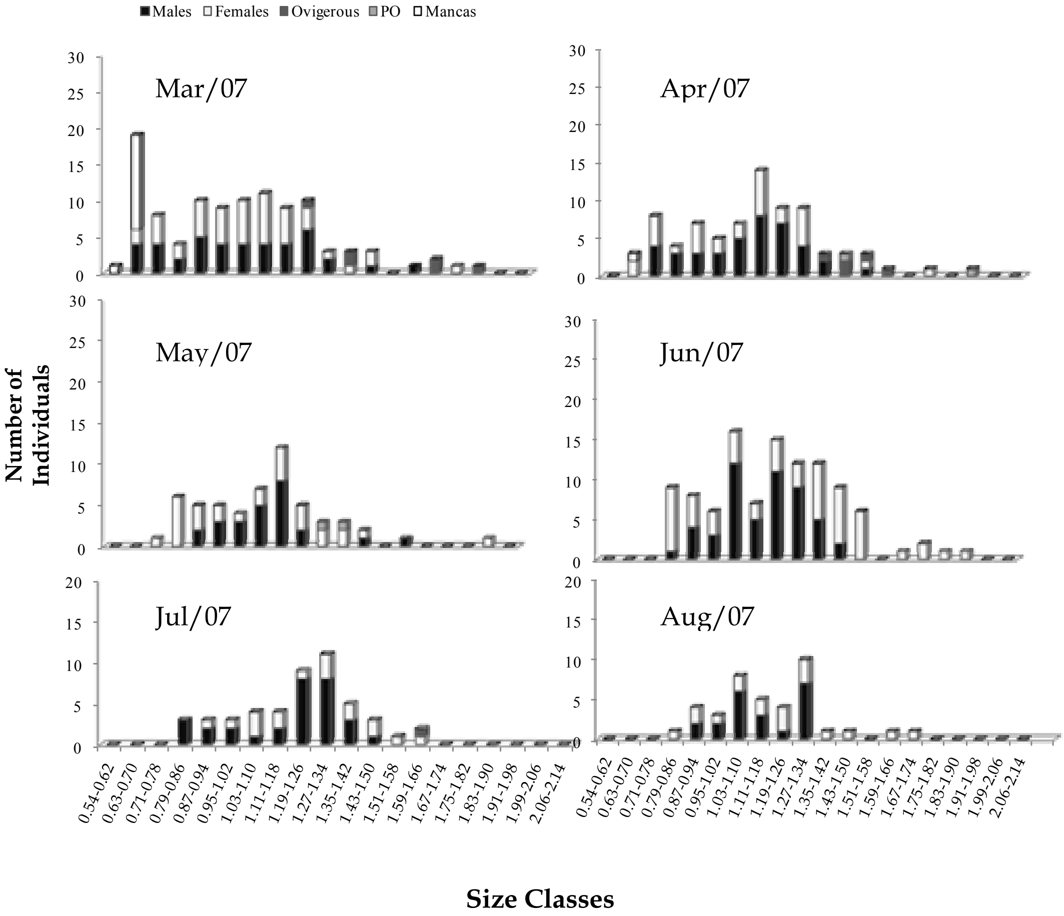

■ Males □ Females ■ Ovigerous ■ PO □ Mancas
Mar/07
Apr/07
May/07
Jun/07
Jul/07
Aug/07
Number of Individuals
Size Classes
0
5
10
15
20
25
30
0.54-0.62
0.63-0.70
0.71-0.78
0.79-0.86
0.87-0.94
0.95-1.02
1.03-1.10
1.11-1.18
1.19-1.26
1.27-1.34
1.35-1.42
1.43-1.50
1.51-1.58
1.59-1.66
1.67-1.74
1.75-1.82
1.83-1.90
1.91-1.98
1.99-2.06
2.06-2.14

