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INSTITUTO DE BIOCIÊNCIAS - RIO CLARO

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS
BIOLOGIA CELULAR E MOLECULAR

MECANISMOS DE DIFERENCIAÇÃO CROMOSSÔMICA EM 13 ESPÉCIES DE ELATERIDAE (COLEOPTERA, POLYPHAGA) ESTABELECIDOS ATRAVÉS DA ANÁLISE DE CÉLULAS MITÓTICAS E MEIÓTICAS

MARIELLE CRISTINA SCHNEIDER

Tese apresentada ao Instituto de Biociências, da Universidade Estadual Paulista "Júlio de Mesquita Filho", Campus de Rio Claro, como parte dos requisitos para obtenção do título de Doutor em Ciências Biológicas (Biologia Celular e Molecular)

RIO CLARO
Estado de São Paulo - Brasil
Fevereiro/2006

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Orientadora: Profa. Dra. Doralice Maria Cella

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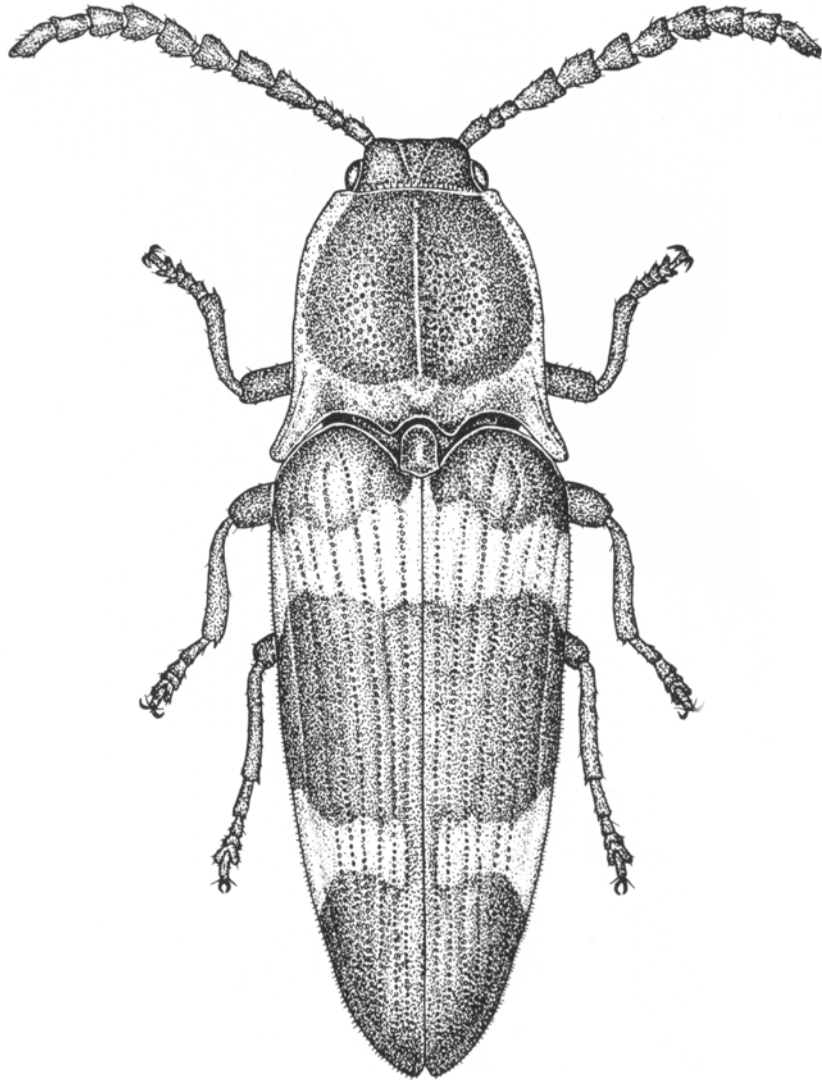
1. RESUMO

A família Elateridae possui cerca de 9.300 espécies descritas taxonomicamente, as quais estão agrupadas em 18 subfamílias; porém, menos de 1% destas foram analisadas sob o ponto de vista citogenético. O objetivo deste trabalho é caracterizar citogeneticamente 13 espécies da família Elateridae, pertencentes à subfamília Agrypninae, tribo Conoderini (*Conoderus dimidiatus*, *Conoderus fuscofasciatus*, *Conoderus malleatus*, *Conoderus rufidens*, *Conoderus scalaris*, *Conoderus stigmosus*, *Conoderus ternarius* e *Conoderus* sp.), subfamília Agrypninae, tribo Pyrophorini (*Pyrearinus candelarius*, *Pyrophorus divergens* e *Pyrophorus puncatissimus*), e subfamília Elaterinae, tribo Agriotini (*Cardiorhinus rufilateris* e *Pomachilus* sp.2), visando estabelecer as principais estratégias de diferenciação cromossômica que ocorreram nestas espécies. O estudo cromossômico de oito espécies da tribo Conoderini evidenciou que sete espécies compartilham um cariótipo similar, ou seja, 2n(♂)=16+X0 e 2n(♀)=16+XX, com a maioria dos cromossomos acrocêntricos. A única exceção foi o número diplóide 2n=14 e o sistema de determinação sexual neoXY/neoXX detectados em *Conoderus stigmosus*, os quais ainda não haviam sido descritos para espécies de Conoderini e provavelmente, são mais diferenciados que aqueles observados em todos os outros representantes da tribo. As três espécies de Pyrophorini exibiram a fórmula cariotípica 2n(♂)=14+X0 e 2n(♀)=14+XX, com cromossomos

predominantemente acrocêntricos. Os dois representantes da subfamília Elaterinae (Agriotini) mostraram cariótipos distintos, isto é, *Cardiorhinus rufilateris* apresentou 2n(♂)=18+X0 e *Pomachilius* sp.2 revelou 2n(♂)=18+Xy_p, com cromossomos submetacêntricos e acrocêntricos. Através da análise de células testiculares meióticas foi possível notar que as 13 espécies de Elateridae apresentam cromossomos com comportamento regular durante a meiose e que o bivalente sexual neoXY de *Conoderus stigmosus* possui um quiasma terminal. O emprego da técnica de bandamento C foi realizado em seis espécies de Conoderini e revelou a ocorrência de grandes blocos de heterocromatina constitutiva na região pericentromérica de quase todos os cromossomos e de blocos adicionais na região terminal ou intersticial de alguns cromossomos. Além disso, heteromorfismo de regiões banda C positivas foi observado nos autossomos de algumas espécies. A técnica de bandamento C também foi útil para mostrar que os cromossomos sexuais neoX e neoY de *Conoderus stigmosus* já estão bastante diferenciados com relação a composição e organização da cromatina, visto que o cromossomo neoX apresenta heterocromatina constitutiva na região pericentromérica e o cromossomo neoY é quase inteiramente heterocromático. Células gonadais mitóticas e meióticas de oito espécies de Agrypninae (Conoderini e Pyrophorini), quando submetidas à impregnação pelo íon prata, revelaram que a maioria das espécies possui 2 RONs localizadas em autossomos de tamanho grande ou médio; porém, duas espécies exibiram 4 RONs autossômicas. O uso da tríplice coloração CMA_3/DA/DAPI nos cromossomos de seis representantes das tribos Conoderini e Pyrophorini evidenciou, em quase todas as espécies, uma região rica em seqüências de base GC associada a RON, e em três espécies de Conoderini, algumas regiões coradas pela Cromomicina A_3 foram coincidentes com a heterocromatina constitutiva. As principais estratégias de diferenciação cromossômica detectadas nas espécies de Elateridae são apresentadas e discutidas neste trabalho.

Palavras-chave: bivalente, cariótipo, citogenética, evolução, heterocromatina constitutiva, nucléolo, quiasma, região organizadora de nucléolo, sistema de determinação sexual



Platycrepidius bicinctus

2. INTRODUÇÃO E REVISÃO BIBLIOGRÁFICA

2.1. CONSIDERAÇÕES GERAIS SOBRE OS COLEOPTERA

A ordem Coleoptera, com aproximadamente 357.899 espécies descritas taxonomicamente, é considerada a mais rica e variada da classe Insecta (Costa, 2003) e compreende cerca de 40% de todos os insetos e 30% dos animais existentes (Lawrence e Britton, 1994). Na região Neotropical, são conhecidas 127 famílias, 6.703 gêneros e 72.476 espécies; porém, este número de espécies certamente será superior a 100.000, após uma revisão do número de representantes existentes em cada família (Costa, 2003).

Uma das características mais importantes que contribuiu para a enorme diversidade e sucesso adaptativo dos coleópteros aos diferentes hábitats foi o aparecimento dos élitros, o que resultou em uma maior proteção das asas. Além disso, outras modificações morfológicas no corpo dos besouros, acompanhadas pela redução de membranas expostas na superfície corpórea foram importantes para a proteção contra predadores e para a exploração de diversos nichos (Lawrence e Britton, 1994).

De acordo com algumas características morfológicas, os representantes da ordem Coleoptera podem ser agrupados em quatro subordens: Archostemata, Myxophaga, Adephaga e Polyphaga (Lawrence e Newton, 1982).

A subordem Archostemata é um grupo que retém algumas características primitivas, apresentando morfologia similar aos primeiros besouros que constam em registros fósseis datados de 250 milhões de anos atrás (Galián e Lawrence, 1993; White, 1983). Esta subordem possui o menor número de representantes dentre os Coleoptera, pois em todo o mundo existe apenas cerca de 30 espécies descritas. No Brasil são conhecidas somente cinco espécies. Os membros desta subordem são encontrados em madeiras semidecompostas de áreas florestadas (Costa, 1999, 2003). Com aproximadamente 75 espécies, a subordem Myxophaga é considerada a segunda menor entre os coleópteros em número de espécies. Na fauna brasileira, os Myxophaga estão representados por 34 espécies. Os representantes desta subordem são extremamente pequenos e vivem associados a ambientes aquáticos, semi-aquáticos ou higropétricos (Costa, 1999, 2003). A subordem Adephaga, com 34.707 espécies, é a segunda maior entre os Coleoptera e possui, em sua maioria, espécies predadoras de outros insetos, tanto no estágio larval como adulto. A fauna brasileira de Adephaga é ainda muito pouco conhecida, na qual encontra-se apenas 1.139 espécies (Costa, 1999, 2003).

A subordem Polyphaga, com 293.293 espécies, inclui mais de 90% dos coleópteros conhecidos e exibe a maior diversidade estrutural e biológica de espécies, quando comparada às outras três subordens (Lawrence e Newton, 1982; Costa, 1999). No Brasil, os Polyphaga são representados por 88 famílias, 4.074 gêneros e 24.687 espécies (Costa, 1999). Dentre os Polyphaga encontra-se a família Elateridae, a qual tem aproximadamente 9.300 espécies descritas, sendo que 590 ocorrem na fauna brasileira (Costa, 2003). Os representantes desta família estão agrupados em 18 subfamílias e são cosmopolitas em distribuição (Lawrence e Newton, 1995; Lawrence *et al.*, 2000).

Os elaterídeos são peculiares pela sua capacidade de saltar e produzir um ruído característico (tec-tec), sendo descritos na literatura como “click-beetles”. Além destas características, algumas espécies apresentam dois pontos luminescentes na margem posterior do protórax, sendo facilmente

reconhecidas à noite, pela emissão de uma luz esverdeada (Borror, 1988; Johnson, 2002). Os indivíduos adultos possuem o corpo alongado, de lados paralelos e forma arredondada em ambas as extremidades; o tamanho é bastante variável. Geralmente são herbívoros, fungívoros e detritívoros, e ocorrem em flores, folhas e sob cascas de árvores. As larvas, delgadas, de corpo duro e brilhante, têm diferentes hábitos alimentares, dependendo da espécie, podendo ser saprófagas, fungívoras, carnívoras e herbívoras, alimentando-se de sementes e raízes de diversas plantas cultivadas. As larvas vivem geralmente no solo ou em troncos de árvores (Borror, 1988; Gillott, 1995; Marinoni *et al.*, 2001).

2.2. CARACTERÍSTICAS CITOGENÉTICAS DOS COLEOPTERA

A ordem Coleoptera possui mais de 3.000 espécies analisadas sob o ponto de vista citogenético (Petitpierre, 1996) e evidencia uma grande diversidade de número cromossômico, sendo o 2n=4 do elaterídeo *Chalcolepidius zonatus* Eschscholtz, 1829 (Ferreira *et al.*, 1984) e o 2n=69 do carabídeo *Ditomus capito* Serville, 1821 (Serrano, 1981), o menor e o maior número diplóide, respectivamente, já verificado entre os coleópteros. Os tipos de sistemas cromossômicos de determinação sexual também são muito variáveis e podem ser aquiasmáticos, como o Xy_p, Xy_c, X0 e X_1+X_2, ou quiasmáticos, como o neoXY, Xy, Xy_r, X_1X_2Y e XY_1Y_2 (Smith e Virkki, 1978).

O cariótipo $2n=20=18+Xy_p$, exibindo todos os cromossomos do complemento metacêntricos, é proposto como ancestral para os besouros da subordem Polyphaga e tem sido encontrado na maioria das famílias que tem alguns representantes estudados citogeneticamente (Smith e Virkki, 1978; Galián e Lawrence, 1993). Além disso, o sistema de determinação sexual do tipo Xy_p já foi observado em alguns gêneros considerados primitivos, tais como *Forsterita* (Chrysomelidae) e *Glaresis* (Glaresidae), bem como em espécies de famílias derivadas, como Brentidae e Curculionidae (Smith e Virkki, 1978).

No sistema de determinação sexual do tipo Xy_p, o X e o y representam os cromossomos sexuais e a letra "p" indica a forma de associação dos cromossomos durante a meiose, a qual foi originalmente comparada a um

pára-quedas por Stevens, em 1906 (*apud* Smith e Virkki, 1978). Neste sistema, o cromossomo sexual X representa o pára-quedas e o cromossomo y, conectado ao X por estruturas semelhantes a dois fios tênues, representa a carga (Stevens 1906 *apud* Smith e Virkki, 1978).

2.2.1. Informações citogenéticas da família Elateridae

As informações citogenéticas da família Elateridae estão restritas a 81 espécies, pertencentes a quatro diferentes subfamílias, Agrypninae, Cardiophorinae, Denticollinae e Elaterinae (Smith e Virkki, 1978; Ferreira *et al.*, 1984; Vidal, 1984; Virkki *et al.*, 1984; Virkki e Denton, 1987; Yadav e Vyas, 1993, 1994; Rozek e Lachowska, 2001; Rozek *et al.*, 2004). A família Elateridae caracteriza-se por apresentar uma grande heterogeneidade de números cromossômicos, os quais variam de 2n=4 (Ferreira *et al.*, 1984) a 2n=23 (Virkki, 1962), e de sistemas cromossômicos de determinação sexual, os quais podem ser do tipo Xy_p, X0, XY, neoXY e X_1X_2Y ($X_p$neoXneoy_p) (Smith e Virkki, 1978; Vidal, 1984; Virkki *et al.*, 1984; Virkki e Denton, 1987; Yadav e Vyas, 1993, 1994; Rozek e Lachowska, 2001; Rozek *et al.*, 2004). Apenas em 15 espécies desta família existem descrições sobre a morfologia cromossômica (Smith, 1956; Banerjee, 1959; Piza, 1960; Agarwal, 1962; Kacker, 1963; Ferreira *et al.*, 1984; Virkki *et al.*, 1984; Virkki e Denton, 1987; Yadav e Vyas, 1993, 1994; Rozek e Lachowska, 2001), nas quais é possível verificar uma predominância de cromossomos acrocêntricos.

Uma explicação para a diversidade cariotípica encontrada na família Elateridae seria a modificação do cariótipo 2n=18+Xy_p, considerado ancestral para os Polyphaga, através de fissões ou fusões autossômicas, levando a um aumento ou diminuição do número cromossômico, respectivamente. Segundo Smith (1950), as fusões entre o cromossomo X e os autossomos ou a simples perda do cromossomo y_p seriam os rearranjos mais freqüentes que originaram os diferentes tipos de sistemas de determinação sexual em Elateridae.

O sistema de determinação sexual do tipo Xy_p é o mais freqüente entre os Coleoptera e ocorre em 22% das espécies de Elateridae estudadas (Smith 1950, 1953, 1960; Smith e Virkki, 1978; Rozek *et al.*, 2004). O cromossomo

sexual X_p deste sistema, geralmente é um metacêntrico de tamanho médio e o cromossomo y_p é um metacêntrico extremamente pequeno. Existem diferentes evidências para explicar a forma de associação do bivalente sexual Xy_p durante a meiose; porém, as opiniões nos primeiros estudos realizados em Coleoptera oscilavam entre uma associação nucleolar ou quiasmática entre estes cromossomos (Smith e Virkki, 1978).

Stevens (1905 *apud* Smith e Virkki, 1978), analisando três espécies de Chrysomelidae portadoras do sistema de determinação sexual Xy_p, inferiu que a configuração em pára-quedas era devido à ocorrência de nucléolo no bivalente sexual. Smith (1951) sugeriu que a associação dos cromossomos sexuais Xy_p durante a meiose seria por emparelhamento de segmentos terminais, isto é, o pára-quedas seria na verdade um bivalente em anel composto por dois cromossomos metacêntricos de tamanhos diferentes.

Observando as células meióticas de alguns besouros portadores do sistema de determinação sexual Xy_p, John e Lewis (1960) propuseram que a existência de material nucleolar entre o X_p e o y_p, formado inicialmente durante a prófase I, poderia garantir a associação destes cromossomos até a anáfase I. Para estes autores, o emparelhamento inicial dos cromossomos sexuais parece envolver uma associação terminal não específica de segmentos heterocromáticos.

Drets *et al.* (1983) verificaram que a associação aquiasmática entre segmentos paracentroméricos e centroméricos, contendo heterocromatina constitutiva, e a associação terminal eucromática entre os braços longos dos cromossomos sexuais, promove a manutenção da configuração do Xy_p em *Epilachna paenulata* (Germar, 1824) (Coccinellidae). Estes pesquisadores propuseram ainda que nesta espécie, a associação do bivalente sexual não está relacionada com a presença de material nucleolar, uma vez que este não foi observado em células meióticas submetidas à impregnação pelo íon prata.

Em *Chelymorpha variabilis* Boheman, 1854 (Chrysomelidae), o estudo de cromossomos meióticos e de microestendidos profásicos demonstrou que o X_p e o y_p são sempre assinápticos no paquíteno e a manutenção da configuração em pára-quedas, dos cromossomos sexuais, é devido a

associação terminal não homóloga entre estes cromossomos, envolvendo blocos de heterocromatina constitutiva e não a associação nucleolar (Postiglioni e Brum-Zorrilla, 1988; Postiglioni *et al.*, 1991).

A análise em espécies de Curculionidae, realizada por Virkki *et al.* (1990), evidenciou que a associação dos cromossomos sexuais Xy_p no diplóteno resultava na configuração típica de pára-quedas. Nestas espécies, a presença de uma substância proteinácea argentofílica entre o X_p e o y_p foi verificada somente nas fases mais tardias da prófase meiótica, permanecendo até a anáfase I. Considerando estes resultados, os autores propuseram que esta substância poderia servir como material de adesão dos cromossomos sexuais, contribuindo com a segregação correta destes cromossomos; porém, esta substância não estaria relacionada com o nucléolo.

O sistema de determinação sexual do tipo X0 é o segundo mais freqüente entre os Coleoptera (Smith e Virkki, 1978) e de maior ocorrência na família Elateridae, sendo encontrado em aproximadamente 70% dos elaterídeos já analisados (Stevens, 1909; Smith, 1953, 1960; Banerjee, 1959; Agarwal, 1960, 1962; Piza, 1960; Virkki, 1962; Kacker, 1963; Manna e Mandrira, 1972; Dasgupta, 1977; Smith e Virkki, 1978; Vidal, 1984; Yadav e Vyas, 1993, 1994).

Existem evidências que mostram que o sistema de determinação sexual X0 originou-se a partir do sistema Xy_p, através da perda do cromossomo y_p. Esta derivação pode ter ocorrido pela progressiva heterocromatinização do cromossomo y_p, tornando-o inerte e geneticamente obsoleto, ou pela "degeneração" do cromossomo y_p, envolvendo transferência de material genético para os autossomos (Smith e Virkki, 1978). O cromossomo X, resultante do sistema Xy_p, em muitas espécies pode permanecer associado ao material nucleolar, como verificado no elaterídeo *Dicrepidus ramicornis* Beauvois, 1805 (Virkki, 1962). Nos gêneros com alta freqüência do sistema de determinação sexual do tipo X0, apesar da perda do cromossomo y_p, o número cromossômico básico permaneceu inalterado, ou seja, a eliminação do y_p não envolveu grandes mudanças autossômicas (Smith e Virkki, 1978).

O sistema de determinação sexual do tipo XY foi estabelecido por Smith (1953) para incluir uma categoria na qual os cromossomos sexuais são indistinguíveis dos autossomos ou não identificáveis pelo seu comportamento durante a meiose. Segundo levantamento realizado por Smith e Virkki (1978), somente cerca de 2% das espécies de Coleoptera analisadas citogeneticamente possuem o sistema de determinação sexual XY. Por outro lado, estes autores não descartaram a possibilidade deste número ser superestimado, devido ao fato de incluir casos em que a prófase não foi muito bem analisada. Na família Elateridae, este tipo de sistema de determinação foi detectado em apenas duas das espécies já estudadas (Virkki e Denton, 1987; Yadav e Vyas, 1993).

O sistema de determinação sexual do tipo neoXY ocorre em aproximadamente 7% das espécies de Coleoptera e geralmente é mais freqüente em famílias que possuem sistema de determinação sexual X0, tal como Carabidae e Passalidae. Porém, nem todas as famílias que possuem sistema do tipo X0 têm sistema neoXY (Smith e Virkki, 1978). Entre os representantes da família Elateridae, o sistema de determinação sexual neoXY foi descrito somente em *Hemirrhipus lineatus* (Olivier, 1790) e *Lacon profusa* Candèze, 1857 (Piza, 1958; Smith e Virkki, 1978).

A origem do sistema neoXY, na maioria dos organismos, ocorre a partir do sistema X0, envolvendo fusão cêntrica entre o cromossomo sexual X e um autossomo, ambos acrocêntricos. O homólogo do autossomo fusionado ao X passa a funcionar como cromossomo Y (Smith e Virkki, 1978). Devido ao fato dos cromossomos metacêntricos serem prevalecentes entre os Coleoptera, uma outra explicação para o surgimento do neoXY seria a fusão dos braços eucromáticos de um autossomo e do cromossomo X, originando o cromossomo sexual neoX. Os braços heterocromáticos dos cromossomos translocados reciprocamente e um dos centrômeros seriam dispensáveis. Caso a origem do sistema de determinação sexual neoXY ocorra a partir do sistema Xy_p, o cromossomo sexual y_p poderia ser eliminado ou os seus genes poderiam ser translocados para o neoY ou para outros autossomos (Smith e Virkki, 1978).