Figure 4

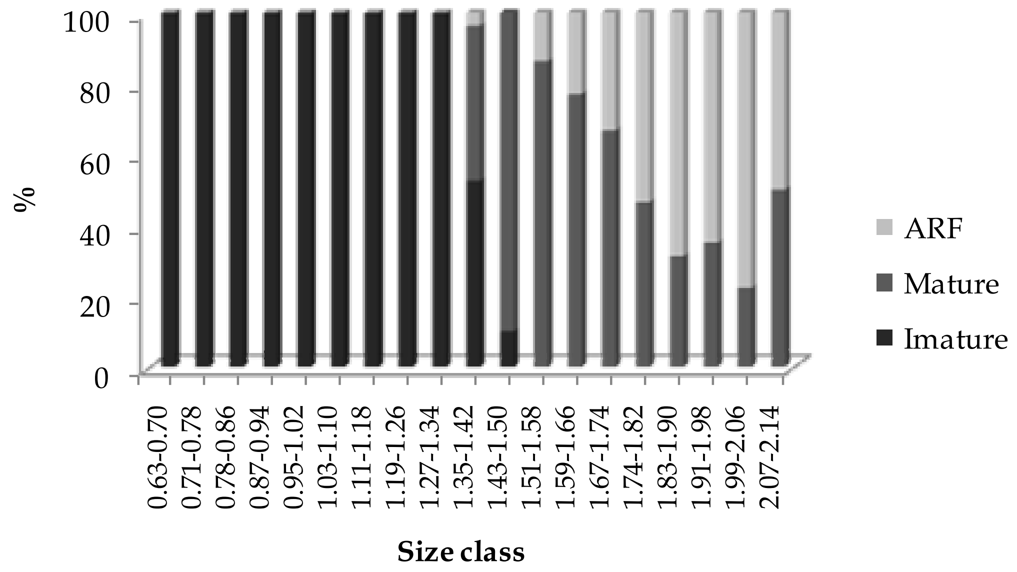

100
80
60
40
20
0
%
0.63-0.70
0.71-0.78
0.78-0.86
0.87-0.94
0.95-1.02
1.03-1.10
1.11-1.18
1.19-1.26
1.27-1.34
1.35-1.42
1.43-1.50
1.51-1.58
1.59-1.66
1.67-1.74
1.74-1.82
1.83-1.90
1.91-1.98
1.99-2.06
2.07-2.14
Size class
ARF
Mature
Imature

Figure 5

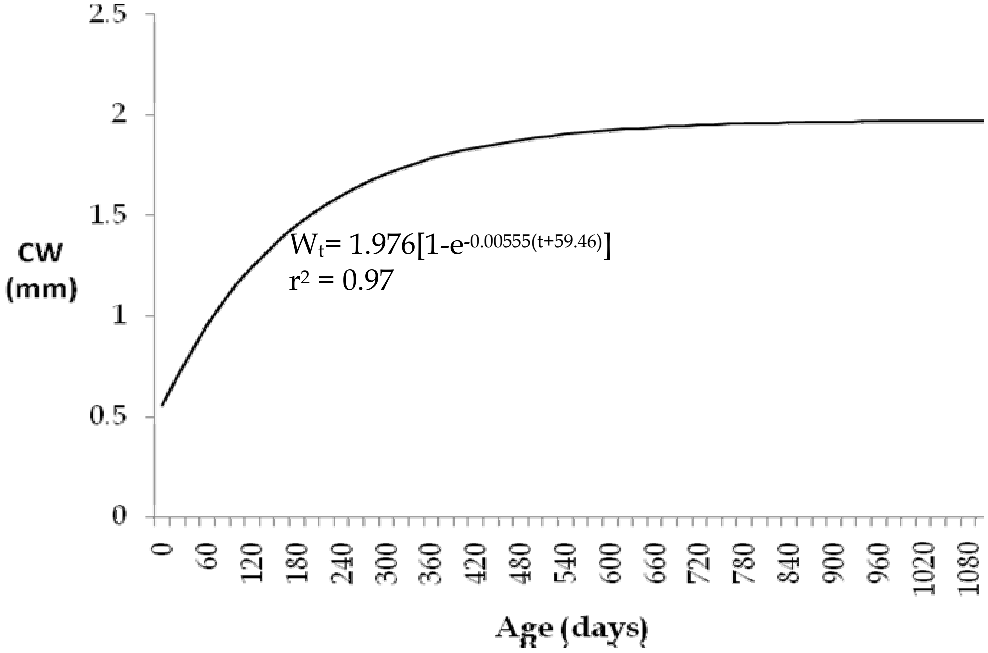


Figure 6a

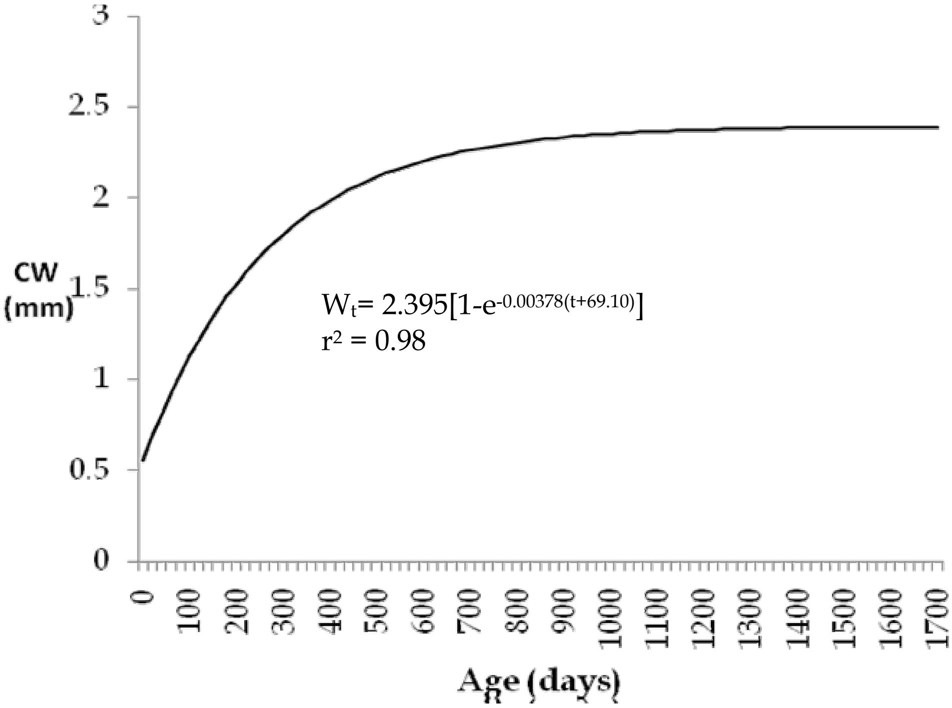


Figure 6b

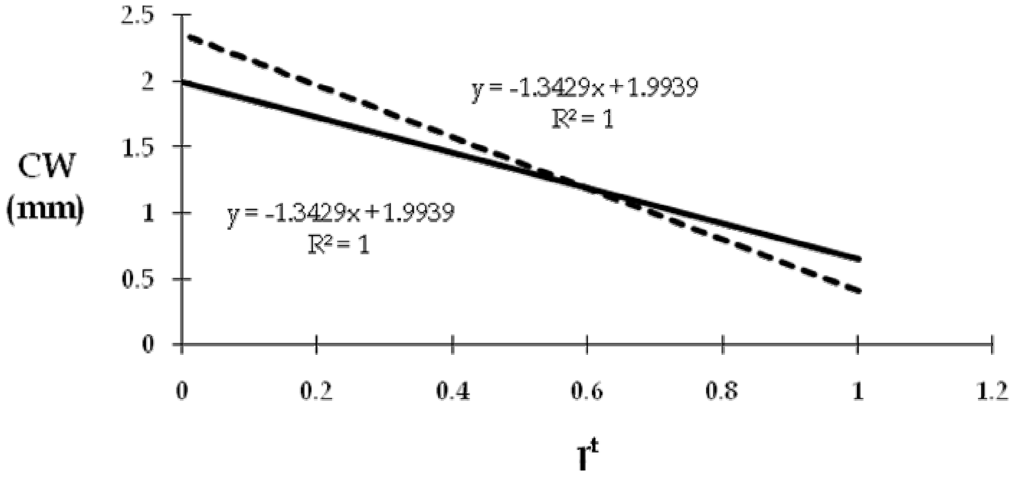

CW
(mm)
2.5
2
1.5
1
0.5
0
y = -1.3429x + 1.9939
R² = 1
y = -1.3429x + 1.9939
R² = 1
0
0.2
0.4
0.6
0.8
1
1.2
I^t

Figure 7

Figure captions

Figure 1: Clustering dendogram (UPGA – Euclidian Distance) of sampling variables collected from March/06 to August/07 in relation to litter water content, soil water content, humidity and air temperature.

Figure 2: Number of males, females, reproductive females and mancas per size classes of *Benthana cairensis* collected during the period of March/2006 to August/2007.

Figure 3: Number of males, females, ovigerous females, post-ovigerous females and mancas of *Benthana cairensis* coolected from March/06 to August/07.

Figure 4: Frequency distribution of cephalothorax width for males, females, Ovigerous females and post ovigerous females (PO) of *Benthana cairensis* collected during the sampling period.

Figure 5: Percentage of immature females, mature females and ARF of *Benthana cairensis* per size clases collected during March/06 to August/07.

Figure 6: **(A)** Growth curve of cephalothorax width (CW) of males of *Benthana cairensis*. **(B)** Growth curve of cephalothorax width (CW) of females of *Benthana cairensis*. W_t represents the cephalothorax width of individuals on time t; $W\infty$ is the cephalothorax maximum mean and t is the age (days). Coefficient of determination from the transformed regression, according with Ford-Walford method (Walford, 1946).

Figure 7: Linearized regression of the cephalothorax width (mm) of males and females of *Benthana cairensis* in relation to transforned ages (r^t).