Em estudos de espécies relacionadas, com sistemas de determinação sexual do tipo X0 e neoXY, pode-se verificar que, nas espécies que possuem o sistema neoXY, o número de pares autossômicos é menor que naquelas que apresentam o sistema X0, confirmando o envolvimento de pares autossômicos na formação do sistema neoXY (Smith e Virkki, 1978).

O último sistema de determinação sexual encontrado na família Elateridae é o tipo $X_p$neoXneoY_p (X_1X_2Y). Este sistema foi descrito em apenas quatro espécies de Coleoptera, *Pityogenes fossifrons* LeConte, 1876 (Curculionidae), *Botanochara angulata* (Germar, 1824) (Chrysomelidae), *Blaps judaeorum* Miller, 1861 (Tenebrionidae) e *Ignelater luminosus* (Illiger, 1807) (descrita erroneamente como *Pyrophorus luminosus* - Elateridae) (Vaio e Postiglioni, 1974; Smith e Virkki, 1978; Virkki *et al.*, 1984).

O sistema $X_p$neoXneoY_p tem origem a partir do sistema Xy_p, no qual uma translocação recíproca entre um segmento do cromossomo y_p e um autossomo difásico (com um braço cromossômico totalmente heterocromático), origina o cromossomo sexual neoY_p; o cromossomo X_p permanece intacto e o homólogo autossômico não fusionado é denominado neoX (Virkki, 1980).

Em *Ignelater luminosus*, verificou-se que a presença de quiasma e de uma substância proteinácea argentofílica está envolvida na associação dos cromossomos sexuais $X_p$neoXneoY_p durante a meiose (Virkki *et al.*, 1984).

2.2.2. Regiões cromossômicas específicas em Elateridae

O estabelecimento do padrão de distribuição da heterocromatina constitutiva e a identificação dos cromossomos portadores das regiões organizadoras de nucléolo (RONs) são muito importantes para o entendimento de como ocorreu a evolução cromossômica nas diferentes famílias de Coleoptera (Petitpierre, 1996; Rozek *et al.*, 2004); contudo, entre os representantes de Elateridae, os dados referentes a estas características são poucos ou até mesmo inexistentes. Além das metodologias de detecção de bandas C e das RONs, o emprego da coloração com fluorocromos base-específicos tem permitido uma melhor diferenciação longitudinal dos cromossomos, especialmente dos insetos, os quais além de possuírem

cromossomos geralmente pequenos, muitas vezes difíceis de serem identificados por técnicas citogenéticas convencionais, não respondem aos tratamentos para obtenção de bandas eucromáticas (Juan *et al.*, 1990; Petitpierre, 1996).

De modo geral, a heterocromatina constitutiva nos Coleoptera localiza-se na região centromérica dos autossomos e do cromossomo sexual X, podendo estar presente também nas regiões intersticiais e teloméricas, porém com menor freqüência. No cromossomo sexual y, a heterocromatina constitutiva tem ocorrência variável, podendo aparecer somente na região pericentromérica ou ao longo de todo o comprimento cromossômico, variando de acordo com o grau de diferenciação deste cromossomo na espécie (Almeida *et al.*, 2000; Rozek *et al.*, 2004).

Na família Elateridae, as informações sobre a distribuição da heterocromatina constitutiva são recentes e restritas a apenas seis espécies. Rozek e Lachowska (2001) e Rozek *et al.* (2004) verificaram que em *Prosternon tesselatum* (Linnaeus, 1758), com 2n=20+Xy, e *Adelocera murina* (Linnaeus, 1758), com $2n=20+Xy_p$, grandes blocos de heterocromatina constitutiva estão presentes na região pericentromérica de todos os autossomos e do cromossomo sexual X. Em *P. tesselatum*, o cromossomo sexual y é totalmente heterocromático, enquanto que em *A. murina*, o cromossomo y_p apresenta-se inteiramente eucromático. Em *Denticollis linearis* (Linnaeus, 1758), *Athous vitattus* (Fabricius, 1792), *Agriotes* sp. e *Ampedus* sp., blocos heterocromáticos bem evidentes foram observados em células profásicas mitóticas e meióticas (Rozek *et al.*, 2004); porém, a localização destes blocos nos cromossomos não foi estabelecida.

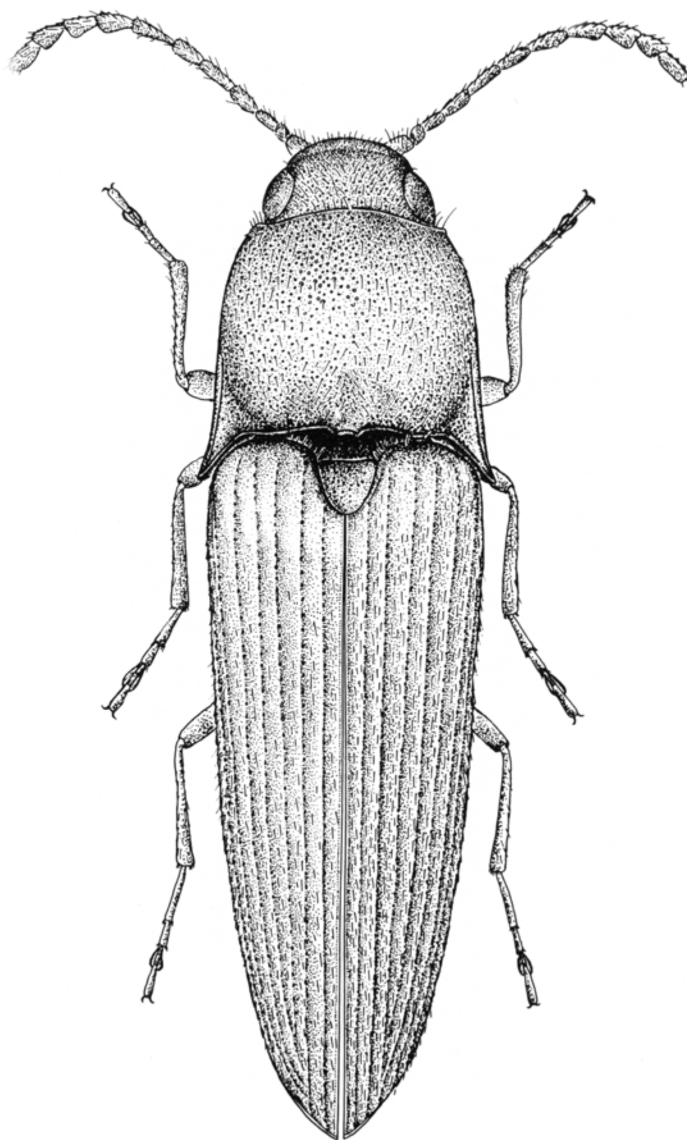
A identificação das RONs é de grande importância para comparações cariotípicas de espécies relacionadas em estudos taxonômicos e evolutivos, no estabelecimento da arquitetura molecular dos cromossomos e para a elucidação do modo de associação de alguns cromossomos sexuais durante a meiose, especialmente daqueles pertencentes ao sistema de determinação sexual do tipo Xy_p (Petitpierre, 1996). Adicionalmente, a determinação do número e da localização das RONs constitui um parâmetro adicional na

caracterização cromossômica das espécies, pois estas regiões estão sempre presentes nos cromossomos e podem apresentar um padrão de distribuição conservado evolutivamente para um grupo de espécies. Alterações no padrão de distribuição das RONs podem ser indicativas de ocorrência de rearranjos cromossômicos e fornecer dados sobre as estratégias de diferenciação cariotípica entre espécies relacionadas (Oliveira, 2004).

Até o presente momento, não existe um padrão de distribuição das RONs bem estabelecido para as diferentes famílias de Coleoptera, estando estas regiões localizadas em pares autossômicos e/ou nos cromossomos sexuais (Almeida *et al.*, 2000).

Ignelater luminosus ($2n=14+X_p neoXneoY_p$) é a única espécie de toda a família Elateridae que teve o padrão de distribuição das RONs estabelecido. Virkki *et al.* (1984) analisando os cromossomos meióticos desta espécie, submetidos à impregnação pelo íon prata, verificaram a presença de 2 RONs localizadas na região terminal de um bivalente autossômico de tamanho grande.

Nos cromossomos de Coleoptera, o uso da coloração por fluorocromos base-específicos, especialmente a CMA_3 (Cromomicina A_3) e o DAPI (4'-6-diamidino-2-fenilindol), está sendo um pouco mais freqüente nos últimos anos; entretanto, estes estudos continuam bastante restritos a algumas famílias, tais como Coccinellidae (Ennis, 1974; Maffei *et al.*, 2001), Geotrupidae (Vitturi *et al.*, 1999; Colomba *et al.*, 2004), Lucanidae (Colomba *et al.*, 2000a), Scarabaeidae (Colomba *et al.*, 1996, 2000b, 2006; Moura *et al.*, 2003; Vitturi *et al.*, 2003; Bione *et al.*, 2005) e Tenebrionidae (Juan *et al.*, 1991; Plohl *et al.*, 1993). Nas espécies pertencentes a estas famílias, os fluorocromos estão sendo úteis para evidenciar particularidades concernentes a composição de base de regiões heterocromáticas constitutivas, de regiões heterocromáticas associadas às RONs, para a identificação de RONs ativas e inativas e até mesmo de regiões da cromatina não diferenciadas pelas técnicas usuais de bandamento C e impregnação pelo íon prata. Até o presente momento, em espécies da família Elateridae, não existem registros sobre o emprego da coloração por fluorocromos base-específicos.



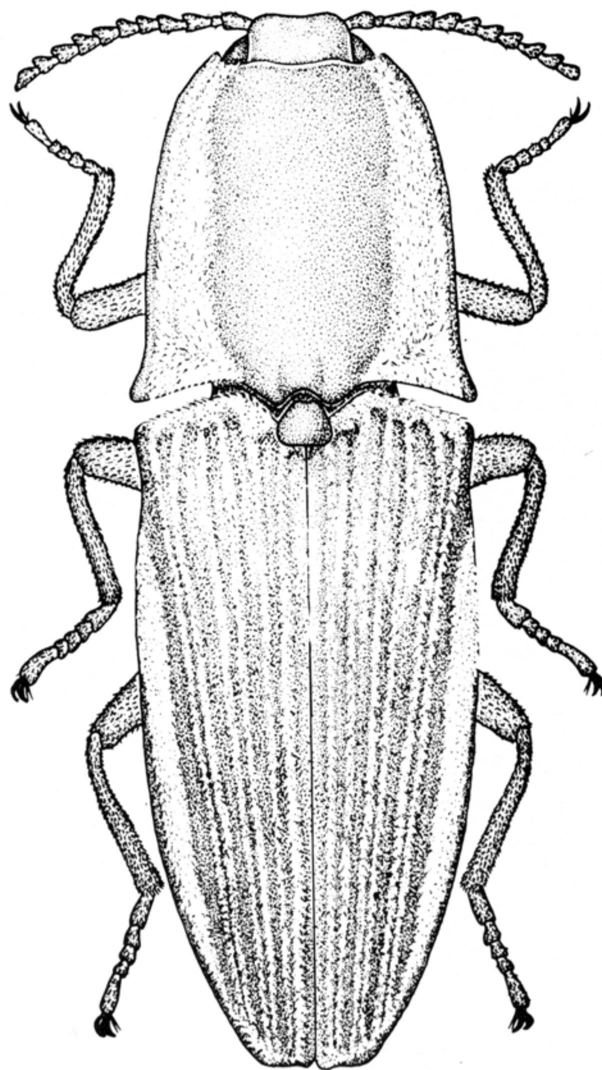
Anchastus brunneofasciatus

3. OBJETIVOS

Considerando as particularidades cariotípicas encontradas na família Elateridae, a grande diversidade de número diplóide e de tipos de sistema de determinação sexual verificada nas espécies desta família previamente estudadas, bem como a escassez de informações citogenéticas com técnicas de coloração cromossômica convencional e diferencial, o presente estudo tem o objetivo de caracterizar citogeneticamente 13 espécies de Elateridae, pertencentes às subfamílias Agrypninae (*Conoderus dimidiatus*, *Conoderus fuscofasciatus*, *Conoderus malleatus*, *Conoderus rufidens*, *Conoderus scalaris*, *Conoderus stigmosus*, *Conoderus ternarius*, *Conoderus* sp., *Pyrearinus candelarius*, *Pyrophorus divergens* e *Pyrophorus punctatissimus*) e Elaterinae (*Cardiorhinus rufilateris* e *Pomachilius* sp.2), visando estabelecer as principais estratégias de diferenciação cromossômica que teriam ocorrido nestas espécies e em outras espécies relacionadas descritas na literatura. Para efetuar-se a caracterização cromossômica, foram determinados:

a. o número diplóide/haplóide de cromossomos, o tipo de sistema de determinação sexual, a morfologia dos cromossomos e o comportamento dos cromossomos durante a meiose.

b. o padrão de distribuição da heterocromatina constitutiva, das regiões organizadoras de nucléolo (RONs), e das regiões de cromatina ricas em seqüências de base GC e AT.



Chalcolepidius zonatus

4. MATERIAL E MÉTODOS

4.1. MATERIAL

O número de exemplares machos e fêmeas adultos das 13 espécies da família Elateridae (Figuras 1-3) analisadas nesta tese e seus respectivos locais de coleta, estão mencionados nas tabelas 1, 2 e 3, conforme a seqüência de resultados apresentada nos artigos.

A identificação taxonômica dos espécimes foi realizada pela Dra. Cleide Costa e pela MSc. Simone Policena Rosa, do Museu de Zoologia, Universidade de São Paulo – USP.

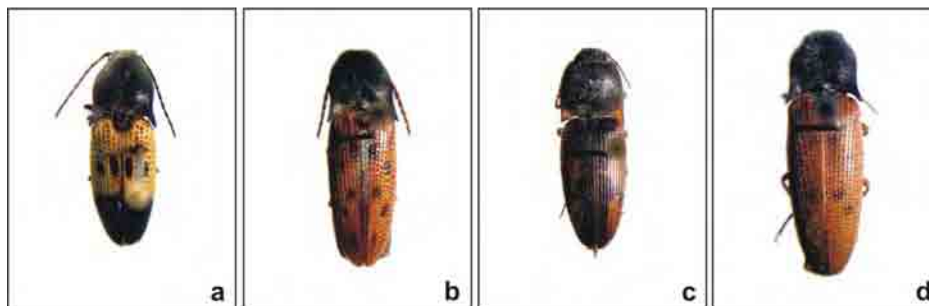


Figura 1 – Exemplares adultos das quatro espécies de Elateridae (Agrypninae, Conoderini) descritas no Artigo I, apresentado nos Resultados. **a.** *Conoderus dimidiatus*, fêmea. **b.** *Conoderus scalaris*, macho. **c.** *Conoderus ternarius*, fêmea. **d.** *Conoderus stigmosus*, macho. Aumento=2x.

Tabela 1 – Espécies de Elateridae (Agrypninae, Conoderini) analisadas no Artigo I dos Resultados, com seus respectivos número de exemplares e locais de coleta.

Espécies	Número de exemplares	Locais de Coleta
Conoderus dimidiatus Germar, 1839	8 machos/3 fêmeas	Ponta Grossa (25°06’S, 50°10’O), PR
Conoderus scalaris (Germar, 1824)	21 machos/5 fêmeas	Rio Claro (22º24’S, 47º33’O), SP
Conoderus ternarius Germar, 1839	6 machos/4 fêmeas	Rio Claro (22º24’S, 47º33’O), SP
Conoderus stigmosus Germar, 1839	3 machos/4 fêmeas	Rio Claro (22º24’S, 47º33’O), SP

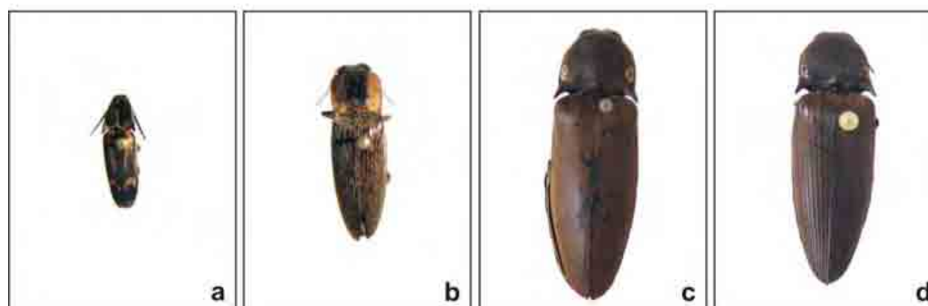


Figura 2 – Exemplares adultos das quatro espécies de Elateridae (Agrypninae) estudadas no Artigo II que consta nos Resultados. **a.** Conoderini. **b-d.** Pyrophorini. **a.** *Conoderus malleatus*, fêmea. **b.** *Pyrearinus candelarius*, macho. **c.** *Pyrophorus divergens*, macho. **d.** *Pyrophorus punctatissimus*, macho. Aumento=1,2x.

Tabela 2 – Espécies, número de espécimes, e locais de coleta de quatro representantes de Agrypninae (Elateridae) utilizados nas análises citogenéticas do Artigo II.

Espécies	Número de exemplares	Locais de Coleta
Conoderini		
Conoderus malleatus (Germar, 1824)	3 machos/2 fêmeas	Ponta Grossa (25°06'S, 50°10'O), PR
Pyrophorini		
Pyrearinus candelarius (Germar, 1841)	12 machos	Rio Claro (22º24'S, 47º33'O), SP
Pyrophorus divergens Eschscholtz, 1829	4 machos	Rio Claro (22º24'S, 47º33'O) e Campinas (22°54'S, 47°04'O), SP
Pyrophorus punctatissimus Blanchard, 1843	12 machos/1 fêmea	Rio Claro (22º24'S, 47º33'O), SP

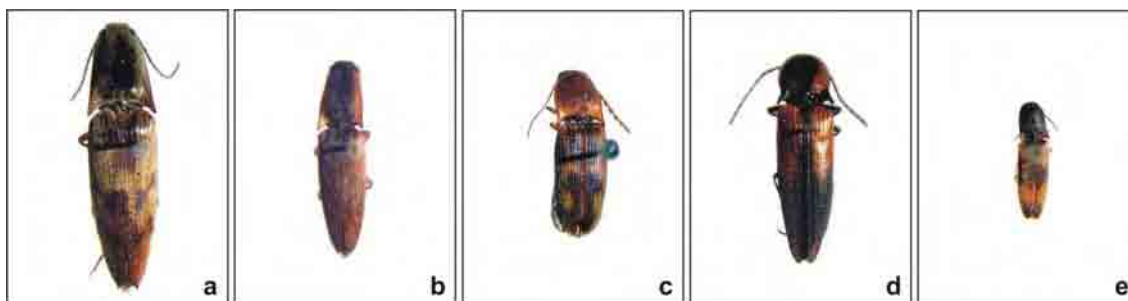


Figura 3 – Exemplares machos adultos das cinco espécies de Elateridae analisadas no Artigo III que integra os Resultados. **a-c.** Agrypninae, Conoderini. **d-e.** Elaterinae, Agriotini. **a.** *Conoderus fuscofasciatus*. **b.** *Conoderus rufidens*. **c.** *Conoderus* sp. **d.** *Cardiorhinus rufilateris*. **e.** *Pomachilius* sp.2. Aumento=2x.

Tabela 3 – Espécies de Elateridae citogeneticamente descritas no Artigo III, com seus respectivos número de exemplares analisados e locais de coleta.

Espécies	Número de exemplares	Locais de Coleta
Agrypninae		
Conoderini		
Conoderus fuscofasciatus Eschscholtz, 1829	8 machos	Ponta Grossa (25°06'S, 50°10'O), PR e Rio Claro (22º24'S, 47º33'O), SP
Conoderus rufidens (Fabricius, 1801)	2 machos	Rio Claro (22º24'S, 47º33'O), SP
Conoderus sp.	3 machos	Rio Claro (22º24'S, 47º33'O), SP
Elaterinae		
Agriotini		
Cardiorhinus rufilateris (Eschscholtz, 1822)	1 macho	Rio Claro (22º24'S, 47º33'O), SP
Pomachilius sp.2	2 machos	Ponta Grossa (25°06'S, 50°10'O), PR e Rio Claro (22º24'S, 47º33'O), SP

4.2. MÉTODOS

4.2.1. Obtenção das preparações cromossômicas

As preparações citológicas, para o estudo dos cromossomos mitóticos e meióticos, foram obtidas de testículos e ovários de indivíduos adultos, de acordo com as técnicas descritas a seguir:

- Sem a utilização de colchicina

a. dissecar o animal em solução fisiológica para insetos, retirar as gônadas e colocá-las em uma placa de Petri, contendo solução hipotônica (água de torneira), durante 2 minutos;

b. transferir o material para uma placa de Petri contendo fixador Carnoy I (metanol e ácido acético – 3:1) e deixar durante 30 minutos, no mínimo;

c. macerar, sobre uma lâmina, uma das gônadas, juntamente com uma gota de ácido acético 40%;

d. secar a lâmina em uma placa de metal à temperatura de 35 a 40º C.

- Com a utilização de colchicina

a. dissecar o animal em solução fisiológica para insetos, retirar as gônadas e transferi-las para um recipiente, contendo colchicina 0,05% (em solução fisiológica para insetos), durante 90 minutos;

b. acrescentar um volume de água de torneira igual ao de colchicina, por 12 minutos;

c. adicionar 10 gotas de fixador Carnoy I (metanol e ácido acétido – 3:1) e deixar durante 1 minuto;

d. transferir as gônadas para uma placa de Petri contendo fixador Carnoy I e deixar durante 30 minutos, no mínimo;

e. macerar, sobre uma lâmina, uma das gônadas, juntamente com uma gota de ácido acético 40%;

f. secar a lâmina em uma placa de metal à temperatura de 35 a 40º C.

4.2.2. Coloração convencional (Giemsa)

a. corar a lâmina em solução de Giemsa a 3% (47 ml de água destilada, 1.5 ml de solução comercial de Giemsa e 1.5 ml de tampão fostato pH 6.8) durante 12 minutos;

b. lavar a lâmina em água destilada e secar ao ar.

4.2.3. Coloração diferencial

Para a determinação das regiões heterocromáticas constitutivas e dos cromossomos portadores das regiões organizadoras de nucléolo, foi utilizada a técnica de obtenção de bandas C, descrita por Sumner (1972) e de impregnação pelo íon prata (AgRON), descrita por Howell e Black (1980), respectivamente. Para o estabelecimento das regiões de DNA ricas em seqüências de bases GC e AT, foi utilizada a técnica de coloração com os fluorocromos base-específicos Cromomicina A_3 (CMA_3) e 4'-6-diamidino-2-fenilindol (DAPI), e de contracoloração com Distamicina A (DA), descrita por Schweizer (1980).

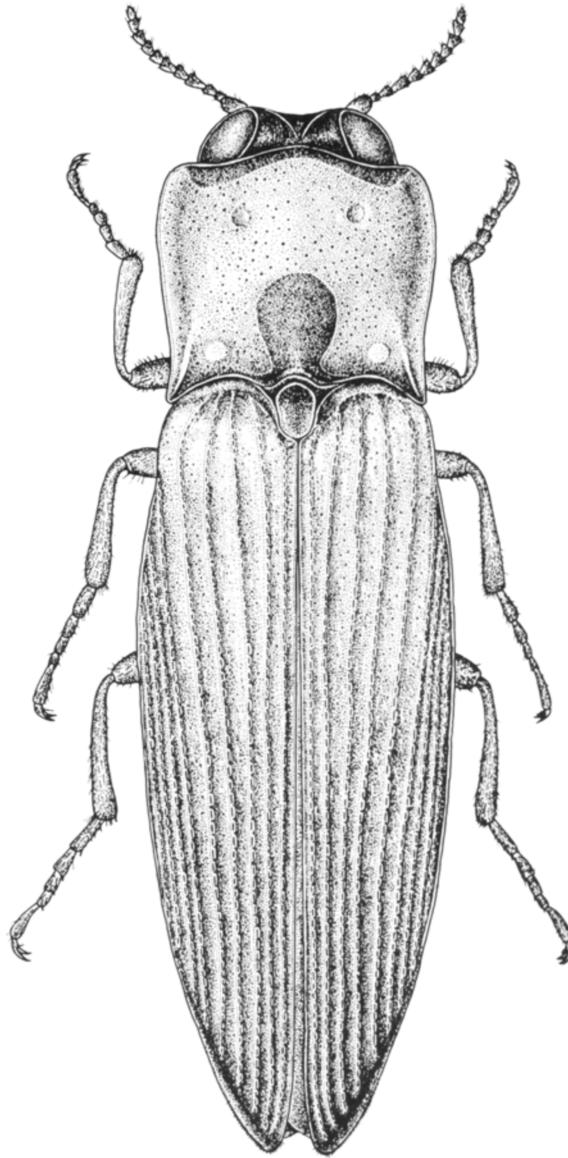
4.2.4. Coloração seqüencial

Algumas preparações cromossômicas foram analisadas com coloração seqüencial, a qual consiste em submeter à preparação cromossômica a técnica de coloração convencional e posteriormente, a técnica de bandamento C e/ou impregnação pelo íon prata.

4.2.5. Análises cromossômicas

As análises dos cromossomos corados convencionalmente ou submetidos à técnica de bandamento C e/ou impregnação pelo íon prata, foram realizadas em microscopia de luz. As melhores células mitóticas e meióticas foram fotografadas em um fotomicroscópio Zeiss, com objetiva 100 de imersão, optovar 1.25 e filtro verde. O filme utilizado foi o Imagelink HQ Microfilm (Kodak), revelado em D72 (1:4 em água) por 5 minutos à temperatura de 18º

C. As células coradas com fluorocromos foram fotografadas em um fotomicroscópio Olympus BX50, objetiva 100 de imersão, com os filtros específicos para o fluorocromo DAPI (360-390 nm) e CMA_3 (430-480 nm). O filme utilizado foi o TMAX-100 (Kodak), revelado em D76 durante 7 minutos à temperatura de 20°C. As ampliações fotográficas foram feitas em papel Kodabromide RC-F3 (Kodak) e reveladas em D72 (1:2 em água). A morfologia dos cromossomos foi determinada seguindo a nomenclatura proposta por Levan *et al.* (1964).



Pyrearinus termitilluminans

5. RESULTADOS

Os resultados obtidos com a análise citogenética de 13 espécies da família Elateridae são apresentados na forma de três artigos, mencionados a seguir:

Artigo I - aceito para publicação no periódico Genética (Netherlands)

Evolutionary chromosomal differentiation among four species of *Conoderus* Eschscholtz, 1829 (Coleoptera, Elateridae, Agrypninae, Conoderini) detected by standard staining, C-banding, silver nitrate impregnation, and CMA_3/DA/DAPI staining.

Artigo II – enviado para publicação no periódico Journal of Zoological Systematics and Evolutionary Research (Germany)

Chromosomal similarities and differences among four Neotropical Elateridae (Conoderini and Pyrophorini) and other related species, with comments on the NORs pattern in Coleoptera.

Artigo III – enviado para publicação no periódico Folia Biologica (Prague)

Singular strategies of karyotype differentiation traced in two subfamilies of Elateridae (Coleoptera, Polyphaga: Agrypninae, Elaterinae).

Artigo I

Evolutionary chromosomal differentiation among four species of *Conoderus* Eschscholtz, 1829 (Coleoptera, Elateridae, Agrypninae, Conoderini) detected by standard staining, C-banding, silver nitrate impregnation, and CMA_3/DA/DAPI staining.

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Resumo

A exuberante fauna brasileira de Elateridae é caracterizada por uma alta diversidade cariotípica, incluindo uma espécie (*Chalcolepidius zonatus* Eschscholtz, 1829) que possui o menor número diplóide entre os Coleoptera. A análise citogenética de *Conoderus dimidiatus* Germar, 1839, *C. scalaris* (Germar, 1824), *C. ternarius* Germar, 1839, e *C. stigmosus* Germar, 1839, através de coloração convencional e diferencial, foi realizada com o objetivo de estabelecer os mecanismos de diferenciação cariotípica nestas espécies. *Conoderus dimidiatus*, *C. scalaris*, e *C. ternarius* apresentaram 2n(♂)=17 e 2n(♀)=18 e sistema de determinação sexual X0/XX, os quais são similares aqueles encontrados na maioria das espécies de Conoderini. O cariótipo de *C. stigmosus* foi caracterizado por apresentar 2n=16 e um sistema de determinação sexual neoXY/neoXX, sendo muito diferenciado em relação aqueles de outras espécies do gênero. Algumas características dos cromossomos mitóticos e meióticos de *C. stigmosus* sugerem a ocorrência de uma fusão entre o cromossomo X ancestral e um autossomo como a causa de origem do sistema neoXY. As técnicas de bandamento C e de impregnação pelo íon prata mostraram que as quatro espécies de *Conoderus* possuem características cromossômicas similares aquelas da maioria das espécies de Polyphaga, incluindo banda C pericentromérica e RONs autossômicas. Técnicas de coloração tríplice com CMA_3/DA/DAPI também proporcionaram informações úteis para diferenciar estas espécies de *Conoderus*. Estas técnicas revelaram uma heterocromatina particular, rica em seqüências de bases GC, associada com as RONs em *C. scalaris* e *C. stigmosus* e heteromorfismo de CMA_3 em *C. scalaris* e *C. ternarius*.

Palavras-chave: bivalente, cariótipo, constrição secundária, fluorocromo, heteromorfismo, meiose, quiasma, RONs, sinapse, sistema de determinação sexual

Abstract

The speciose Brazilian Elateridae fauna is characterized by high karyotypic diversity, including one species (*Chalcolepidius zonatus* Eschscholtz, 1829) with the lowest diploid number within any Coleoptera order. Cytogenetic analysis of *Conoderus dimidiatus* Germar, 1839, *C. scalaris* (Germar, 1824), *C. ternarius* Germar, 1839, and *C. stigmosus* Germar, 1839 by standard and differential staining was performed with the aim of establishing mechanisms of karyotypic differentiation in these species. *Conoderus dimidiatus*, *C. scalaris*, and *C. ternarius* have diploid numbers of 2n(♂)=17 and 2n(♀)=18, and a X0/XX sex determination system, similar to that encountered in the majority of Conoderini species. The karyotype of *C. stigmosus* was characterized by a diploid number of 2n=16 and a neoXY/neoXX sex determination system that was highly differentiated from other species of the genus. Features of some mitotic and meiotic chromosomes suggest an autosome/ancestral X chromosome fusion as the cause of the neoXY system origin in *C. stigmosus*. C-banding and silver impregnation techniques showed that the four *Conoderus* species possess similar chromosomal characteristics to those in most Polyphaga species, including pericentromeric C band and autosomal NORs, respectively. Triple staining techniques including CMA_3/DA/DAPI also provided useful information for differentiating these *Conoderus* species. These techniques revealed unique GC-rich heterochromatin associated with NORs in *C. scalaris* and *C. stigmosus* and CMA_3-heteromorphism in *C. scalaris* and *C. ternarius*.

Key words: bivalent, chiasma, karyotype, fluorochrome, heteromorphism, meiosis, NORs, secondary constriction, sex determination system, synapse

Introduction

"Click-beetles" are members of the family Elateridae, composed of approximately 9.300 species (Costa, 1999) distributed in 18 subfamilies (Lawrence & Newton, 1995). Cytogenetic analysis within the Elateridae has been restricted to the 4 subfamilies, Agrypninae, Cardiophorinae, Denticollinae, and Elaterinae, involving a total of 83 species (Smith & Virkki, 1978; Ferreira et al., 1984; Vidal, 1984; Virkki, Flores & Escudero, 1984; Virkki & Denton, 1987; Yadav & Vyas, 1993; 1994; Rozek & Lachowska, 2001; Rozek et al., 2004). The subfamily Agrypninae shows the highest karyotypic diversity among all Elateridae, revealing 10 different karyotypes from cytogenetic analysis of 23 different species (Table 1). Diploid numbers of the Agrypninae vary between 2n=22 (Rozek et al., 2004) and 2n=4 (Ferreira et al., 1984). The latter example comprises one of the lowest chromosomal numbers known in the order Coleoptera. The type of sex determination system of the Agrypninae is also very heterogeneous, including simple systems, such as Xy_p, Xy, X0, and neoXY (Smith & Virkki, 1978; Vidal, 1984; Virkki & Denton, 1987; Yadav & Vyas, 1994; Rozek et al., 2004), or multiple systems, such as X_1X_2Y ($X_p$neoXneoy$_p$) (Virkki, Flores & Escudero, 1984). In the Agrypninae species, there is a predominance of acrocentric chromosomes, atypical for the Coleoptera.

In the Agrypninae, all described species of the Conoderini tribe are karyotypically uniform in chromosome numbers (2n=17), chromosomal morphology, predominance of acrocentrics, and an X0 sex determination system (Virkki, 1962; Vidal, 1984; Yadav & Vyas, 1994). However, analysis of several *Heteroderes macroderes* Candèze, 1859 revealed 2n=19 with a predominance of metacentric chromosomes (Agarwal, 1960; 1962), suggesting intraspecific chromosomal variation in this group (Table 1).

The remarkable karyotypic conservatism of Conoderini species may be a consequence of the methodological procedures used in previous cytogenetic analyses that involved only standard staining techniques, as well as the small number of species analyzed. The goal of this study is to use other cytogenetic techniques that are capable of revealing constitutive heterochromatin and

nucleolar organizer regions (NORs) in order to reveal higher resolution chromosomal differentiation among Conoderini species.

The major goal of this work is to characterize karyotypic diversity in four species of *Conoderus*, *C. dimidiatus*, *C. scalaris*, *C. ternarius*, and *C. stigmosus*, using standard and differential staining to determine mechanisms in chromosomal differentiation of these species, during the evolutionary process. Specifically, diploid chromosome numbers, chromosomal morphology, type of sex determination system, chromosomal behaviour during meiosis, C-banding patterns, AT- and GC-rich DNA regions, and chromosomal location of the NOR were all documented.

Table 1. The cytogenetically known Agrypninae species, with their respective diploid number, sex determination system, and chromosomal morphology. A=acrocentric. M=metacentric. SM=submetacentric.

Tribe	Species	Chromosomal formula (2n males)	Chromosomal morphology	References
Agrypnini				
	Adelocera colonicus (Candèze, 1881)	17=16+X0	----	Smith & Virkki, 1978
	Adelocera modesta (Candèze, 1857)	17=16+X0	----	Smith & Virkki, 1978
	Adelocera murina (Lineu, 1758)	22=20+Xyp	----	Rozek et al., 2004
	Adelocera rectangularis (Say, 1825)	17=16+X0	----	Smith, 1953
	(?) *Adelocera* sp.	17=16+X0	----	Manna & Mandrira, 1972
	Agrypnus fuscipes (Fabricius, 1775)	17=16+X0	16A+XA	Banerjee, 1959; Dasgupta, 1977
	Agrypnus sp.	11=10+X0	6M+?	Kacker, 1963
	Colaulon lezeleuci (Candèze, 1857)	17=16+X0	----	Virkki, 1962
	Lacon profusa Candèze, 1857	14=12+neoXY	----	Smith & Virkki, 1978
Conoderini				
	Conoderus dimidiatus Germar, 1839	17=16+X0	16A+XA	this report
	Conoderus pilatei (Candèze, 1859)	17=16+X0	----	Virkki, 1962
	Conoderus rodriguezi (Candèze, 1881)	17=16+X0	----	Virkki, 1962
	Conoderus scalaris (Germar, 1824)	17=16+X0	16A+XA	this report
	Conoderus stigmosus Germar, 1839	16=14+neoXY	2SM+12A+neoXA+neoYA	this report
	Conoderus ternarius Germar, 1839	17=16+X0	4M+12A+XA	this report
	Heteroderes lenis (Candèze, 1859)	17=16+X0	2M+14A+XA	Yadav & Vyas, 1994
	Heteroderes macroderes Candèze, 1859	19=18+X0	12M+6A+XM	Agarwal, 1960; 1962
	Heteroderes macroderes Candèze, 1859	17=16+X0	4M+12A+XA	Yadav & Vyas, 1994
	Heteroderes modestus Candèze, 1859	17=16+X0	2M+14A+XA	Yadav & Vyas, 1994
	Heteroderes sericeus Candèze, 1859	17=16+X0	2SM+14A+XA	Yadav & Vyas, 1994
	Monocrepidius sp. (=*Conoderus* sp.)	17=16+X0	----	Vidal, 1984
Hemirhipini				
	Chalcolepidius silbermani Chevrolat, 1835	12=10+Xy	10A+XA+yA	Virkki & Denton, 1987
	Chalcolepidius zonatus Eschscholtz, 1829	4	2M+2A	Ferreira et al., 1984
	Hemirrhipus lineatus (Olivier, 1790)	10=8+neoXY	----	Piza, 1958
Pyrophorini				
	Pyrophorus luminosus Illiger, 1807 (now in *Ignelater* Costa, 1975)	17=14+X1X2Y	----	Virkki, Flores & Escudero, 1984
	Pyrophorus nyctophanus Germar, 1841 (= *P. phosphorescens* Castelnau, 1840)	15=14+X0	2M+X?	Piza, 1960
	Pyrophorus pellucens Eschscholtz, 1829 (=*P. phosphorescens* Castelnau, 1840, pars)	15=14+X0	----	Smith, 1960
	Pyrophorus radians Champion, 1895 (now in *Deilelater* Costa, 1975)	11=10+X0	----	Virkki, 1962

Material and methods

The number of individuals analyzed and collection localities for the *Conoderus* species studied are listed in Table 2. Vouchers were deposited in the Departamento de Biologia, UNESP, Rio Claro, SP, Brazil. Chromosomal preparations were obtained from adult male and female gonadal tissues. The gonads were dissected in insect saline solution, placed in hypotonic solution (tap water) for 2 min, and fixed in Carnoy I (3 methanol: 1 acetic acid) for 30 min. Metaphase chromosomes were prepared from some dissected gonads immersed in a 0.05% colchicine solution for 90 min. For slide preparations, the gonads were macerated in 40% acetic acid and the slides were dried on a hot plate at 40° C. All chromosomal preparations were sequentially stained using Giemsa/C-banding/AgNOR. A 3% Giemsa solution was used for standard staining for 12 min. Constitutive heterochromatin was detected by C-banding (Sumner, 1972) and nucleolar organizer regions (NORs) were identified by silver nitrate impregnation (AgNOR) following Howell & Black (1980). For verifying AT- and GC-rich chromatin regions, the chromosomes were stained with base-specific CMA_3/DA/DAPI fluorochromes, according to Schweizer (1980). Chromosomal morphology was determined according to the nomenclature proposed by Levan, Fredga & Sandberg (1964).

Table 2. Number of specimens analyzed and the collection localities of the *Conoderus* species.

Species	Number of individuals	Collection localities
Conoderus dimidiatus Germar, 1839	8 males/3 females	Ponta Grossa (25°06'S, 50°10'W), Paraná State, Brazil
Conoderus scalaris (Germar, 1824)	21 males/5 females	Rio Claro (22º24'S, 47º33'W), São Paulo State, Brazil
Conoderus ternarius Germar, 1839	6 males/4 females	Rio Claro (22º24'S, 47º33'W), São Paulo State, Brazil
Conoderus stigmosus Germar, 1839	3 males/4 females	Rio Claro (22º24'S, 47º33'W), São Paulo State, Brazil

Results

Mitotic chromosomes

Analysis of standard stained mitotic cells of *C. dimidiatus*, *C. scalaris*, and *C. ternarius* revealed diploid numbers of 2n=17 for males and 2n=18 for females consistent with a X0/XX sex chromosome system. All chromosomes were acrocentric, except the 6th and 8th pairs of *C. ternarius*, which were metacentric (Figure 1a, c, e, g, i, and j). In these three species, the autosomal chromosomes varied in size, and the X chromosome was intermediate in size compared to the 3rd and 4th autosomal pairs. In some cells of *C. ternarius*, the chromosomes were less condensed with a prominent secondary constriction in the distal region of the long arm of pair 1 (Figure 1i).

The mitotic metaphases of *C. stigmosus* were 2n=16=14+neoXY in males (Figure 2a) and 2n=16=14+neoXX in females (Figure 2c). The chromosomal morphology was acrocentric in six autosomal pairs and sex chromosomes, and submetacentric in the 4th pair. In this species, the autosomal pairs also varied in size, but the sex chromosomes were the largest elements of the karyotype: the neoX was slightly larger than the neoY chromosome.

C-bands of spermatogonial and oogonial mitotic metaphases of all these species–revealed constitutive heterochromatin blocks in the pericentromeric region of all chromosomal elements, with the exception of the 2nd and 5th pairs of *C. dimidiatus* and *C. scalaris*, and the 2nd pair of *C. ternarius* and *C. stigmosus*, in which C bands were lacking (Figure 1b, d, f, h, k and Figure 2b and d). Additionally, telomeric C bands occur in the long arm of some chromosomes. Chromosomal polymorphism in relation to the pericentromeric and/or telomeric C bands was noted in the analyzed sample of all four *Conoderus* species.

The distribution of constitutive heterochromatin in *C. dimidiatus* mitotic metaphases showed that the chromosomes of all specimens possess similar C band patterns, including pericentromeric blocks in the 1, 3, 4, 7, and 8 autosomal pairs and X chromosome, and telomeric bands in the 7th and 8th pairs and X chromosomes (Figure 1b and d). However, a constitutive heterochromatin polymorphism, involving the 1st and 6th pairs, was found in one

male and one female of this species. The presence or absence of pericentromeric C bands in pair 6 and telomeric C bands in pairs 1 and 6 was observed in homomorphic and heteromorphic condition (Figure 1b and d).

The chromosomes of *C. scalaris* exhibited a C band pattern similar to that found in *C. dimidiatus*, but telomeric bands occurred only in the 3rd autosomal pair and in the X chromosome of *C. scalaris* (Figure 1f and h). Telomeric constitutive heterochromatin polymorphism in the 1st and 6th pairs was also detected in three males and one female of *C. scalaris*. Heterochromatin polymorphism in the pericentromeric region of the 6th pair, like that observed in *C. dimidiatus*, was not encountered in *C. scalaris*.

The mitotic cells of *C. ternarius* revealed constitutive heterochromatin in the pericentromeric region of the 1, 3, 4, 5, 6, 7, and 8 pairs, and X chromosomes. Telomeric C bands were evident in the long arm of the acrocentric pairs 4, 5, 7 and in both arms of the metacentric pairs 6 and 8. Additionally, the X chromosome possessed a tenuous C band in the long arm interstitial region (Figure 1k). In *C. ternarius*, constitutive heterochromatin polymorphism was only verified in telomeric regions of two females on the 1st pair; this polymorphism exhibited similar features to those noted in *C. dimidiatus* and *C. scalaris*.

The constitutive heterochromatin of *C. stigmosus* is located in the pericentromeric region of the autosomal pairs 1, 3, 4, 5, 6, and 7. In one female, additional telomeric C bands appeared in pairs 4, 5, 6, and 7. Constitutive heterochromatin also occurred in the pericentromeric region of the sex chromosomes, extending along the entire length of the neoY long arm, with exception of the terminal region of this chromosome, which was C band negative (Figure 2b and d).

The mitotic metaphases of the four *Conoderus* species used in Giemsa/C-banding/AgNORs sequential staining allowed for the verification of NORs, which only occur on autosomal pairs, occupying the telomeric region (Figure 3). In *C. dimidiatus*, 4 NORs on the short arm of the 2nd pair and the long arm of the 4th pair were observed (Figure 3a). *Conoderus scalaris* possessed 2 NORs on the long arm of the 4th pair of chromosomes (Figure 3b).

In *C. ternarius*, 2 NORs on the long arm of the 1st pair were observed coincident with the secondary constriction (Figure 3c). *Conoderus stigmosus* possessed 2 NORs on the short arm of the 2nd pair (Figure 3d).

When mitotic cells of one individual of each *Conoderus* species were stained with CMA_3/DA/DAPI fluorochromes, a similar CMA_3/DA labelling pattern was detected in the pericentromeric region on pair 2 of *C. scalaris*, *C. ternarius*, and *C. stigmosus* (Figure 4b, c, and d). This pericentromeric labelling appeared to extend up to the short arm telomeric region of the acrocentric 2nd pair; however, the CMA_3/DA labelled 2nd pair of *C. scalaris* and *C. ternarius* were shown to be heteromorphic in relation to the presence of the CMA_3 positive region in all the cells analyzed (Figure 4b and c). It is worth pointing out that the 2nd pair of all these species was wholly C band negative. The use of CMA_3/DA fluorochrome permitted the identification of other labelled chromosomes, such as pair 6 in *C. dimidiatus* and *C. ternarius*, pair 8 in C*. ternarius*, and the neoY chromosome in *C. stigmosus* (Figure 4a, c, and d). The CMA_3/DA positive pericentromeric region on the 6th and 8th pairs coincided with C bands and the interstitial regions of the neoY long arm were partly included in the C banded region. The DA/DAPI produced homogeneous staining with no particular bright regions in the chromosomes of the four *Conoderus* species.

Meiotic chromosomes

The standard stained pachytenic testicular cells of *C. dimidiatus*, *C. scalaris*, and *C. ternarius* exhibited 8 autosomal bivalents plus 1 sexual univalent (Figure 5a, b, and c). In these cells, the sexual univalent usually appeared highly condensed and positively heteropycnotic. The pachytene cells of *C. stigmosus* showed 8 filamentous structures, which were similar in thickness, and 1 precociously condensed and positive heteropycnotic block that is probably the neoX chromosome (Figure 5d). The neoY chromosome was the largest compared to the other 7 autosomal bivalents.