Considerações Finais

Frente as mais de 120 espécies de Oniscidea existentes no Brasil, uma porção muito pequena destas teve aspectos de sua biologia estudados; quando iniciaram-se as pesquisas com esse grupo os trabalhos concentraram-se em descrições de espécies e novos registros de ocorrência. Recentemente, os estudos acerca desses animais no país estão focados nas pesquisas das espécies do sul, mas especificamente do estado do Rio Grande do Sul, aumentando o conhecimento sobre morfologia, sistemática, ecologia e comportamento.

O estudo da biologia básica de uma espécie é de extrema importância, servindo de subsídio para estudos futuros, inclusive de biologia aplicada. Considerando-se a importânca dos isópodos terrestres no ecossistema da fauna de solo, o conhecimento de novas espécies e a descrição de suas características e dinâmica populacional torna-se uma ferramenta essencial para o avanço dos estudos nesse grupo de crustáceos.

Quando se iniciaram os estudos com as espécies brasileiras as comparações entre os padrões de desenvolvimento, morfologia, ecologia populacional eram feitas com espécies de ocorrência principalmente no hemisfério norte, onde os estudos com isópodos começaram a ser reliazados há muito mais tempo e há mais informações a respeito de sua biologia. Com o avanço dos estudos das espécies existentes na região subtropical foi possível realizar comparações entre padrões dessas espécies com as de regiões temperadas, além das áridas e tropicais, sendo possível assim a caracterização de um padrão diferenciado, principalmente no que se refere a características de história de vida de espécies, daquelas vivendo em regiões temperadas.