Despite analysis of various pachytene cells in *C. stigmosus*, it was not possible to determine the type of synapsis (total, partial, or terminal) between the neoX and neoY chromosomes. It was unclear whether the neoX

corresponded only to the heteropycnotic block or was composed of an additional chromatin segment synapsed with the neoY chromosome. This last proposition could explain the presence of a neoY chromosome with a thickness similar to those of the autosomal bivalents.

The diplotene cells of the four *Conoderus* species showed only one chiasma per autosomal bivalent, interstitial or terminal (Figure 5e, f, g, and h). In these cells, the X univalent of *C. dimidiatus*, *C. scalaris*, and *C. ternarius* remained highly condensed and was positively heteropycnotic (Figure 5e, f, and g). The neoXY bivalent of *C. stigmosus* showed one terminal chiasma, confirming the presence of a synapsis between the sex chromosomes. The chiasma between the neoX and neoY chromosomes probably involves the long arm terminal region of both chromosomes that lacks constitutive heterochromatin. In all the diplotene cells analyzed, the neoXY bivalent is isopycnotic in relation to the autosomal bivalents (Figure 5h). Furthermore, diplotene spermatocytes showed the presence of secondary constrictions in one and in three autosomal bivalents of *C. scalaris* and *C. stigmosus*, respectively (Figure 5f and h).

Metaphase I cells revealed the chromosome formula 2n=8II+X0 in *C. dimidiatus*, *C. scalaris*, and *C. ternarius* (Figure 6a, b, and c) and 2n=7II+neoXY in *C. stigmosus* (Figure 6d). In these spermatocytes, the autosomal bivalents and the sex chromosomes were condensed and isopycnotic in appearance.

The anaphase I cells of the four *Conoderus* species showed that the autosome bivalents and sexual chromosomes undergo reductional behaviour during the first meiotic division (Figure 6e and f). The metaphasic II cells of *C. dimidiatus*, *C. scalaris*, and *C. ternarius* had haploid numbers of n=9=8+X and n=8 (Figure 6g and h). In cells with n=9, a differentially condensed chromosomal element was identified as the X chromosome. The metaphase II cells of *C. stigmosus* exhibited n=8=7+neoX (Figure 6i) and n=8=7+neoY. In this species, the sex chromosomes were recognized as the largest elements of the haploid complement. The study of the metaphase II cells of the four *Conoderus* species confirmed the reductional behaviour and the regular segregation of all the chromosomes in the preceding anaphase I cells.

Meiotic cells of all the species were subjected to Giemsa/AgNOR sequential staining; however, only *C. scalaris* spermatocytes revealed complementary information about NOR patterns in relation to those obtained in spermatogonial metaphases. The spermatocytes exhibited 4 NORs on the 2nd and 4th bivalents (Figure 7a), whereas spermatogonial metaphases showed 2 NORs on pair 4 (Figure 3b). The NORs on the 4th bivalent were coincident with the secondary constrictions (Figure 7a and b).

Due to the small number of slides obtained from individuals of each species, only *C. scalaris* and *C. stigmosus* meiotic cells were studied with CMA_3/DA/DAPI staining. Diakinesis and metaphase I cells in *C. scalaris* showed CMA_3/DA bright fluorescence in the pericentromeric/telomeric region of only one 2nd bivalent chromosomal element (Figure 7c). This CMA_3-heteromorphism was similar to that observed in mitotic cells (Figure 4b). The *C. stigmosus* meiotic cells revealed CMA_3/DA labelling, but the slightly brilliant fluorescence made the identification of the GC-rich regions pattern difficult to observe. The results obtained with the use of C banding, silver nitrate impregnation, and CMA_3/DA/DAPI staining techniques on mitotic and meiotic chromosomes of the four *Conoderus* species are summarized in Figure 8.

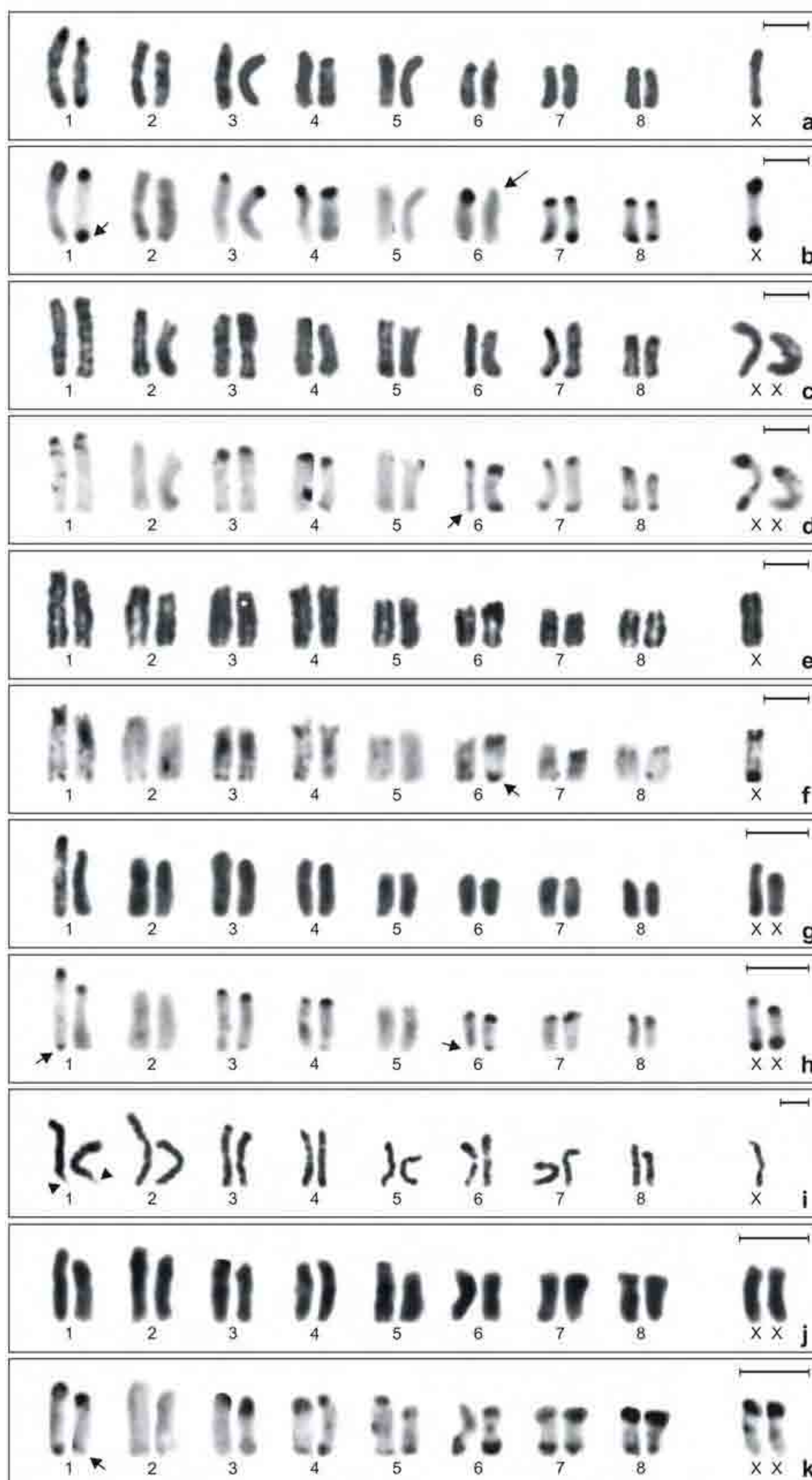

Figure 1. *Conoderus* species karyotypes with 2n(♂)=17=16+X0 (**a**, **b**, **e**, **f**, and **i**) and 2n(♀)=18=16+XX (**c**, **d**, **g**, **h**, **j**, and **k**) submitted to Giemsa (**a**, **c**, **e**, **g**, **i**, and **j**) and C-banding technique (**b**, **d**, **f**, **h**, and **k**) sequential staining. **a-d.** *Conoderus dimidiatus*. **e**-**h.** *Conoderus scalaris*. **i**-**k.** *Conoderus ternarius*. Note that some chromosome pairs possess pericentromeric (large arrow) and telomeric (small arrow) C band

heteromorphism. Pair 2 of all species is C band negative. The arrowhead indicates secondary constriction. Bar=5µm.

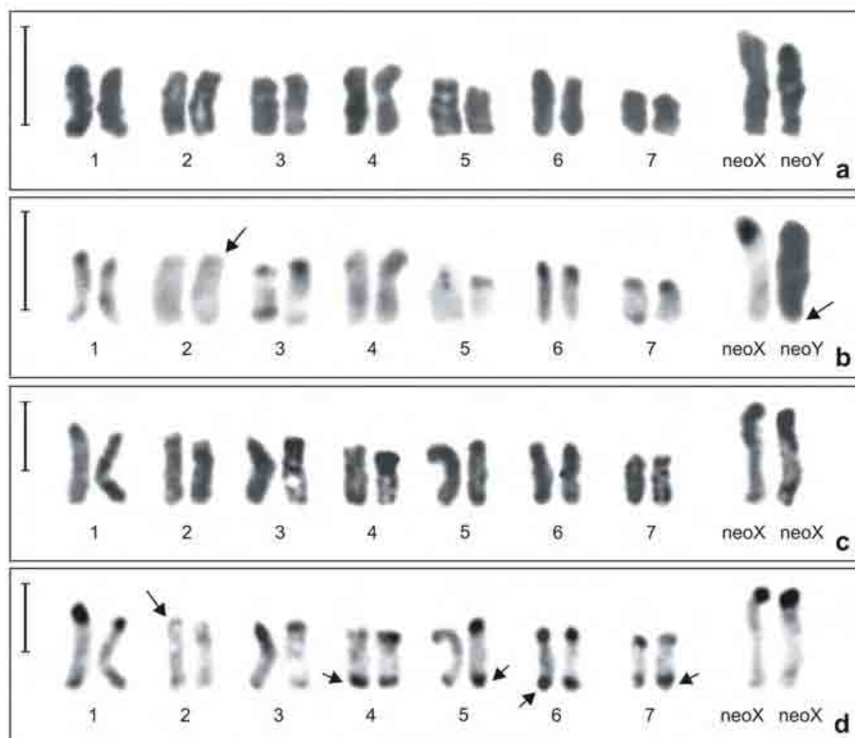

Figure 2. *Conoderus stigmosus* karyotypes subjected to both standard staining (**a** and **c**) and C-banding (**b** and **d**). **a**-**b.** Male, with 2n=16=14+neoXY. **c**-**d.** Female, with 2n=16=14+neoXX. Observe that pair 2 and the terminal region of neoY chromosome long arm are C band negative (large arrow). The small arrows indicate telomeric C bands in some female autosomal pairs. Bar=5µm.

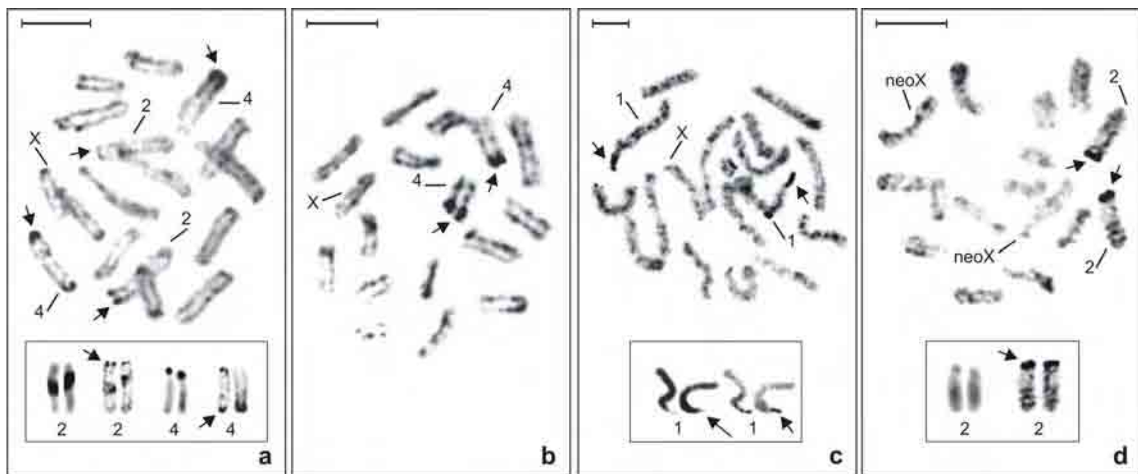

Figure 3. *Conoderus* species mitotic metaphases analyzed by silver nitrate impregnation, demonstrating the chromosome carriers of the NORs. **a.** *Conoderus dimidiatus*, 2n(♂)=16+X0, with 4 NORs on pairs 2 and 4. The insert shows the C-banding/AgNOR sequential staining of pairs 2 and 4. **b.** *Conoderus scalaris*, 2n(♂)=16+X0, with 2 NORs on pair 4. **c.** *Conoderus ternarius*, 2n(♂)=16+X0, with 2 NORs on pair 1. In detail, pair 1 submitted to both Giemsa/AgNOR. **d.** *Conoderus stigmosus*, 2n(♀)=14+neoXX, with 2 NORs on pair 2. In focus, pair 2 subjected to C-banding/AgNOR sequential staining. The small arrow indicates NORs. The large arrow shows secondary constriction. Bar=5μm.

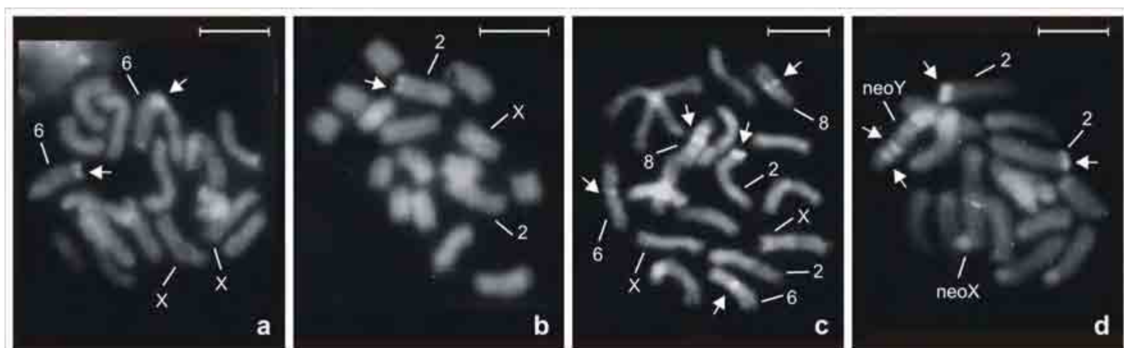

Figure 4. *Conoderus* species mitotic cells stained with CMA_3/DA/DAPI, showing GC-rich chromatin (CMA_3 positive). **a.** *Conoderus dimidiatus*, 2n(♀)=16+XX, with bright fluorescence in pair 6 pericentromeric region. **b.** *Conoderus scalaris*, 2n(♂)=16+X0, with CMA_3-heteromorphism in pair 2 pericentromeric/telomeric region. **c.** *Conoderus ternarius*, 2n(♀)=16+XX, with heteromorphic labelling in pair 2 pericentromeric/telomeric region and brilliant fluorescence in the pericentromeric region of pairs 6 and 8. **d.** *Conoderus stigmosus*, 2n(♂)=14+neoXY, with positive fluorescence in pair 2 pericentromeric/telomeric region and in neoY long arm interstitial region. The arrow indicates CMA_3 positive region. Bar=5μm.

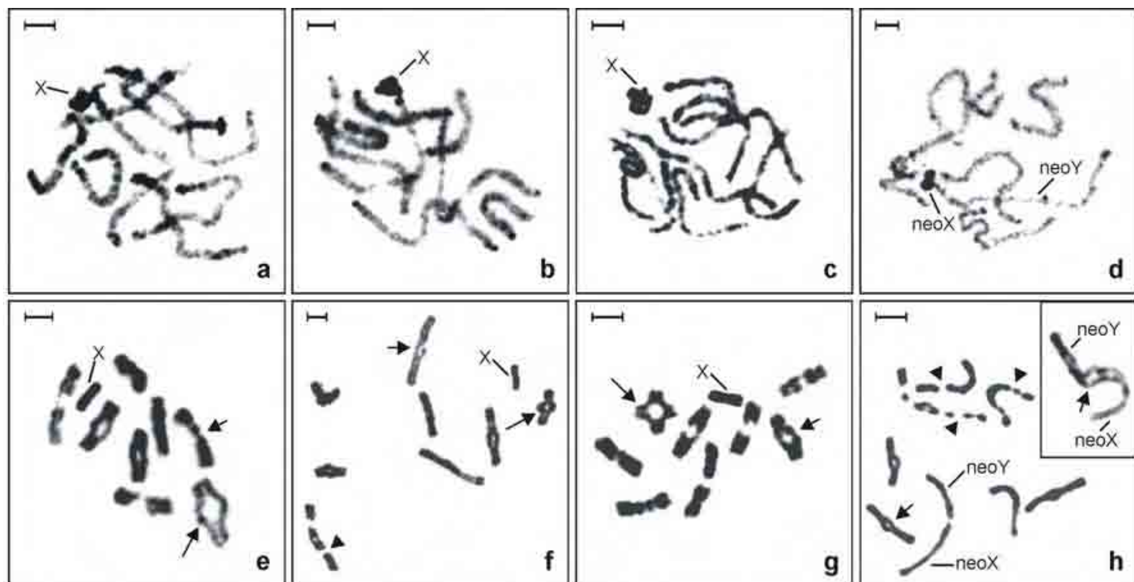

Figure 5. Prophase I spermatocytes of *Conoderus dimidiatus* (**a** and **e**), *Conoderus scalaris* (**b** and **f**), *Conoderus ternarius* (**c** and **g**), and *Conoderus stigmosus* (**d** and **h**) stained with Giemsa. **a-c.** Pachytenes, with 2n=8II+X0. **d.** Pachytene, with 2n=7II+neoXY. **e-g.** Diplotenes, showing the formula 2n=8II+X0. **h.** Diplotene, with 2n=7II+neoXY. In the diplotene cells, observe the bivalents with one terminal (small arrow) or interstitial (large arrow) chiasma. The insert in **h** shows the terminal chiasma in neoXY bivalent. The arrowhead indicates secondary constriction. Bar=5μm.

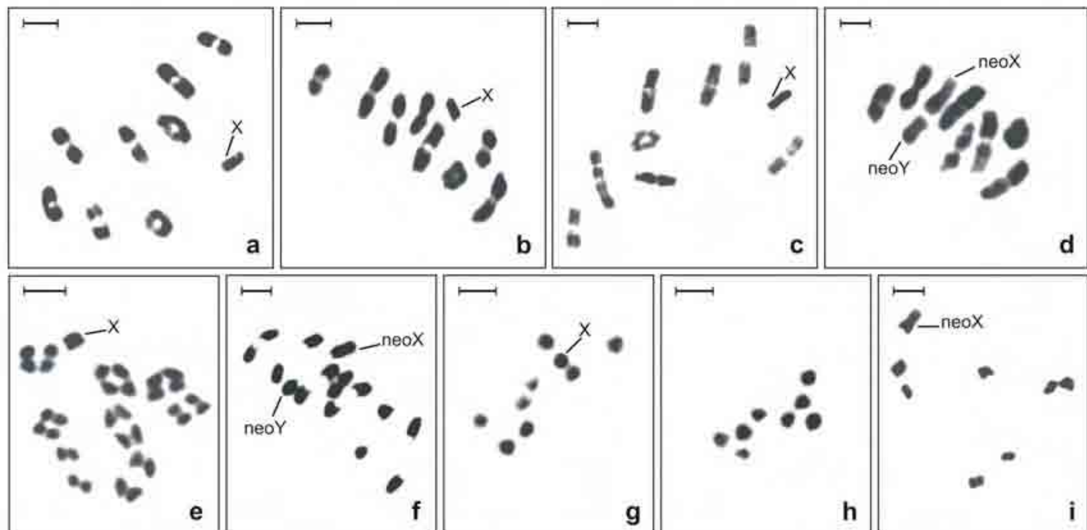

Figure 6. Male meiocytes of *Conoderus dimidiatus* (**a**), *Conoderus scalaris* (**b**, **e**, **g**, and **h**), *Conoderus ternarius* (**c**), and *Conoderus stigmosus* (**d**, **f**, and **i**) stained by Giemsa. **a**-**c**. Metaphases I, 2n=8II+X0. **d.** Metaphase I, 2n=7II+neoXY. **e** and **f.** Anaphases I, showing the reductional behaviour of all chromosomes. **g** and **h.** Metaphases II, with n=8+X and n=8, respectively. **i.** Metaphase II, with n=7+neoX. Bar=5μm.

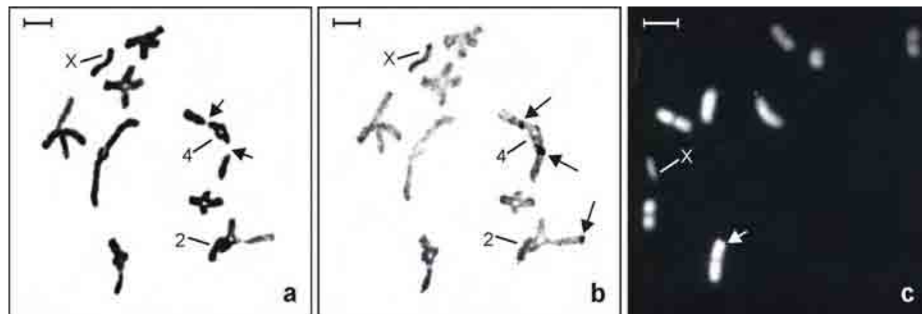

Figure 7. *Conoderus scalaris* spermatocytes, with 2n=8II+X0. **a** and **b.** Diplotene cell analyzed with both Giemsa staining (**a**) and silver impregnation (**b**), showing the secondary constriction (small arrow) on bivalent 4 in **a** and NORs (large arrow) on bivalent 2 and 4 in **b**. **c.** Metaphase I stained by CMA_3/DA, exhibiting CMA_3-heteromorphic labelling (arrow) in bivalent 2. Bar=5μm.

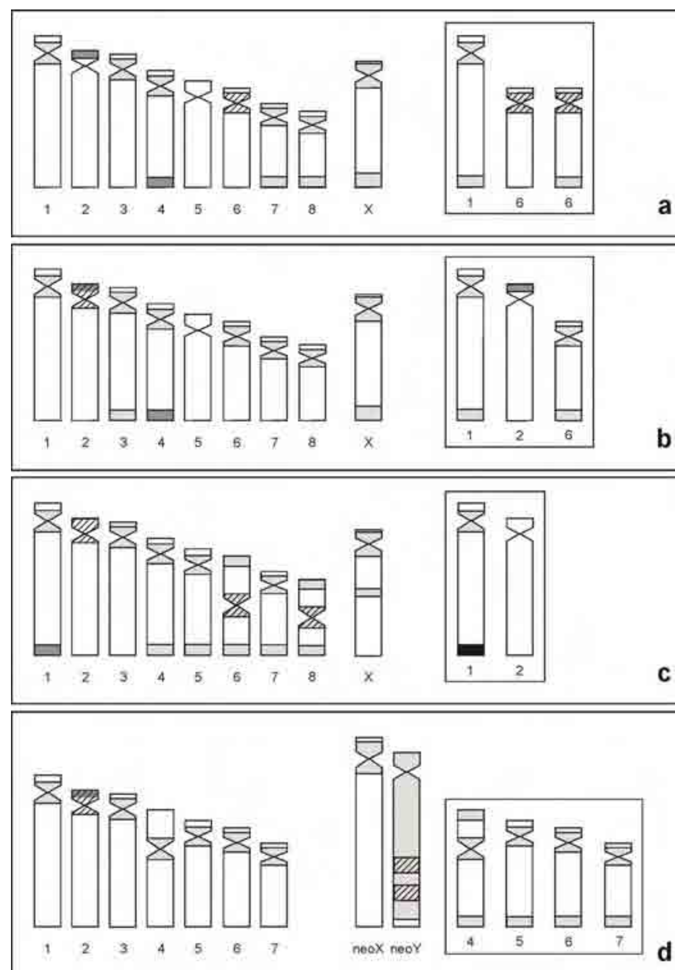

Figure 8. Ideograms of the four *Conoderus* species, representing the C-banding, NORs, and CMA_3/DA pattern. **a.** *Conoderus dimidiatus*. **b.** *Conoderus scalaris*. **c.** *Conoderus ternarius*. **d.** *Conoderus stigmosus*. The inserts show the autosomal elements with C band and/or CMA_3/DA heteromorphism.
□= C band. ■= NORs. ▨= CMA_3/DA labelling. ■= C band and NORs coincident region. ▨= C band and CMA_3/DA coincident region. ▨= NORs and CMA_3/DA coincident region.

Discussion

The high diversity of diploid numbers and types of sex determination systems in the subfamily Agrypninae (Table 1) is probably derived from the karyotype $2n=18+Xy_p$, with all metacentric chromosomes. This has been proposed as the basic karyotype for the coleopteran suborder Polyphaga and has been recorded in some species belonging to three other Elateridae subfamilies (Smith & Virkki, 1978). Karyotypic evolution in the subfamily Agrypninae has been achieved mainly by reduction in chromosome numbers by autosomal fusions, resulting in diploid numbers lower than 2n=20 chromosomes, and loss of the y_p chromosome, yielding the X0 sex determination system. Moreover, some species of this subfamily seem to have undergone additional karyotypic differentiation, involving fusions between autosomes and sex chromosomes suggested by the occurrence of derived sex determination systems, such as neoXY or X_1X_2Y.

Cytogenetic analysis of these *Conoderus* species shows that *C. dimidiatus*, *C. scalaris*, and *C. ternarius* possess a diploid number and type of sex determination system similar to those reported for two other species of the genus (Virkki, 1962), *C. pilatei* (Candèze, 1859) and *C. rodriguezi* (Candèze, 1881), and for almost all Conoderini species (Vidal, 1984; Yadav & Vyas, 1994). Furthermore, prevalence of acrocentric chromosomes in the four *Conoderus* species studied was expected given previous descriptions of the majority of the cytogenetically known Conoderini species (Agarwal, 1960; 1962; Yadav & Vyas, 1994). However, variations in the number of acrocentric chromosomes among these species involved autosomes and rarely the X chromosome (Table 1). These could be due to the occurrence of pericentric inversions that converted meta/submetacentric chromosomes to acrocentrics or vice versa. Surprisingly, cytogenetic study of *C. stigmosus* evidenced diploid number not recorded for the subfamily Agrypninae so far and a type of sex determination system still not found among the Conoderini species. The karyotypic characteristics of this species are probably more differentiated than those observed in other representatives of this genus.

The novel *C. stigmosus* diploid number and type of sex determination system could be derived from a karyotype with 2n=16+X0. Taking into account this karyotype, the neoX sex chromosome of *C. stigmosus* probably originated from a tandem fusion between the long arm telomeric region of the original X chromosome and the long arm proximal region of a small autosomal element that had lost its centromeric region. The homologue of the autosome fused with the X chromosome became the neoY sex chromosome. The neoX chromosome retained the pericentromeric C band, while the neoY chromosome became heterochromatic along most of its entire length, with the exception of the long arm terminal region, which is C band negative. Furthermore, addition and/or duplication of the constitutive heterochromatin regions could be responsible for the increase of the *C. stigmosus* neoY chromosome size. This hypothesis of the neoXY sex determination system origin is supported by the reduction in the diploid number and the large size of the neoX sex chromosome in *C. stigmosus*. The presence of a neoXY sex determination system has been reported in two other Agrypninae species, *Hemirrhipus lineatus* (Olivier, 1790) and *Lacon profusa* Candèze, 1857 (Table 1). Piza (1958) also inferred that in *H. lineatus*, fusions between autosomes and sex chromosomes could have formed the extremely large neoX and neoY chromosomes and reduced the diploid number to 2n=10.

The meiotic chromosomes of the four *Conoderus* species exhibited behavioural patterns similar to those encountered in a majority of beetles species (Smith & Virkki, 1978) and in two other *Conoderus* species (Virkki, 1962). Although *C. stigmosus* meiotic chromosomes showed some characteristics similar to those of other *Conoderus* species, a singular and synaptic neoXY bivalent with a prominent terminal chiasma was noted in late prophase I cells. This sex bivalent chiasmatic association probably occurs between the autosomal portion of the neoX chromosome and the ancient homologous autosome that originated the neoY chromosome, as proposed by Piza (1958) for *H. lineatus*.

Chromosomes with a large amount of constitutive heterochromatin, mainly in the pericentromeric region, are probably characteristic of the

Elateridae family, considering the C-banding pattern obtained in the species analyzed in this work, as well as in *Adelocera murina* (Lineu, 1758) and *Prosternon tesselatum* (Lineu, 1758) (Rozek & Lachowska, 2001; Rozek et al., 2004). Additional C bands in the interstitial and/or telomeric regions, as verified in the *Conoderus* species, have not been described for Elateridae species, but are occasionally encountered in other beetle species (Juan & Petitpierre, 1989; Rozek, 1998; Almeida, Zacaro & Cella, 2000; Schneider, Artoni & Almeida, 2002; Zacaro et al., 2004). These additional heterochromatic regions could have originated from multiples duplications of small repetitive DNA segments and/or from the transfer of constitutive heterochromatic material among equidistant sites of non-homologous chromosomes (Schweizer & Loidl, 1987).

Heterochromatin polymorphism is common in a wide variety of eukaryote species (John, 1988; John & King, 1983; Sumner, 2003). In the *Conoderus* species examined here, heteromorphism of C band distributions could be the result of constitutive heterochromatin addition or deletion. Alternatively, due to the fact that the autosomal elements of heteromorphic pairs showed no size disparity, the conversion of euchromatin into heterochromatin might explain the origin of this heteromorphism. The predominance of C band heteromorphism in some autosomal pairs suggests that these chromosomes are tolerant to losses or gains in constitutive heterochromatic material (John, 1988). According to Sumner (1990), the lack of C band positive regions on certain chromosomes can be attributed to the methodology used, because it does not reveal all types of heterochromatin.

There was a considerable discrepancy between the *C. stigmosus* sex chromosomes in relation to the constitutive heterochromatin content. Despite the region of homology between-the neoX and neoY chromosomes confirmed by the presence of chiasma, these chromosomes are almost wholly differentiated in their chromatin composition and organisation. This result points to an ancient origin of the neoXY sex determination system of *C. stigmosus*.

Interspecific variation in number and location of the NORs was independent of the diploid number and type of sex determination system encountered in the four *Conoderus* species. However, the NORs were located

in autosomes and occurred predominantly on the 2nd pair. Virkki, Flores & Escudero (1984) also noted the occurrence of 2 NORs on large autosomes in *Pyrophorus luminosus* Illiger, 1807 (now in *Ignelater* Costa, 1975), when the meiotic cells of this species were impregnated with silver nitrate. Additional NORs were detected on the 4th chromosome pair of *C. dimidiatus* and *C. scalaris*, whereas these regions were only observed on the 1st pair of *C. ternarius*, showing NOR pattern variation. The increased number of chromosome pairs bearing the NORs and the varied location of these regions on chromosomes could occur due to the partial or total translocations of the rDNA genes. Translocations related to the interspecific numerical and positional variation of the NORs were also proposed by Bione, Camparoto & Simões (2005) for some Scarabaeidae coleopteran species. According to Sánchez-Gea, Serrano & Galián (2000), transposition has also been indicated as the cause of NOR pattern variability. On the other hand, the variation of autosomal pair numbers bearing NORs could arise from the use of the silver nitrate impregnation technique, which reveals only the NORs that were active in the preceding interphase (Galián et al., 1995; Petitpierre, 1996).

Despite the fact that chromosomes of the four *Conoderus* species exhibited very prominent C band positive regions, the majority of the constitutive heterochromatic regions were not compartmentalised in AT- or GC-rich DNA sequences, because only some of these regions revealed CMA_3 bright fluorescence and were homogeneously DAPI stained. In *C. scalaris* and *C. stigmosus*, CMA_3 labelling on chromosome pair 2 seemed to include the NORs. In other species, the CMA_3 fluorochrome has been useful in detecting both active and inactive NORs because rDNA is usually GC-rich (Schweizer et al., 1983; Schmid & Guttenbach, 1988). However, because pair 2 of *C. ternarius* showed CMA_3 bright fluorescence and did not carry any NORs, and one *C. scalaris* and *C. ternarius* chromosome pair revealed CMA_3-heteromorphism, CMA_3 labelling on pair 2 of *C. scalaris* and *C. stigmosus* could represent unique heterochromatin associated with NORs. In other coleopteran species (Vitturi et al., 1999; 2003), e.g., *Pentodon bidens punctatum* Villers, 1899 (Scarabaeidae)

and *Thorectes intermedius* (Costa, 1827) (Geotrupidae), heterochromatin associated with NORs and labelled with CMA_3 was observed.

In conclusion, chromosomal analysis of four Brazilian *Conoderus* species suggests dramatic karyotypic evolution among species. Karyotypic differentiation has been more rapid for autosomes than for sex chromosomes. Interspecific autosomal differentiation has involved the distribution and quantity of constitutive heterochromatin and GC-rich regions, as well as the number and location of NORs. In the sex chromosomes, the most remarkable change was the ancient X chromosome/autosome fusion, from which the derivative sex determination system of the neoXY type originated, at least in one *Conoderus* species. Considering the wide variety of Elateridae species, principally in the Brazilian fauna, characterization of a greater number of “click-beetle” representatives is needed to provide a clearer understanding of elaterid chromosomal evolution.

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Artigo II

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Chromosomal similarities and differences among four Neotropical Elateridae (Conoderini and Pyrophorini) and other related species, with comments on the NORs pattern in Coleoptera.

Running title: Chromosomal similarities and differences among four Elateridae species

Marielle Cristina Schneider[1], Simone Policena Rosa[2], Mara Cristina de Almeida[3], Cleide Costa[2], and Doralice Maria Cella[1]

Resumo

Este trabalho trata da análise citogenética comparativa de quatro espécies de Elateridae neotropicais e mostra, pela primeira vez, uma revisão sobre o padrão das regiões organizadoras de nucléolo (RONs) nos cromossomos de Coleoptera. A caracterização citogenética de *Conoderus malleatus* (Conoderini), *Pyrearinus candelarius*, *Pyrophorus divergens*, e *Pyrophorus punctatissimus* (Pyrophorini) foi realizada através do estudo de células mitóticas e meióticas submetidas à coloração convencional (Giemsa) e diferencial (impregnação pela prata e coloração com fluorocromos CMA_3, específico para GC, e DAPI, específico para AT). A análise de células espermatogoniais evidenciou 2n=17 em *C. malleatus* e 2n=15 em *Pyrearinus candelarius*, *Pyrophorus divergens*, e *Pyrophorus punctatissimus*. Nestas espécies, observou-se sistema de determinação sexual do tipo XO e a morfologia acrocêntrica de quase todos os cromossomos. O estudo de células meióticas das quatro espécies revelou a ocorrência de sinapse total entre os autossomos, a presença de um quiasma terminal ou intersticial na maioria dos bivalentes, e o comportamento reducional e segregação regular de todos os cromossomos. Embora as três espécies de Pyrophorini tenham mostrado muitas características cariotípicas similares, existe uma discrepante, a qual foi notada em células diplotênicas e é concernente ao número de bivalentes com dois quiasmas, isto é, *Pyrearinus candelarius* possuía somente um bivalente, *Pyrophorus divergens* mostrou dois bivalentes, e *Pyrophorus punctatissimus* exibiu até quatro bivalentes com dois quiasmas. Células testiculares impregnadas com nitrato de prata evidenciaram 2 RONs terminais localizadas no 4º par autossômico da espécie de Conoderini e no 2º par autossômico dos três representantes de Pyrophorini. O emprego da coloração CMA_3/DA/DAPI nos cromossomos de *Pyrearinus candelarius* e *Pyrophorus punctatissimus* revelou que as regiões marcadas com CMA_3 eram coincidentes com as RONs. As estratégias principais de diferenciação cariotípica que ocorreram entre as quatro espécies de Elateridae e outras espécies relacionadas, e as tendências gerais das mudanças das RONs durante a evolução cromossômica dos Coleoptera são discutidas neste trabalho.

Palavras-chave: bivalente, cariótipo, fluorocromo, meiose, quiasma, sistema de determinação sexual

Abstract

This work deals with the comparative cytogenetic analysis of four Neotropical Elateridae species and shows for the first time, a review about the nucleolar organizer regions (NORs) pattern on Coleoptera chromosomes. The cytogenetic characterization of *Conoderus malleatus* (Conoderini), *Pyrearinus candelarius*, *Pyrophorus divergens*, and *Pyrophorus punctatissimus* (Pyrophorini) was accomplished through the study of mitotic and meiotic cells submitted to the standard (Giemsa) and differential staining (silver impregnation and GC-specific CMA_3 and AT-specific DAPI fluorochromes). The analysis of spermatogonial cells evidenced the diploid number 2n=17 in *C. malleatus* and 2n=15 in *Pyrearinus candelarius*, *Pyrophorus divergens*, and *Pyrophorus punctatissimus*. In these species, the sex determination system of the X0 type and the acrocentric morphology of almost all chromosomes were noted. The study of meiotic cells of the four species revealed the occurrence of total synapsis between the autosomes, the presence of one terminal or interstitial chiasma in the majority of the bivalents, and the reductional behaviour and regular segregation of all chromosomes. Although the three Pyrophorini species have disclosed many similar karyotypical characteristics, there is one discrepant, which were noted in diplotene cells and is concerning the number of bivalents with two chiasmata, that is, *Pyrearinus candelarius* only possessed one bivalent, *Pyrophorus divergens* showed two bivalents, and *Pyrophorus punctatissimus* exhibited until four bivalents with two chiasmata. Testicular cells impregnated with silver nitrate evidenced 2 terminal NORs located on 4th autosomal pair of the Conoderini species and on 2nd autosomal pair of the three Pyrophorini representatives. The employment of the CMA_3/DA/DAPI staining in the *Pyrearinus candelarius* and *Pyrophorus punctatissimus* chromosomes revealed that the CMA_3 labelled regions were coincident with the NORs. The main strategies of karyotypical differentiation that have occurred among the four Elateridae species and other related species, and the general trends of the NORs shifts during the Coleoptera chromosomal evolution are discussed in this work.

Key words: bivalent, chiasma, fluorochrome, karyotype, meiosis, sex determination system

Introduction

The family Elateridae has about 9300 described species (Costa 2003) and is the eighth largest of the suborder Polyphaga, which comprises 148 families worldwide (Lawrence and Newton 1995). The Elateridae are cosmopolitan in distribution and are included in 18 subfamilies (Lawrence and Newton 1995; Lawrence et al. 1999). A review on Elateridae disclosed that cytogenetic data are still scarce and are restricted to less than 1% of the species, belonging to the Neartic, Neotropical, Oriental, and Paleartic regions (Smith and Virkki 1978; Ferreira et al. 1984; Vidal 1984; Virkki et al. 1984; Virkki and Denton 1987a; Yadav and Vyas 1993, 1994; Rozek and Lachowska 2001; Rozek et al. 2004; Schneider et al. under submission).

The Elateridae fauna of the Neotropical region is interesting cytogenetically due to its richness in terms of species number, possessing approximately 2100 representatives (Costa 2003), and the highest karyotypical diversity in relation to those documented for other biogeographic regions. This karyotypical diversity is related to the great variety of chromosome number and type of sex determination systems described to date. In the Neotropical Elateridae, the diploid number ranges from 2n=4 (Ferreira et al. 1984) to 2n=23 (Virkki 1962), being these the lowest and highest chromosomal numbers, respectively, already found in this family. The sex determination system of the Xy_p type, which has been presumed to be the ancestral for Polyphaga (Smith and Virkki 1978), was not yet observed in the Neotropical elaterid species; however, the occurrence of derivative sex determination systems, such as the X0, Xy, neoXY, and X_1X_2Y has been documented in this group (Smith 1960; Virkki 1962; Vidal 1984; Virkki et al. 1984; Virkki and Denton 1987a; Schneider et al. under submission). The singular cytogenetical characteristics of the Neotropical elaterids are surprising and can contribute to the knowledge of the chromosomal evolution strategies among related species.

The chromosomes of the Elateridae as well as of the majority of the Coleoptera families have been occasionally examined with differential staining techniques, which evidence specific chromosomal regions. The employment of methodologies that detect the nucleolar organizer regions (NORs), such as the

silver nitrate impregnation and the fluorescence *in situ* hybridization with a rDNA probe, is of particular interest in the Coleoptera cytogenetic studies, considering that it can supply data about the chromosomal molecular organization, the possible shifts of the NORs during the karyotypic differentiation of related species, and about the mode of association between sex chromosomes in some types of sex determination system (Petitpierre 1996). Currently, more than 3000 Coleoptera species have already been cytogenetically described (Petitpierre 1996), but only 186 species of the suborder Adephaga and Polyphaga have their NORs distribution pattern established (Table 1). Of these species, approximately 35 are from Neotropical region (Almeida et al. 2000; Bione et al. 2005a,b; De la Rúa et al. 1996; Drets et al. 1983; Maffei et al. 2001a,b,c; Moura et al. 2003; Postiglioni and Brum-Zorrilla 1988; Proença et al. 2002a,b, 2004; Schneider et al. 2002; Virkki 1983; Virkki and Denton 1987a,b; Virkki and Sepúlveda 1990; Zacaro et al. 2004).

Considering that there are few Neotropical coleopteran species cytogenetically analyzed and there is not yet a more frequent NORs distribution pattern established for beetles, the present work has two main purposes: first, to determine the karyotype, the chromosome behaviour during the meiosis, the NORs distribution pattern, and the chromosomal regions AT- and GC-rich of four Neotropical Elateridae species from subfamily Agrypninae, being one of the tribe Conoderini, *Conoderus malleatus,* and the three other of the tribe Pyrophorini, *Pyrearinus candelarius*, *Pyrophorus divergens*, and *Pyrophorus punctatissimus*; second, to compile the data about the coleopteran NORs distribution pattern and to outline the general trends of the NORs shifts during the Coleoptera chromosomal evolution.

Material and methods

A sample of 34 adult individuals collected in the states of Paraná (PR) and São Paulo (SP), Brazil, were analyzed: 3 males and 2 females of *Conoderus malleatus*, from Ponta Grossa (25°05'S, 50°09'W), PR; 12 males of *Pyrearinus candelarius*, from Rio Claro (22°24'S, 47°33'W), SP; 4 males of

Pyrophorus divergens, from Rio Claro (22°24'S, 47°33'W) and Campinas (22°54'S, 47°04'W), SP, and Ponta Grossa (25°05'S, 50°09'W), PR; 12 males and 1 female of *Pyrophorus punctatissimus*, from Rio Claro (22°24'S, 47°33'W), SP. The voucher specimens were deposited in the Departamento de Biologia, UNESP, Rio Claro, SP.

Gonads of adult specimens of both sexes were used for chromosomal preparations. After animal dissection in insect salt solution, the gonads were removed and placed in 0.05% colchicine solution, during 90 minutes. For hypotonic treatment, an equal volume of tap water was added to the material and left for 2 minutes. For obtaining all meiosis phases, the testes of some males were not submitted to the colchicine treatment. All the gonads were fixed in Carnoy I, during 30 minutes. Cell suspension was obtained by macerating the gonads in 40% acetic acid on the slides surface. The slides were dried on a metal plate at 40° C. The majority of the chromosomal preparations were submitted to standard/AgNOR sequential staining, using 3% Giemsa solution, during 12 minutes, and silver nitrate impregnation according to Howell and Black (1980). For detection of the AT- and GC-rich chromatin regions, the fluorochromes 4'-6-diamidino-2-phenylindole (DAPI) and chromomycin A_3 (CMA_3) with counterstain distamycin A (DA) were used, according to the technique described by Schweizer (1980). The chromosomal morphology followed the nomenclature proposed by Levan et al. (1964).

Results

Gonadal cells of *C. malleatus* (Conoderini), *Pyrearinus candelarius*, *Pyrophorus divergens*, and *Pyrophorus punctatissimus* (Pyrophorini) were analyzed with standard staining and silver nitrate impregnation. Additionally, testicular cells of *Pyrearinus candelarius* and *Pyrophorus punctatissimus* were submitted to the CMA_3/DA/DAPI staining.

Standard staining (Giemsa)

The study of mitotic metaphase cells of *C. malleatus* showed 2n=17 for males and 2n=18 for females (Fig. 1a-b). The analysis of spermatogonial cells of *Pyrearinus candelarius*, *Pyrophorus divergens*, and *Pyrophorus punctatissimus* revealed that these species possess the same diploid number, that is, 2n=15 chromosomes (Fig. 1c-e). Oogonial metaphases were only obtained from *Pyrophorus punctatissimus*, which evidenced 2n=16 chromosomes (Fig. 1f). In the four analyzed species, a similar sex determination system of the X0/XX type was observed.

The karyotype analysis of *C. malleatus* disclosed that the chromosomes gradually decrease in size and the X sex chromosome possesses intermediate size between the 3rd and 4th autosomal pairs (Fig. 1a-b). On the other hand, the chromosomes of *Pyrearinus candelarius*, *Pyrophorus divergens*, and *Pyrophorus punctatissimus* can be classified in three groups according their size: large (pairs 1 and 2), medium (pairs 3, 4, and 5), and small (pairs 6 and 7). In these last three species, the X sex chromosome is a small element of intermediate size between the 6th and 7th autosomal pairs (Fig. 1c-f).

In relation to the chromosomal morphology, *C. malleatus*, *Pyrearinus candelarius*, *Pyrophorus divergens*, and *Pyrophorus punctatissimus* showed that almost all chromosomes are acrocentrics; however, there is variation in the number of submetacentric autosomal pairs and in the morphology of the X chromosome among the studied species. The submetacentric condition was detected in the pairs 4, 6, and 8 of *C. malleatus*, pairs 4, 6, 7, and X chromosome of *Pyrearinus candelarius*, and pairs 6, 7, and X chromosome of *Pyrophorus divergens* and *Pyrophorus punctatissimus* (Fig. 1). In some *C. malleatus* cells, whose chromosomes were less condensed, a prominent constriction in the elements of the 6th autosomal pair was noted, which corresponds to the centromeric region of these chromosomes.