A descrição de mais uma espécie de *Benthana* amplia o número de espécies do gênero descritas para o Estado do Rio Grande do Sul, onde são conhecidas 6 espécies, o que representa 25% das espécies conhecidas so gênero.

As informações adquiridas durante o desenvolvimento dessa tese não representam conclusões finais, e sim representam indagações essenciais que levam a novas investigações. A indicação de que fêmeas ovígeras possam procurar locais diferenciados para abrigar-se, mostrando um padrão diferente dos outros membros da população, traz um número de questões que podem vir a ser exploradas, como por exemplo se a proteção oferecida por esses abrigos realmente compensaria o gasto energético de procurar tais locais, que são mais raros no ambiente em que vivem; se fêmeas que não acham tais locais têm alguma desvantagem no que se refere ao "fitness" em relação às fêmeas que os encontram. A mesma questão poderia ser aplicada a indivíduos que iniciam seu desenvolvimento nesses sítios específicos e aqueles que se desenvolvem na serapilheira circundante.

Outra questão que poderia ser explorada em relação aos caracteres sexuais secundários dos machos é o fato de apresentarem dimorfismo em relação ao exópodo do pleópodo 1 e uma quantidade muito maior de setas nos quatro primeiros pares de pereiópodos. A projeção lateral presente no primeiro pleópodo dos machos é uma autapomorfia do gênero *Benthana* e o dimorfismo sexual presente nas setas dos pereiópodos é um padrão comum em diversas espécies do gênero. No entando não há registo de estudos que tenham buscado inferir alguma relação dessas características com o sucesso reprodutivo dos machos, se são estruturas que auxiliam de alguma forma no momento da cópula, se constituem algum tipo de atrativo para as fêmeas ou se conferem somente algum tipo de proteção para o aparelho copulatório, no caso do exópodo do pleópodo 1.

Enfim, a finalização de uma tese é o início de uma gama de opções para a realização de estudos diversos, ainda mais quando se trata de uma espécie que ainda não possuia nenhum aspecto de sua história de vida estudado.

A seguir, os principais resultados encontrados no presente trabalho:

- *Benthana cairensis* é diferenciada de *B. olfersii*, espécie mais assemelhada, por não apresentar um lobo com setas no mero do pereiópodo 7 dos machos, característica marcante de *B. orfersii*. Ainda, essa última é incluída do subgênero *Benthanoscia* pelo fato dos machos apresentarem dimorfismo nos ramos dos urópodos.
- A espécie apresenta três estágios de manca. MI é o estágio mais curto, com duração aproximada de 4 horas e que apresenta a morfologia mais simples, não mostrando ainda características que permitam a identificação do gênero.
- Os estágios de MII e MII já apresentam características genéricas o que facilita sua identificação, mesmo se tratando de um indivíduo com características juvenis.

- Os estágios de juvenil são diferenciados sexualmente e os machos podem ser diferenciados pela presença da papila genital e diferenças morfológicas dos endópodos e exópodos dos pleópodos 1.
- A completa diferenciação sexual no que se refere ao dimorfismo sexual das setas dos pereiópodos só é completamente evidente quando os machos atingem 1.2 mm de largura de cefalotórax; o que pode ser considerado o início da maturidade sexual.
- A população de *B. cairensis* do Sítio Cairé é caracterizada pela seguinte proporção em relação à composição etária e de sexo: 4.96% de mancas, 45.26% de machos, 43.18% de fêmeas e 6.6% de fêmeas reprodutivas.
- A proporção sexual operacional é de 1:1; machos são menores e vivem menos que as fêmeas, as quais atingem um maior tamanho corporal.
- Há fêmeas reprodutivas durante todas as estações do ano e elas investem menos em uma única prole quando comparadas com fêmeas de espécies que apresentam reprodução sazonal.
- O estratégia reprodutiva da espécie parece ser a de uma reprodução contínua, com fêmeas aptas para a reprodução em todas as épocas do ano, podendo essas apresentar mais de um evento reprodutivo.