The analysis of the meiotic testicular cells of the four species confirmed the chromosome number and the type of sex determination system established in mitotic cells. The submetacentric morphology of the *Pyrearinus candelarius*,

Pyrophorus divergens, and *Pyrophorus punctatissimus* X chromosome was clearly evident in diplotene and diakinesis cells (Fig. 2g; Fig. 3b-c).

The prophase I and metaphase I spreads revealed the meioformula 2n=8II+X0 in *C. malleatus* and 2n=7II+X0 in *Pyrearinus candelarius*, *Pyrophorus divergens*, and *Pyrophorus punctatissimus* (Fig. 2; Fig. 3a-e). The metaphasic II spermatocytes evidenced the haploid chromosomal number n=8+X or n=8 for *C. malleatus* and n=7+X or n=7 for *Pyrearinus candelarius*, *Pyrophorus divergens*, and *Pyrophorus punctatissimus*, indicating that the autosomes and X sex chromosome possess reductional behaviour and regular segregation in the preceding anaphase (Fig. 3f-j). In the majority of the meiosis phases, the X chromosome was easily identified due to its highest degree of condensation in relation to the autosomes.

The study of prophasic I spermatocytes also provided data about the synaptic behaviour as well as the number and distribution of chiasmata in the bivalents of the analyzed species. The pachytene cells showed that the autosomal bivalents are synapsed along their entire chromosomal length whereas the X chromosome is a single element, precociously condensed, and positive heteropycnotic (Fig. 2a-d). All autosomal diplotenic bivalents in *C. malleatus* and the majority of them in *Pyrearinus candelarius*, *Pyrophorus divergens*, and *Pyrophorus punctatissimus* have only one interstitial or terminal chiasma (Fig. 2e-h); but, among the Pyrophorini representatives, the analysis of approximately 30 diplotenic cells per species showed particularities concerning to the number of bivalents that possess two chiasmata, as follows: *Pyrearinus candelarius* disclosed 1 medium size bivalent, *Pyrophorus divergens* showed 2 large or medium size bivalents, and *Pyrophorus punctatissimus* revealed until 4 large and medium size bivalents (Fig. 2f-h). These chiasmata can be interstitial and/or terminal.

Silver nitrate impregnation

The analysis of testicular cells of *C. malleatus*, *Pyrearinus candelarius*, *Pyrophorus divergens*, and *Pyrophorus punctatissimus* submitted to the Giemsa/AgNOR sequential staining evidenced that all four species possess 2

autosomal NORs (Fig. 4; Fig. 5). The diakinesis spermatocytes of *C. malleatus* showed that the NORs are located on terminal region of the 4th autosomal bivalent short arm (Fig. 4a-b). Pachytene and diplotene cells of *Pyrearinus candelarius*, *Pyrophorus punctatissimus*, and *Pyrophorus divergens* disclosed NORs on the terminal region of the 2nd bivalent short arm (Fig. 4c-f; Fig. 5c-h). The *Pyrophorus divergens* meiotic NORs pattern was also confirmed in the mitotic metaphase cells (Fig. 5a-b).

Heteromorphic NORs were detected in the late prophase I cells (diplotene and diakinesis) of all analyzed species (Fig. 4f; Fig. 5h). This heteromorphism involves the presence of the labelled region or the intensity of the labelling by silver nitrate between the chromosomal elements of the bivalent. Due to the fact of this NOR heteromorphism has been only encountered in some cells of the same individual, it is probably related with the differential activity of the NORs between the chromosomes of the bivalent.

The use of the silver impregnation in the meiotic chromosomes also revealed the presence of an argentophilic material on X chromosome of the four species (Fig. 4d, f; Fig. 5f, h). As this material was only noted in some meiotic cells and was non-specific, occurring on entire length or on a small region of the X chromosome, it was interpreted as not related with the nucleolar activity.

CMA_3/DA/DAPI staining

Spermatogonial metaphases of *Pyrearinus candelarius* and *Pyrophorus punctatissimus* with CMA_3/DA/DAPI staining exhibited bright CMA_3 labelling on pair 2 short arm (Fig. 6a, c). This GC-rich region is coincident to the NORs site. Brilliant CMA_3 labelling was also observed in the terminal region of the 2nd autosomal bivalent short arm of the *Pyrearinus candelarius* spermatocytes (Fig. 6b). The chromosomes of both species were homogeneously DAPI stained, with no particular brilliant region.

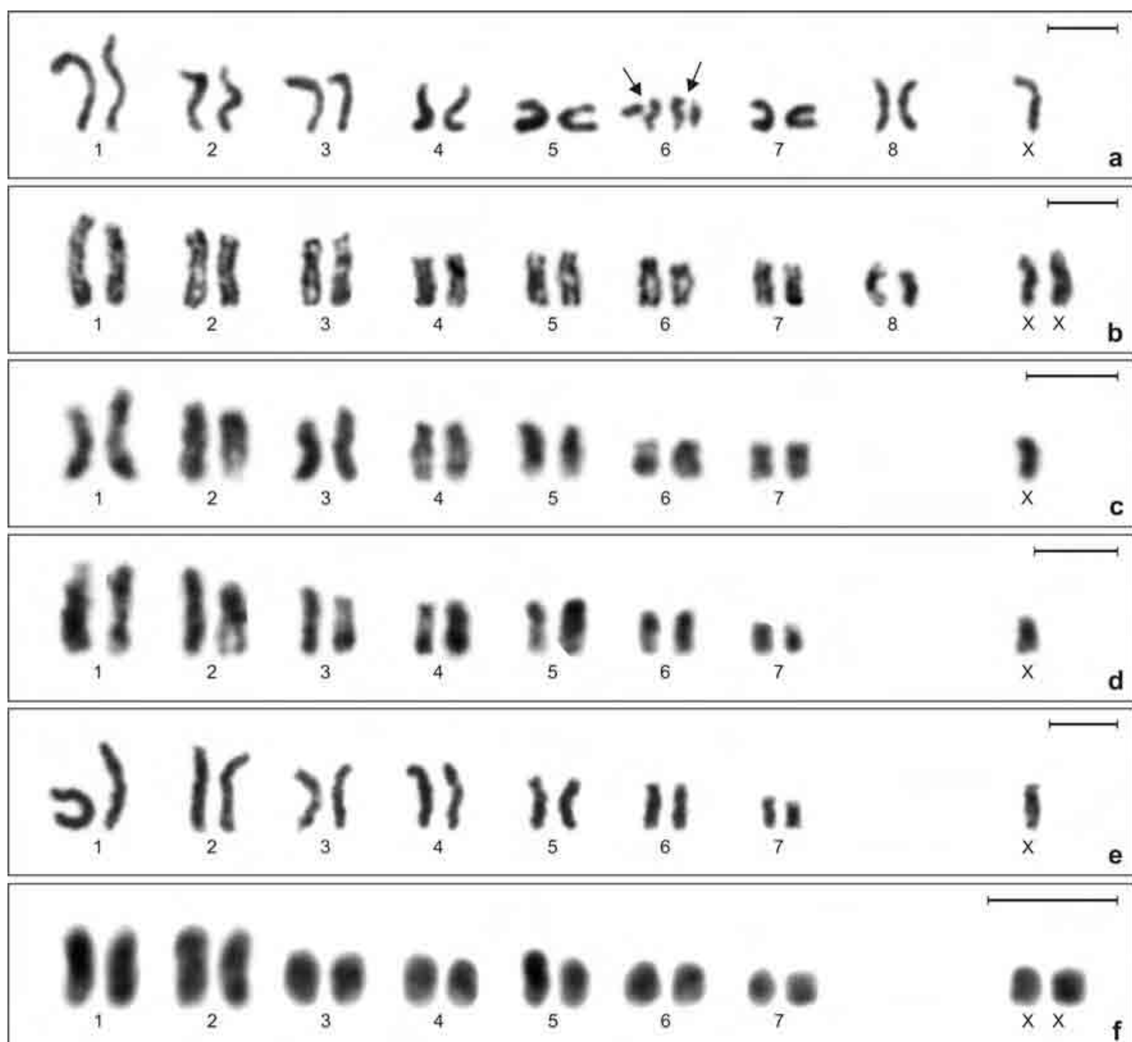

Figure 1. Standard stained Elateridae karyotypes. (**a**-**b**) *Conoderus malleatus*, with 2n(♂)=17=16+X0 and 2n(♀)=18=16+XX, respectively. (**c**) *Pyrearinus candelarius*, 2n(♂)=15=14+X0. (**d**) *Pyrophorus divergens*, 2n(♂)=15=14+X0. (**e**-**f**) *Pyrophorus punctatissimus*, with 2n(♂)=15=14+X0 and 2n(♀)=16=14+XX, respectively. The arrows indicate prominent centromere constrictions. Bar=5μm.

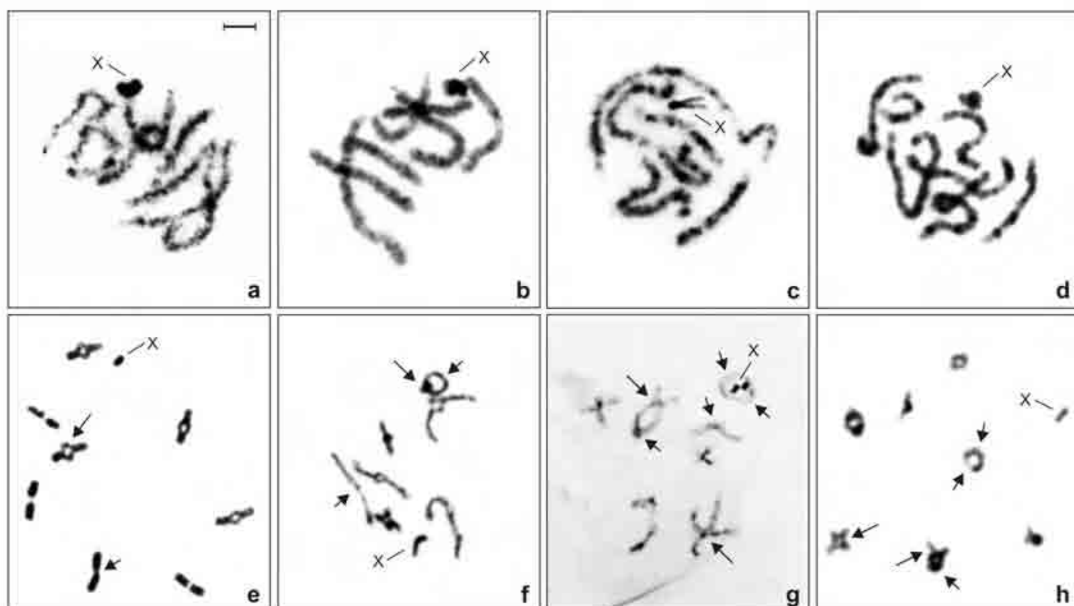

Figure 2. Prophase I testicular cells of *Conoderus malleatus* (**a**, **e**), *Pyrearinus candelarius* (**b**, **f**), *Pyrophorus divergens* (**c**, **g**), and *Pyrophorus punctatissimus* (**d**, **h**) standard stained. (**a**) Pachytene, 2n=8II+X0. (**b**-**d**) Pachytenes, 2n=7II+X0. (**e**) Diplotene, 2n=8II+X0, evidencing one chiasma per autosomal bivalent. (**f**-**h**) Diplotenes, 2n=7II+X0, exhibiting one or two chiasmata per autosomal bivalent. The large arrow indicates interstitial chiasma. The small arrow shows terminal chiasma. Observe in **g** the submetacentric morphology of the X chromosome. Bar=5μm.

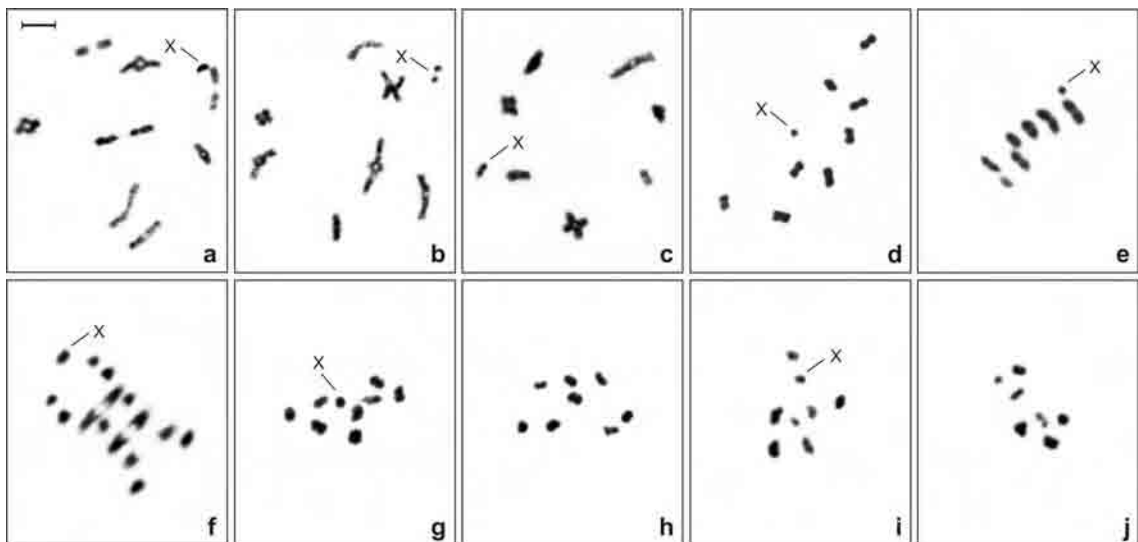

Figure 3. Spermatocytes of *Conoderus malleatus* (**a**, **d**, **g**, **h**), *Pyrearinus candelarius* (**b**, **f**), *Pyrophorus divergens* (**e**), and *Pyrophorus punctatissimus* (**c**, **i**, **j**) subjected to standard staining. (**a**) Diakinesis, with the meioformula 2n=8II+X0. (**b**-**c**) Diakineses, evidencing the meioformula 2n=7II+X0 and the submetacentric morphology of the X chromosome. (**d**-**e**) Metaphases I, with 2n=8II+X0 and 2n=7II+X0, respectively. (**f**) Anaphase I, showing the reductional behavior of all chromosomes. (**g**-**h**) Metaphases II, with n=9=8+X and n=8, respectively. (**i**-**j**) Metaphases II, with n=8=7+X and n=7, respectively. Bar=5μm.

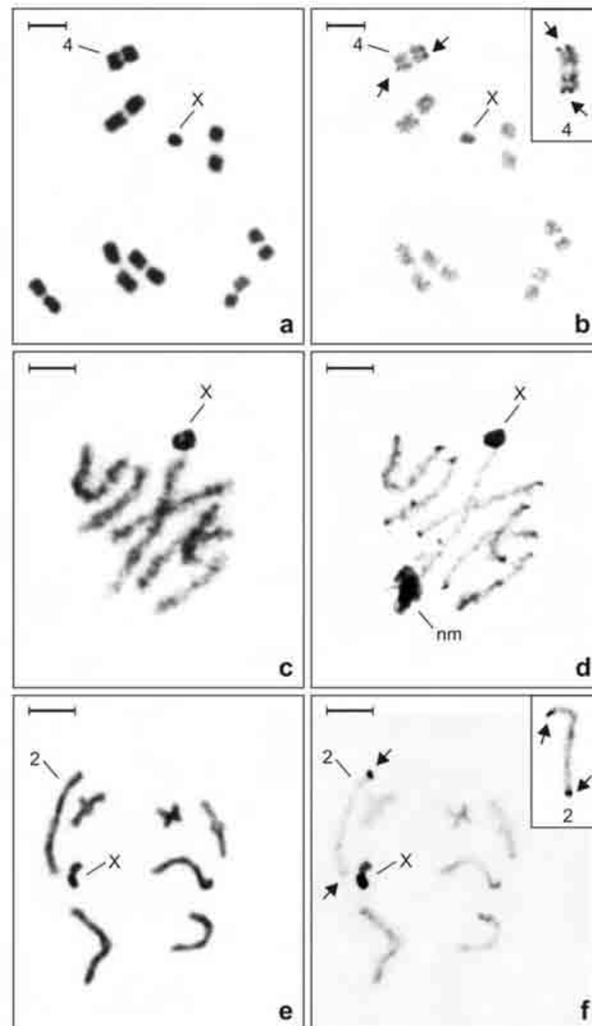

Figure 4. NOR pattern in prophase I spermatocytes of *Conoderus malleatus*, 2n=8II+X0 (**a**-**b**), and *Pyrearinus candelarius*, 2n=7II+X0 (**c**-**f**) obtained with Giemsa (**a**, **c**, **e**) and AgNOR (**b**, **d**, **f**) sequential staining. (**a**-**b**) Diakinesis, revealing NORs (arrows) on terminal region of the 4th bivalent. In detail, the 4th autosomal bivalent bearing the NORs (arrows). (**c**-**d**) Pachytene, evidencing nucleolar material (nm) on terminal region of the 2nd bivalent. (**e**-**f**) Diplotene, exhibiting bivalent 2 with heteromorphic NOR (arrows). In focus, the homomorphic NORs (arrows) on terminal region of the 2nd bivalent. Bar=5μm.

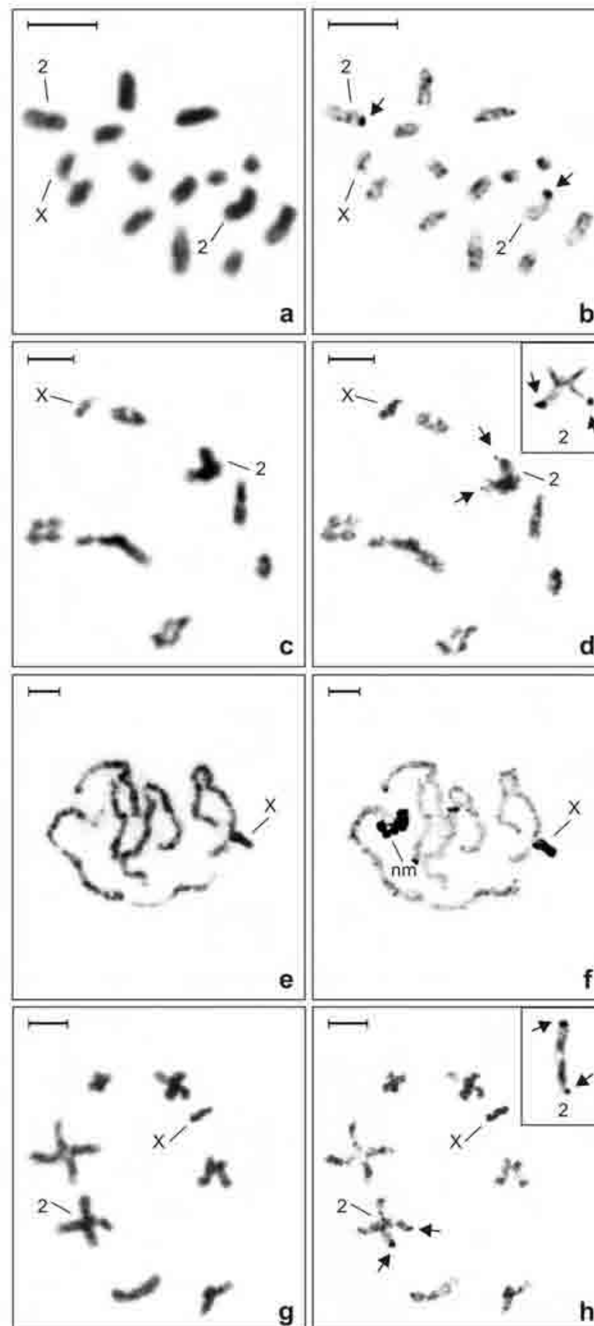

Figure 5. NOR pattern in testicular cells of *Pyrophorus divergens* (**a**-**d**) and *Pyrophorus punctatissimus* (**e**-**h**) obtained with Giemsa (**a**, **c**, **e**, **g**) and silver impregnation (**b**, **d**, **f**, **h**) sequential staining. Observe that the NORs (arrows) are always located on terminal region of the 2nd pair or bivalent. (**a**-**b**) Mitotic metaphase, 2n=15=14+X0. (**c**-**d**) Diplotene, 2n=7II+X0. In detail, bivalent 2 with deeply impregnated NORs. (**e**-**f**) Pachytene, 2n=7II+X0, showing nucleolar material (nm) on 2nd autosomal bivalent. (**g**-**h**) Diplotene, 2n=7II+X0. In the insert, bivalent 2 with heteromorphic NORs. Bar=5μm.

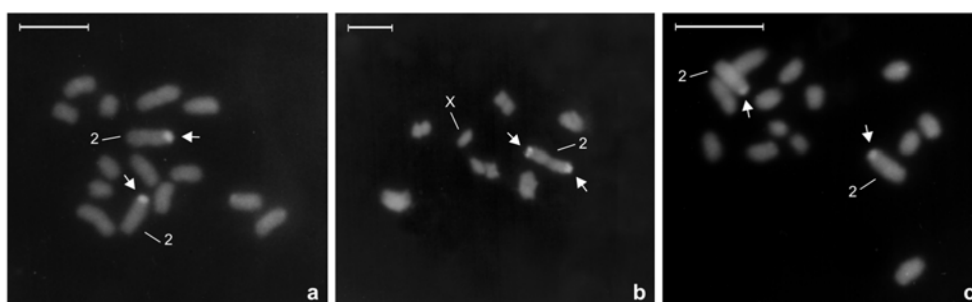

Figure 6. CMA_3/DA staining of testicular cells of *Pyrearinus candelarius* (**a**-**b**) and *Pyrophorus punctatisismus* (**c**), showing GC-rich DNA on terminal region of the 2nd pair or bivalent (arrows). (**a**) Mitotic metaphase, 2n=15=14+X0. (**b**) Diakinesis, 2n=7II+X0. (**c**) Mitotic metaphase, 2n=15=14+X0. Bar=5μm.

Discussion

In the family Elateridae only species of four subfamilies (Agrypninae, Cardiophorinae, Denticollinae, and Elaterinae) were cytogenetically analyzed (Smith and Virkki 1978; Ferreira et al. 1984; Vidal 1984; Virkki et al. 1984; Virkki and Denton 1987a; Yadav and Vyas 1993, 1994; Rozek and Lachowska 2001; Rozek et al. 2004; Schneider et al. under submission). The subfamily Agrypninae possesses 30 currently studied species and contains a large number of cytogenetic data of representatives of the Neartic, Neotropical, Oriental, and Paleartic regions (Smith and Virkki 1978; Ferreira et al. 1984; Vidal 1984; Virkki et al. 1984; Virkki and Denton 1987a; Yadav and Vyas 1994; Rozek et al. 2004; Schneider et al. under submission). The sample of the analyzed species in the present work belongs to the subfamily Agrypninae and to the tribes Conoderini and Pyrophorini.

The representatives of the tribe Conoderini have evidenced a remarkable karyotypic uniformity, which is shared among the Neotropical and Oriental species. This karyotypic conservatism is related to the occurrence of diploid number 2n=17 in males, sex determination system of the X0 type, chromosomal morphology predominantly acrocentric, and symmetric size of the chromosomes. These characteristics were observed in the Conoderini species examined in this work, *Conoderus malleatus*, in six other species of this same genus previously described in the literature, *C. dimidiatus*, *C. pilatei*, *C. rodriguezi*, *C. scalaris*, *C. ternarius*, and *Conoderus* sp. (under *Manocrepidius* sp.) (Virkki 1962; Vidal 1984; Yadav and Vyas 1994; Schneider et al. under submission), and also in four species of the *Heteroderes* genus, *H. lenis*, *H. macroderes*, *H. modestus*, and *H. sericeus* (Agarwal 1960, 1962; Yadav and Vyas 1994). Only one already described Conoderini species (*Conoderus stigmosus*, 2n=14+neoXY) has disclosed different chromosomal formula from that one predominant in the tribe (Schneider et al. under submission) and in *H. macroderes*, an interpopulational karyotypic variation (2n=16+X0 and 2n=18+X0) was verified (Agarwal 1960, 1962; Yadav and Vyas 1994).

The analysis of all information above-mentioned suggests that the 2n=16+X0 with the majority of the chromosomes acrocentric could be

considered the ancestral karyotype for the Conoderini species and that different situations could indicate chromosomal derivations. The 2n=16+X0=8II+X0 presumably has evolved from the karyotype that has been proposed by Smith and Virkki (1978) to be the basic one for Polyphaga, $2n=18+Xy_p=9II+Xy_p$, with metacentric chromosomes, through three main events: first, chromosomal fusions, reducing the number of autosomal pairs to 8; second, loss of the y_p chromosome, giving rise to the sex determination system of the X0 type; third, pericentric inversions, changing the chromosomal morphology from metacentric to acrocentric, involving invariably the X chromosome. However, considering that the number of autosomal pairs with acrocentric morphology can differ among the species, it is still premature to speculate if pericentric inversions have occurred independently in the autosomes of these species and have altered the metacentric to acrocentric morphology or vice versa.

Thus, further cytogenetic studies in other Conoderini species, mainly the genera *Aeolus* and *Deronocus*, not yet analyzed cytologically certainly will provide additional evidences for testing the ancestrally of the 2n=16+X0 for this tribe.

In contrast to the Conoderini, the species of the tribe Pyrophorini evidenced interspecific karyotypical diversity. The Pyrophorini are endemic to Neotropical region and south of the Neartic region, and possesses only three species cytogenetically examined, *Deilelater radians* (under *Pyrophorus radians*), with 2n=10+X0 (Virkki 1962), *Ignelater luminosus* (under *Pyrophorus luminosus*), with $2n=14+X_1X_2Y$ (Virkki et al. 1984), and *Pyrophorus phosphorescens* (under *Pyrophorus nyctophanus* and *Pyrophorus pellucens*, *pars*), with 2n=14+X0 and one metacentric autosomal pair (Piza 1960; Smith 1960)

The obtained results in *Pyrearinus candelarius*, *Pyrophorus divergens*, and *Pyrophorus punctatissimus* (Pyrophorini) revealed that karyotypical characteristics found in these three species is agree with those ones previously reported for *Pyrophorus phosphorescens* (Piza 1960; Smith 1960), e.g., diploid number 2n=15 in males, sex determination system of the X0 type, chromosomes classified in groups according to their size, and small size of the

X sex chromosome. The only karyotypic difference observed among the studied species is concerned with the morphology of the 4th autosomal pair, which is submetacentric in *Pyrearinus candelarius* and acrocentric in *Pyrophorus divergens* and *Pyrophorus punctatissimus*. Chromosomal rearrangements of the pericentric inversions type could have lead to morphological variation in this autosomic pair. In *Pyrophorus phosphorescens*, the chromosomal morphology was only described for the 1st pair (Piza 1960), being impossible to use this information in the comparison with related species.

Taking into account the karyotypical heterogeneity and the low number of the studied Pyrophorini species up to now, the ancestral karyotype for this tribe can not be yet proposed; however, the Pyrophorini basic karyotype is probably different of that 2n=16+X0 proposed for Conoderini, in view of the presence of $2n=14+X_1X_2Y$ (under $2n=14+X_p neoXneoY_p$) in *Ignelater luminosus* (under *Pyrophorus luminosus*) (Virkki et al. 1984), whose origin can only be explained from $2n=18+Xy_p$ by fusion events between the y_p sex chromosome and an autosomal element, and between two autosomal pairs.

In the four studied species, the chromosomal behaviour during the meiosis is very similar to that one already encountered in other Elateridae species (Stevens 1909; Piza 1958, 1960; Banerjee 1959; Virkki 1962; Virkki et al. 1984; Virkki and Denton 1987a; Yadav and Vyas 1993; Schneider et al. under submission) and in the majority of the beetles (Smith and Virkki 1978), that is, total synapsis between the autosomes, precocious condensation and positive heteropycnosis of the X sex chromosome in early prophase I cells, presence of one chiasma per autosomal bivalent, and regular segregation of all chromosomes during the meiosis. Only one exception to these was encountered in *Pyrearinus candelarius*, *Pyrophorus divergens*, and *Pyrophorus punctatissimus*, which showed bichiasmate bivalents and exhibited differences in relation to the number of these bivalents.

According to Alegre and Petitpierre (1990), closely related species and populations of a same species could be distinguished in relation to chiasma distribution and frequency. The occurrence of bivalents with more than one chiasma does not seem to be a rare event among the Elateridae

representatives or exclusive of a particular group of species, considering that bivalents with two or even three chiasmata were already observed in species of the subfamilies Agrypninae (Agarwal 1962; Kacker 1963; Ferreira et al. 1984; Yadav and Vyas 1994) and Elaterinae (Smith 1956). In these species, as well as in *Pyrearinus candelarius*, *Pyrophorus divergens*, and *Pyrophorus punctatissimus*, the bivalents of large size are those that always have a high number of chiasmata. John (1990) proposed that the chiasma number is partly dependent on the chromosome length and that once one chiasma is formed, it could interfere in the formation of a second chiasma in a proximate site. This last proposition justifies the absence of two chiasmata in the small size bivalents of the studied species.

An overview of the NORs pattern on Coleoptera chromosomes (Table 1) shows that the autosomal NORs are widespread in the Adephaga and Polyphaga species. This autosomal NORs pattern could be representative of an ancestral state and/or the most stable evolutionary condition for the representatives of these two suborders, and distinct circumstances, such as the presence of NORs on autosomal and sexual chromosomes or only on sex chromosomes, could be indicative of karyotypical differentiations.

Considering that the previously cited karyotypical differentiations are verified in unrelated families or subfamilies of Adephaga and Polyphaga (Table 1), e.g., Cicindelinae, Collyrinae, Scaritinae (Adephaga), Chrysomelidae, Coccinelidae, Scarabaeidae, and Tenebrionidae (Polyphaga), probably the shifts in the NORs location from autosomes to sex chromosomes have taken place as independent events in the different groups of species and should often have occurred during the karyotypical evolution of the species. It is worth pointing out that in the sample of Adephaga and Polyphaga species, all the representatives of a same family or subfamily have never disclosed NORs only on sex chromosomes, corroborating the ancestrally of the autosomal NORs for these two Coleoptera suborders.

Alterations in the NORs distribution pattern can be responsible for karyotypical differentiations of the described species, occurring as a unique event or concomitant with variations in the diploid number and type of sex

determination system. The last mentioned situation seems to have occurred in the subfamily Cicindelinae (Adephaga), in which the chromosomal derivations, from the basal to the derivative taxa, have given rise to multiple sex determination systems and have shifted the NORs from autosomes to sex chromosomes (Galián and Hudson 1999; Galián et al. 2002; Proença et al. 2002b; Proença and Galián 2003; Proença et al. 2004). Gómez-Zurita et al. (2004) analyzing some representatives of the *Timarcha* genus (Chrysomelidae, Polyphaga) also verified the presence of NOR on sex chromosome, but only in the species that possesses derivative sex determination system of the neoXY type.

The employment of the silver nitrate impregnation technique on chromosomes of the species that possess sex determination system of the Xy_p type frequently evidences silver precipitation on sexual chromosomes (Table 1), such as verified in representatives of the Curculionidae, Meloidae, Scarabaeidae, and Tenebrionidae families (Polyphaga); however, in many cases, this silver precipitation is not representative of the NOR, but is an argyrophilous substance, which might have function in the association and disjunction of the sex chromosomes during the meiosis (Virkki et al. 1990; Petitpierre 1996). Therefore, the occurrence of NOR on sex chromosomes of species with Xy_p sex determination system only can be confirmed using the fluorescence *in situ* hybridization technique with rDNA probe.

Other characteristic that has been reported with a surprising frequency in the Adephaga and Polyphaga species is the presence of 2 NORs located on elements of one autosomal pair (Table 1). In some beetle groups, such as Carabini, Trechinae (Adephaga), and Hydrophilidae (Polyphaga), the number of NORs seems to be directly linked to the stability of both diploid number and sex determination system; but in the majority of the coleopteran, the number of NORs seems to remain unchanged despite the karyotypical variations. In the karyotypic derivation process, change in the number of NORs among autosomes appears to be more common than alteration in the NORs location from autosomes to sex chromosomes, considering that many groups of closely

related species predominantly exhibit differences in the number of autosomic NORs.

Taking into account that 2 NORs are the most frequent pattern in the Coleoptera representatives analyzed up to now, the karyotypical derivations seems to have led to an increase of the number of NORs. However, some researchers have proposed that in Cicindelinae (Adephaga) the more derivative species possess a lowest number of NORs (Galián et al. 2002; Proença et al. 2002b; Proença et al. 2004). Thus, the study of a high variety of beetles species is still necessary for the establishment of the strategies of karyotypical derivations and determination of the relationship between these strategies and the number of NORs.

The literature data about the NORs location on Coleoptera chromosomes evidenced that the more frequent pattern is NORs associated to the chromosome ends, but NORs on chromosome interstitial or proximal sites have been already registered (Table 1). According to Sumner (2003), the NORs on chromosome distal regions are also predominant in many animal species. Perhaps the NORs on chromosome ends of Coleoptera represent a basic condition and discrepancies in relation to this location are indicative of karyotype differentiation.

The autosomal NORs pattern established in the four Elateridae species analyzed in the present work is in agreement with that one observed in other representatives of this family (Table 1). In the Elateridae, the autosomal NORs also seems to be representative of the ancestral state, and at least in the analyzed species so far, this pattern is constant, despite the interspecific variations of chromosomal number and type of sex determination system.

The described Conoderini species showed 2 or 4 telomeric NORs located on pair 1 long arm of *C. ternarius*, on pair 2 short arm of *C. stigmosus*, and on pair 2 short arm and pair 4 long arm of *C. dimidiatus* and *C. scalaris* (Schneider et al. under submission). The unique Conoderini representative studied in this work, *C. malleatus*, disclosed 2 NORs on the 4th autosomal pair, but in contrast to *C. dimidiatus* and *C. scalaris*, these are located on chromosomal elements short arm. In these Conoderini species, the variation in

the number of NORs can be due to the employment of the silver impregnation technique, which reveals only the NORs that were transcriptionally active in the preceding interphase (Galián et al. 1995; Petitpierre 1996). Alternatively, the differences in relation to the number and location of the NORs could be consequence of chromosomal rearrangements of the translocation type. These translocations could have involved the total rDNA, leading to shifts in the NORs location, or could have involved only part of the rDNA, occasioning an increase of the NORs number. Only the FISH technique may help to choose between these two hypotheses.

The tribe Pyrophorini possesses description of the NORs distribution pattern for only one species, *Ignelater luminosus* (under *Pyrophorus luminosus*), which showed 2 NORs associated with an autosomal pair of large size (Virkki et al. 1984). In the three Pyrophorini species analyzed in this work, *Pyrearinus candelarius*, *Pyrophorus divergens*, and *Pyrophorus punctatissimus*, a similar pattern to that one of *Ignelater luminosus* (under *Pyrophorus luminosus*) was encountered, that is, 2 telomeric NORs located on short arm of the 2nd autosomal pair. The data about the NORs pattern in Conoderini and Pyrophorini reveal that in the species of these two tribes, the NORs conservatism refers to its presence on autosomal chromosomes and its location on pair 2. Schneider et al. (under submission) also stated that the NORs associated to the 2nd autosomal pair could be representative of a basic karyotypical characteristic for Conoderini species and discrepant conditions, such as the occurrence of NORs on pairs 1 and 4, could be result of karyotypical derivations.

In the family Elateridae, only four species belonging to the tribe Conoderini have their chromosomes submitted to the staining with base-specific fluorochromes. In these species, the labelled CMA_3 regions can be coincident with some C bands and/or with an special type of heterochromatin associated to the NORs; moreover, the GC-specific CMA_3 fluorochrome revealed chromosomal regions that were not detected by C-banding and silver impregnation techniques (Schneider et al. under submission). In the two analyzed Pyrophorini species, *Pyrearinus candelarius* and *Pyrophorus*

punctatissimus, the GC-rich sequences of DNA are correspondent with the NORs. In various vertebrate and invertebrate species, the CMA_3 fluorochrome was useful for detecting the NORs due to the fact of the rDNA generally containing GC-rich sequences (Schweizer et al. 1983; Schmid and Guttenbach 1988). Other possibility in the studied Pyrophorini representatives could be that the CMA_3 fluorochrome had not evidenced the NORs, but a special type of GC-rich heterochromatin associated to this region. This fact was already verified in some coleopteran (Vitturi et al. 1999, 2003; Colomba et al. 2004), e.g., *Anoplotrupes stercorosus*, *Thorectes intermedius* (Geotrupidae), and *Pentodon bidens punctatum* (Scarabaeidae). Furthermore, the obtained data with the fluorochrome staining revealed that in the Pyrophorini species, there is a lower quantity of chromatin regions compartmentalized in GC-rich sequences than those of Conoderini described by Schneider et al. (under submission).

Additional information obtained of the NORs pattern review on Coleoptera chromosomes is that there is a coincidence of NORs labelling in many species whose chromosomes were submitted to both silver impregnation and FISH techniques (Table 1). Considering that the NORs pattern has been established in less than 1% of the beetles species, which belongs to only 12 of the 166 described families (Lawrence and Newton 1995), and that the silver impregnation has supplied satisfactory results to detect the NORs on coleopteran chromosomes, the employment of this simple and non expensive methodology in a high number and variety of species, surely will provide interesting data that will improve the understanding of the NORs shifts on the beetles chromosomes during the evolutionary process.

Table 1. List of karyotyped Coleoptera species, with data on nucleolar organizer regions (NORs). B=supernumerary chromosome; M=metacentric; Sm=submetacentric; St=subtelocentric; A=acrocentric; Sa=subacrocentric; CH=constitutive heterochromatin; AgNOR=NOR impregnated by silver nitrate; FISH=fluorescence *in situ* hybridization using rDNA probe; SC=spreading cell for synaptonemal complex visualization. The species taxonomy was based on Lawrence and Newton (1995).

Species	*Chromosomal formula (2n males)*	*Number of NORs*	*Chromosome bearing the NORs*	*Technique*	*References*
ADEPHAGA					
Carabidae					
Carabinae					
Carabini					
Carabus cancellatus Illiger, 1798	28=26+XY	2	1 autosomal pair of large size	Ag NOR and FISH	De la Rúa et al. 1996
Carabus coarctatus Brulle, 1838	28=26+XY	2	1 autosomal pair of large size	Ag NOR and FISH	De la Rúa et al. 1996
Carabus guadarramus La Ferté-Sénectère, 1846	28=26+XY	2	1 autosomal pair of large size	Ag NOR and FISH	De la Rúa et al. 1996
Carabus granulatus Linnaeus, 1758	27=26+X0	2	1 autosomal pair of large size	Ag NOR and FISH	De la Rúa et al. 1996
Carabus lusitanicus Fabricius, 1801	28=26+XY	2	1 autosomal pair of large size	Ag NOR and FISH	De la Rúa et al. 1996
Carabus macrocephalus Dejean, 1826	28=26+XY	2	1 autosomal pair of large size	Ag NOR and FISH	De la Rúa et al. 1996
Carabus melancholicus Fabricius, 1798	28=26+XY	2	1 autosomal pair of large size	Ag NOR and FISH	De la Rúa et al. 1996
Carabus morbillosus Fabricius, 1792	28=26+XY	2	1 autosomal pair of large size	Ag NOR and FISH	De la Rúa et al. 1996
Carabus nemoralis Müller, 1764	28=26+XY	2	1 autosomal pair of large size	Ag NOR and FISH	De la Rúa et al. 1996
Carabus problematicus Herbst, 1786	28=26+XY	2	1 autosomal pair of large size	Ag NOR and FISH	De la Rúa et al. 1996
Carabus rugosus Fabricius, 1775	28=26+XY	2	1 autosomal pair of large size	Ag NOR and FISH	De la Rúa et al. 1996
Carabus violaceus Linnaeus, 1758	28=26+XY	2	1 autosomal pair of large size	Ag NOR and FISH	De la Rúa et al. 1996
Calosoma maderae (Fabricius, 1775)	28=26+XY	2	1 autosomal pair of large size	Ag NOR and FISH	De la Rúa et al. 1996
Calosoma sycophanta (Linnaeus, 1758)	28=26+XY	2	1 autosomal pair of large size	Ag NOR and FISH	De la Rúa et al. 1996
Ceroglossini					
Ceroglossus chilensis Eschscholtz, 1829	30=28+XY/41=38+III/ 42=40+XY	2	1 autosomal pair of large size	Ag NOR and FISH	De la Rúa et al. 1996; Galián et al. 1996
Cychrini					
Cychrus caraboides (Linnaeus, 1758)	35=34+X0(Bs)	2-4	------	Ag NOR	De la Rúa et al. 1996
		4	2 autosomal pairs of medium size-distal region	FISH	
Cicindelinae					

Cicindelini					
Cicindela (Brasiella) argentata Fabricius, 1801	$21=18+X_1X_2Y$	2	2 autosomal chromosomes of medium size	FISH	Proença et al. 2004
Cicindela (Cosmodela) aurulenta Fabricius, 1801	$22=18+X_1X_2X_3Y$	4	1 autosomal pair and 2 X	FISH	Proença et al. 2004
Cicindela campestris Linnaeus, 1758	$22=18+X_1X_2X_3Y$	2	multivalent sexual	Ag NOR	Serrano 1981; Serrano and Collares-Pereira 1992; Galián et al. 1995
Cicindela deserticoloides Codina, 1931	22=18+XXXY	2	multivalent sexual	Ag NOR	Galián et al. 1995
Cicindela flexuosa Fabricius, 1787	$22=18+X_1X_2X_3Y$	2	multivalent sexual	Ag NOR	Galián et al. 1995
Cicindela flexuosa Fabricius, 1787	$22=18+X_1X_2X_3Y$	2	1 autosomal pair or 2 sex chromosomes	Ag NOR and FISH	Proença and Galián 2003
Cicindela germanica Linnaeus, 1758	16=14+XY	2-4	autosomal	FISH	Galián et al. 2002
Cicindela littoralis Fabricius, 1792	$22=18+X_1X_2X_3Y$	3 or 2	1 autosomal pair and X-distal region or 1 autosomal pair of small size-distal region	Ag NOR and FISH	Proença and Galián 2003
Cicindela littorea Forsk, 1775	$22=18+X_1X_2X_3Y$	2	multivalent sexual	Ag NOR	Serrano et al. 1986; Galián et al. 1995
Cicindela maura Linnaeus, 1758	22=18+XXXY	2	multivalent sexual	Ag NOR	Galián et al.,1995
Cicindela melancholica Fabricius, 1798	22=18+XXXY	2	multivalent sexual	Ag NOR	Galián et al.,1995
		2	X-interstitial region and Y-distal region	FISH	
Cicindela (Cylindera) paludosa Dufour, 1820	15=14+X0	2	1 autosomal pair	Ag NOR and FISH	Serrano et al. 1986; Galián et al. 1990; Galián et al. 1995
Cicindela suturalis Fabricius, 1798	$23=18+X_1X_2X_3X_4Y$	2	X_3 and X_4	FISH	Proença et al. 2004
Cicindela (*Rivacindela*) *cardinalba* Sumlin, 1987	$24=20+X_1X_2X_3Y$	2	2 sex chromosomes-distal region	Ag NOR and FISH	Galián and Hudson 1999
Cicindela (*Rivacindela*) *gillesensis* Hudson, 1994	$26=22+X_1X_2X_3Y$	2	2 sex chromosomes-distal region	Ag NOR and FISH	Galián and Hudson 1999
Cicindela (*Rivacindela*) sp. (*saetigera* Horn, 1893 group)	$26=22+X_1X_2X_3Y$	2	2 sex chromosomes-distal region	Ag NOR and FISH	Galián and Hudson 1999
Odontocheila confusa Dejean, 1825	22=20+XY	2	1 autosomal pair of medium size	Ag NOR and FISH	Proença et al. 2002a
Odontocheila nodicornis (Dejean, 1825)	35=34+X0	2	1 autosomal pair of medium size	Ag NOR and FISH	Proença et al. 2002a
Prothymia sp.	24=20+XXXY	2	X and Y	FISH	Galián et al. 2002
Pentacomia sp.	21=20+X0	2	autosomal	FISH	Galián et al. 2002
Therates sp.	23=20+XXY	2	X and Y	FISH	Galián et al. 2002
Manticorini					
Mantichora mygaloides Thomson, 1857	38=36+XY	8	autosomal	FISH	Galián et al. 2002
Megacephalini					
Omus californicus Eschscholtz, 1829	36=34+XY	6	autosomal	FISH	Galián et al. 2002
Omus dejeani Reiche, 1838	36=34+XY	6	autosomal	FISH	Galián et al. 2002
Amblycheila baroni Rivers, 1890	44=42+XY	8	autosomal	FISH	Galián et al. 2002
Megacephala brasiliensis Kirby, 1818	12=10+XY	2	1 autosomal pair of medium size-proximal region	FISH	Proença et al. 2002b
Megacephala euphratica Latreille & Dejean,	31=30+X0	1-4	------	Ag NOR	Serrano et al. 1986; Galián et al.

1822		6	3 autosomal pairs of medium size	FISH	1995
Megacephala whelani Sumlin, 1992	26=24+XY	6	3 autosomal pairs of medium and small size-telomeric region	FISH	Galián and Hudson 1999
Collyrinae					
Ctenostoma (Procephalus) ornatum ornatum Klug, 1834	17=14+X_1X_2Y	2	pair 7-proximal region	FISH	Zacaro et al. 2004
Neocollyris sp.	28=24+XXXY	2	X and Y	FISH	Galián et al. 2002
Harpalinae					
Harpalini					
Acinopus picipes (Olivier, 1795)	37=36+X0	2	1 autosomal pair-distal region	FISH	Martínez-Navarro et al. 2004
Carterus fulvipes (Latreille, 1817)	57=56+X0	2	1 autosomal pair-distal region	FISH	Serrano 1981; Martínez-Navarro et al. 2004
Cryptophonus tenebrosus (Dejean, 1829)	37	2	1 autosomal pair-distal region	FISH	Martínez-Navarro et al. 2004
Dicheirotrichus obsoletus (Dejean, 1829)	37	2	1 autosomal pair-distal region	FISH	Martínez-Navarro et al. 2004
Ditomus tricuspidatus (Fabricius, 1792)	59	2	1 autosomal pair-distal region	FISH	Martínez-Navarro et al. 2004
Dixus capito (Serville, 1821)	69=68+X0	2	1 autosomal pair-distal region	FISH	Martínez-Navarro et al. 2004
Dixus clypeatus (Rossi, 1790)	45=44+X0	4	2 autosomal pairs-distal region	FISH	Martínez-Navarro et al. 2004
Dixus sphaerocephalus (Olivier, 1795)	55=54+X0	4	2 autosomal bivalents	FISH	Martínez-Navarro et al. 2004
Egadroma marginatum (Dejean, 1829)	39=38+X0	2	1 autosomal pair-distal region	FISH	Martínez-Navarro et al. 2004
Egadroma piceus (Guérin-Méneville, 1830)	37=36+X0	4	2 autosomal pairs-distal region	FISH	Martínez-Navarro et al. 2004
Eocarterus amicorum Wrase, 1993	41?=40+X0	2	1 autosomal pair-distal region	FISH	Martínez-Navarro et al. 2004
Harpalus affinis (Schrank, 1781)	38	2	1 autosomal pair-distal region	FISH	Martínez-Navarro et al. 2004
Harpalus anxius (Duftschmid, 1812)	37=36+X0	2	1 autosomal pair-distal region	FISH	Martínez-Navarro et al. 2004
Harpalus contemptus Dejean, 1829	37=36+X0	2	1 autosomal pair-distal region	FISH	Martínez-Navarro et al. 2004
Harpalus decipiens Dejean, 1829	37=36+X0	2	1 autosomal pair-distal region	FISH	Martínez-Navarro et al. 2004
Harpalus distinguendus (Duftschmid, 1812)	37=36+X0	2 or 4	1 or 2 autosomal pairs	FISH	Martínez-Navarro et al. 2004
Harpalus fuscipalpis Sturm, 1818	38	4	4 chromosomes	FISH	Martínez-Navarro et al. 2004
Harpalus honestus (Duftschmid, 1812)	37=36+X0	2	1 autosomal pair-distal region	FISH	Martínez-Navarro et al. 2004
Harpalus microthorax salinator Motschulsky, 1849	37=36+X0	2 or 4	1 or 2 autosomal pairs	FISH	Martínez-Navarro et al. 2004
Harpalus nevadensis K. Daniel & J. Daniel, 1898	?	2	1 autosomal pair-distal region	FISH	Martínez-Navarro et al. 2004
Harpalus rubripes (Duftschmid, 1812)	37=36+X0	2	1 autosomal pair-distal region	FISH	Martínez-Navarro et al. 2004
Harpalus rufipalpis Sturm, 1818	30=28+XY	2	1 autosomal pair-distal region	FISH	Martínez-Navarro et al. 2004
Harpalus sp.	?	2	1 autosomal pair-distal region	FISH	Martínez-Navarro et al. 2004
Harpalus serripes (Quenzel, 1806)	37=36+X0	2	1 autosomal pair-distal region	FISH	Martínez-Navarro et al. 2004
Harpalus wagneri Schauberger, 1926	30=28+XY	2	1 autosomal pair-distal region	FISH	Martínez-Navarro et al. 2004
Lecanomerus sp.	37	2	1 autosomal pair-distal region	FISH	Martínez-Navarro et al. 2004
Nesarpalus fortunatus (Wollaston, 1863)	37	2	1 autosomal pair-distal region	FISH	Martínez-Navarro et al. 2004
Odontocarus cephalotes (Dejean, 1826)	40?	2	1 autosomal pair-distal region	FISH	Martínez-Navarro et al. 2004
Ophonus (Hesperophonus) azureus (Fabricius, 1775)	37?	2	1 autosomal pair-distal region	FISH	Martínez-Navarro et al. 2004
Ophonus (Hesperophonus) cribricollis (Dejean,	37?	2	1 autosomal pair-distal region	FISH	Martínez-Navarro et al. 2004

1829)					
Ophonus (Hesperophonus) pumilio (Dejean, 1829)	37=36+X0	2	1 autosomal pair-distal region	FISH	Martínez-Navarro et al. 2004
Ophonus (Ophonus) ardosiancus (Lutshnik, 1922)	37=36+X0	2	1 autosomal pair-distal region	FISH	Martínez-Navarro et al. 2004
Ophonus (Ophonus) sabulicola hispanicus (Schauberger, 1926)	37	6	6 autosomal chromosomes	FISH	Martínez-Navarro et al. 2004
Parophonus hespericus Jeanne, 1985	38	2	1 autosomal pair-distal region	FISH	Martínez-Navarro et al. 2004
Parophonus hispanus (Rambur, 1838)	37?	2	1 autosomal pair-distal region	FISH	Martínez-Navarro et al. 2004
Pseudoophonus griseus (Panzer, 1797)	37	2	1 autosomal pair-distal region	FISH	Martínez-Navarro et al. 2004
Pseudoophonus rufipes (DeGeer, 1774)	37=36+X0	2	1 autosomal pair-distal region	FISH	Martínez-Navarro et al. 2004
Pseudoophonus (Platus) calceatus (Duftschmid, 1812)	?	2	1 autosomal pair-distal region	FISH	Martínez-Navarro et al. 2004
Stenolophus abdominalis Gene, 1836	37=36+X0	2	1 autosomal pair-distal region	FISH	Martínez-Navarro et al. 2004
Zabrini					
Zabrus (*Zabrus*) *ignavus* Csiki, 1907	47?	4	4 chromosomes	FISH	Sánchez-Gea et al. 2000
Zabrus (*Platyzabrus*) *pecoudi* Colas, 1942	49	4	2 autosomal pairs of medium size-distal region	FISH	Sánchez-Gea et al. 2000
Zabrus (*Iberozabrus*) *ambiguus* Rambur, 1838	59=58+X0	8 or 8-10 or 10-11 or 9-12	4 pairs-whole arm or 8-12 chromosomes	FISH	Galián et al. 1991; Sánchez-Gea et al. 2000
Zabrus (*Iberozabrus*) *angustatus* Rambur, 1838	59	2	1 pair of medium size	FISH	Sánchez-Gea et al. 2000
Zabrus (*Iberozabrus*) *castroi* Martínez & Saez, 1833	59=58+X0(Bs)	6 or 6-8 or12	3-4 pairs or 6 pairs	FISH	Sánchez-Gea et al. 2000
Zabrus (*Iberozabrus*) *coiffaiti* Jeanne, 1981	59=58+X0	8	2 pairs of large size-whole arm and 2 pairs of medium size-distal region	FISH	Sánchez-Gea et al. 2000
Zabrus (Iberozabrus) *curtus arragonensis* Heyden, 1883	59=58+X0	4-6 or 6	2 or 3 autosomal pairs	FISH	Sánchez-Gea et al. 2000
Zabrus (*Iberozabrus*) *curtus neglectus* Schaum, 1864	59=58+X0	2	1 pair	FISH	Sánchez-Gea et al. 2000
Zabrus (*Iberozabrus*) *eserensis* Bolívar, 1918	------	6	3 pairs	FISH	Sánchez-Gea et al. 2000
Zabrus (*Iberozabrus*) *marginicollis* Dejean, 1828	57?	4	2 pairs of medium size-whole arm	FISH	Sánchez-Gea et al. 2000
Zabrus (*Iberozabrus*) *obesus* Audinet-Serville, 1821	59?	4	1 pair of large size-distal region and 1 pair of medium size-whole arm	FISH	Sánchez-Gea et al. 2000
Zabrus (*Iberozabrus*) *rotundatus* Rambur, 1838	------	7-8	7-8 chromosomes; 1 chromosome-interstitial region	FISH	Sánchez-Gea et al. 2000
Zabrus (*Iberozabrus*) *seidlitzi gredosanus* Jeanne, 1970	57=56+X0	5 or 5-6 or 6	------	FISH	Sánchez-Gea et al. 2000
Zabrus (*Iberozabrus*) *seidlitzi laurae* Toribio, 1989	------	5-6 or 6-7 or 8	------	FISH	Sánchez-Gea et al. 2000
Zabrus (*Iberozabrus*) *seidlitzi seidlitzi* Schaum, 1864	57=56+X0	5 or 6	------	FISH	Sánchez-Gea et al. 2000
Zabrus (*Iberozabrus*) *silphoides* Dejean, 1828	59=58+X0	8	4 pairs-whole arm	FISH	Sánchez-Gea et al. 2000
Zabrus (*Iberozabrus*) *theveneti* Chevrolat, 1874	59?	2	1 pair of medium size	FISH	Sánchez-Gea et al. 2000

Zabrus (*Iberozabrus*) *urbionensis* Jeanne, 1970	60?	4	1 pair of large size-distal region and 1 pair of medium size-whole arm	FISH	Sánchez-Gea et al. 2000
Zabrus (*Iberozabrus*) *vasconicus* Uhagón, 1904	63=62+X0(Bs)	10 or 8-12	5 pairs/8-12 chromosomes	FISH	Sánchez-Gea et al.2000
Scaritinae					
Distichus planus (Bonelli, 1813)	39=38+X0	5	2 autosomal pairs and X	FISH	Galián et al. 1999
Scarites (*Scallophorites*) *buparius* (Forster, 1771) (*pyracmon* Bonelli, 1813)	39-37=34+X_1X_2Y(Bs)	4	4 autosomal chromosomes	FISH	Serrano 1980; Galián et al. 1999
Scarites (*Scallophorites*) *hespericus* Dejean, 1831 (*impressus* Fabricius, 1801)	53=52+X0	4	4 autosomal chromosomes	FISH	Galián et al. 1999
Scarites (*Scallophorites*) *occidentalis* Bedel, 1895 (*cyclops* Crotch, 1871)	41=38+X_1X_2Y	4	4 autosomal chromosomes	FISH	Serrano 1984; Galián et al. 1999
Scarites (*Scarites*) *eurytus* (Fischer, 1825)	45=44+X0	6	3 autosomal pairs of large size	FISH	Galián et al. 1999
Scarites (*Parallelomorphus*) *laevigatus* Fabricius, 1792	61=60+X0	4	4 autosomal chromosomes	FISH	Galián et al. 1999
Scarites (*Parallelomorphus*) t*erricola* Bonelli, 1813	57=56+X0	4	2 autosomal pairs of large and medium size-distal region	FISH	Galián et al. 1999
Trechinae					
Bembidion lampros (Herbst, 1784)	23=22+X0	2	1 autosomal pair-distal region	Ag NOR	Rozek 1998a
Bembidion properans (Stephens, 1828)	23=22+X0	2	1 autosomal pair-distal region	Ag NOR	Rozek 1998a
Trechus latus Putzeys, 1847	23=22+X0	2	1 autosomal pair-distal region	Ag NOR	Rozek 1998b
Trechus pilisensis Csiki, 1907	23=22+X0	2	1 autosomal pair-distal region	Ag NOR	Rozek 1998b
Trechus pulchellus Putzeys, 1846	23=22+X0	2	1 autosomal pair-distal region	Ag NOR	Rozek 1998b
Trechus quadristriatus (Schrank, 1781)	23=22+X0	2	1 autosomal pair-distal region	Ag NOR	Rozek 1998b
POLYPHAGA					
HYDROPHILOIDEA					
Hydrophilidae					
Helophorinae					
Helophorus aequalis Thomson, 1868	18=16+XY	2	pair 6	Ag NOR	Angus 1982
Helophorus grandis Illiger, 1798	18=16+XY	2	pair 6	Ag NOR	Angus 1983
SCARABAEOIDEA					
Geotrupidae					
Thorectes intermedius (Costa, 1827)	22=20+XY	------	coincident with CH	Ag NOR	Vitturi et al. 1999
		4 and 1 specimen with 1 or 4	pairs 1 and 3-distal region	FISH	
Anoplotrupes stercorosus (Scriba, 1791)	22=20+XY	------	XY and coincident with CH	Ag NOR	Colomba et al. 2004
		2	2 chromosomes of medium-large size	FISH	
Lucanidae					

Lucaninae					
Dorcus parallelopipedus (Linnaeus, 1758)	18=16+XY	------	coincident with CH	Ag NOR	Colomba et al. 2000a
		2	pair 2-distal region	FISH	
Scarabaeidae					
Dynastinae					
Lygirus ebenus De Geer, 1774	$20=18+Xy_p$	2	1 autosomal pair	Ag NOR	Bione et al. 2005b
Pentodon bidens punctatum Villers, 1899	19=18+X0	------	X-distal region and coincident with CH	Ag NOR	Vitturi et al. 2003
		1	X-distal region	FISH	
Strategus surinamensis hirtus Sternberg, 1910	$20=18+Xy_p$	------	Xy_p	Ag NOR	Bione et al. 2005b
Melolonthinae					
Lyogenys fuscus Blanchard, 1850	$20=18+Xy_p$	------	Xy_p and coincident with CH	Ag NOR	Moura et al. 2003
		1	X	FISH	
Phyllophaga (*Phyllophaga*) *aff capillata* Blanchard, 1850	$20=18+XY_p$	------	1 autosomal bivalent and coincident with CH	Ag NOR	Moura et al. 2003
		2	1 autosomal bivalent of small size	FISH	
Phyllophaga (*Phytalus*) *vestita* Moser, 1918	$20=18+Xy_p$	------	Xy_p and coincident with CH	Ag NOR	Moura et al. 2003
		1	X	FISH	
Rutelinae					
Geniates borelli Camerano, 1894	$20=18+Xy_p$	------	Xy_p	Ag NOR	Bione et al. 2005b
		1	X	FISH	
Macraspis festiva Burmeister, 1844	$18=16+Xy_p$	------	Xy_p	Ag NOR	Bione et al. 2005b
		1	X	FISH	
Pelidnota pallidipennis Bates, 1904	$20=18+Xy_p$	------	Xy_p	Ag NOR	Bione et al. 2005b
		1	X	FISH	
Scarabaeinae					
Bubas bison (Linnaeus, 1767)	20=18+XY	8	coincident with CH	Ag NOR	Colomba et al. 1996, 2006
			and 8 chromosomes	FISH	
Diabroctis mimas (Linnaeus, 1767)	$20=18+Xy_p$	------	Xy_p and coincident with CH	Ag NOR	Bione et al. 2005a
		5	2 autosomal pairs of medium size and X	FISH	
Glyphoderus sterquilinus (Westwood, 1837)	18=16+XY	------	coincident with CH	Ag NOR	Colomba et al. 1996
Gymnopleurus sturmi MacLeay, 1821	20=18+XY	------	coincident with CH	Ag NOR	Colomba et al. 2000b
		4 or 5	4 or 5 chromosomes of medium size	FISH	
Isocopris inhiata (Germar, 1824)	$18=16+Xy_p$	------	Xy_p and coincident with CH	Ag NOR	Bione et al. 2005a
		2	1 autosomal pair of medium size	FISH	
BUPRESTOIDEA					
Buprestidae					

Acmaeoderella boryi (Brulle, 1832)	18=16+Xy_r	------	some bivalents and y	Ag NOR	Karagyan 2001
Acmaeoderella flavofasciata (Piller & Mitterpacher, 1783)	18=16+Xy_r	1	Y	Ag NOR	Karagyan 2001
Acmaeoderella gibbulosa (Menetries, 1832)	18=16+Xy_r	------	Xy_r	Ag NOR	Karagyan 2001
Acmaeoderella vetusta (Menetries, 1832)	18=16+Xy_r	1	Y	Ag NOR	Karagyan 2001
Sphenoptera mesopotamica Marseul, 1865	24=22+Xy_p	2	1 bivalent of large size	Ag NOR	Karagyan 2001
Sphenoptera scovitzi Faldermann, 1835	46?	4 or 6	2 or 3 bivalents of large size	Ag NOR	Karagyan 2001
Stigmodera (*Stigmodera*) *goryi* Hope, 1836	22=20+Xy_p	2	pair 7 or 8-distal region	probably Ag NOR	Gardner 1988
Stigmodera (*Stigmodera*) *porosa* Carter, 1916	22=20+Xy_p	2	pair 7 or 8-proximal region	probably Ag NOR	Gardner 1988
Stigmodera (*Themognatha*) *alternata* Lumholtz, 1889	20=18+Xy_p	2	pair 8-distal region	probably Ag NOR	Gardner 1988
Stigmodera (*Themognatha*) *donovani* Castelnau & Gory, 1838	22=20+Xy_p	2	pairs 6, 7 or 8-distal region	probably Ag NOR	Gardner 1988
Stigmodera (*Themognatha*) *nickerli* Obenberger, 1922	20=18+Xy_p	2	pair 8-distal region	probably Ag NOR	Gardner 1988
Stigmodera (*Themognatha*) *tricolorata* Waterhouse, 1874	22=20+Xy_p	2	pair 7 or 8-distal region	probably Ag NOR	Gardner 1988
Stigmodera (*Themognatha*) *variabilis* (Donovan, 1805)	22=20+Xy_p	2	pairs 7, 8 or 9-distal region	probably Ag NOR	Gardner 1988
ELATEROIDEA					
Elateridae					
Agrypninae					
Conoderini					
Conoderus malleatus (Germar, 1824)	17=16+X0	2	pair 4-distal region	Ag NOR	This report
Conoderus dimidiatus Germar, 1839	17=16+X0	4	pair 2 and pair 4-distal region	Ag NOR	Schneider et al. under submission
Conoderus scalaris (Germar, 1824)	17=16+X0	4	pair 2 and pair 4-distal region	Ag NOR	Schneider et al. under submission
Conoderus stigmosus Germar, 1839	16=14+neoXY	2	pair 1-distal region	Ag NOR	Schneider et al. under submission
Conoderus ternarius Germar, 1839	17=16+X0	2	pair 2-distal region	Ag NOR	Schneider et al. under submission
Pyrophorini					
Ignelater luminosus Costa, 1975 (under *Pyrophorus luminosus* Illiger, 1807)	17=14+$X_p$neoXneoy_p	2	1 bivalent of large size	Ag NOR	Virkki et al. 1984
Pyrearinus candelarius (Germar, 1841)	15=14+X0	2	pair 2-distal region	Ag NOR	This report
Pyrophorus divergens Eschscholtz, 1829	15=14+X0	2	pair 2-distal region	Ag NOR	This report
Pyrophorus punctatissimus Blanchard, 1843	15=14+X0	2	pair 2-distal region	Ag NOR	This report
CUCUJOIDEA					
Coccinelidae					
Coccinelinae					
Cycloneda sanguinea (Linnaeus, 1763)	20=18+Xy_p	2	1 autosomal pair	Ag NOR	Maffei et al. 2001c
Eriopis connexa (Germar, 1824)	20=18+Xy_p	2	1 autosomal pair (♀)/ 1 bivalent and Xy_p(♂)	Ag NOR	Maffei et al. 2001a
Olla v-nigrum (Mulsant, 1866)	20=18+Xy_p	------	Xy_p	Ag NOR and FISH	Maffei et al. 2001b

Epilachninae					
Epilachna paenulata (Germar, 1824)	$18=16+Xy_p$	-----	autosomal	Ag NOR	Drets et al. 1983
TENEBRIONOIDEA					
Meloidae					
Epicauta atomaria (Germar, 1821)	$20=18+Xy_p$	------ 2	non-specific bivalent 7 and Xy_p	Ag NOR SC	Almeida et al. 2000; Zacaro et al. 2003
Tenebrionidae					
Tenebrioninae					
Blaps gibba Castelnau, 1840	$38=30+X_1X_2X_3X_4X_5X_6X_7Y$	------	multivalent sexual	Ag NOR	Vitturi et al. 1996
Blaps gigas Linnaeus, 1767	$35=30+X_1X_2X_3X_4Y$	------	multivalent sexual	Ag NOR	Vitturi et al. 1996
Misolampus goudoti Guérin-Méneville, 1834	$20=18+Xy_p$	2 2	**1 BIVALENT AND XY_P** **1 AUTOSOMAL PAIR OF MEDIUM SIZE**	Ag NOR FISH	Juan et al. 1993
Palembus dermestoides (Fairmaire, 1893)	$20=18+Xy_p$	------ 3	non-specific bivalent 3, 7 and Xy_p	Ag NOR SC	Almeida et al. 2000; Zacaro et al. 2003
Tenebrio molitor Linnaeus, 1758	$20=18+Xy_p$	1 6	Xy_p, 2 autosomal pairs of medium size, X, and y-distal region	Ag NOR FISH	Juan et al. 1993
CHRYSOMELOIDEA					
Chrysomelidae					
Chrysomelinae					
Chrysolina americana (Linnaeus, 1758)	$24=22+Xy_p$	2	pair 1	FISH	Petitpierre 1975, 1996
Chrysolina bankii (Fabricius, 1775)	23=22+X0	2	pair 1	FISH	Petitpierre 1975, 1996
Timarcha aurichalcea Bechyné, 1948	18=16+neoXY	1	neoX	Ag NOR and FISH	Gómez-Zurita et al. 2004
Timarcha espanoli Bechyné, 1948	$26=24+Xy_p$	2	1 autosomal pair	Ag NOR and FISH	Petitpierre 1970; Gómez-Zurita et al. 2004
Timarcha fallax Pérez, 1865	$20=18+Xy_p$	2	pair 4-distal region	FISH	Petitpierre 1970; Gómez-Zurita et al. 2004
Timarcha granadensis Bechyné, 1948	$22=20+Xy_p$	2	1 bivalent	Ag NOR	Gómez-Zurita et al. 2004
Timarcha lugens Rosenhauer, 1856	$20=18+Xy_p$	2	1 autosomal pair	FISH	Petitpierre 1976; Gómez-Zurita et al. 2004
Timarcha marginicollis Rosenhauer, 1856	$20=18+Xy_p$	2	1 bivalent	Ag NOR	Petitpierre 1976; Gómez-Zurita et al. 2004
Timarcha perezi Fairmaire, 1884	$20=18+Xy_p$	2	pair 4-distal region	FISH	Petitpierre 1970; Gómez-Zurita et al. 2004
Timarcha punctella Marseul, 1870	$28=26+Xy_p$	2	1 bivalent	Ag NOR	Gómez-Zurita et al. 2004
Zygogramma bicolorata Pallister, 1953	$24=22+Xy_p$	4	4 chromosomes-proximal and distal region	Ag NOR	Yadav et al. 1992

Galerucinae					
Alagoasa bicolor (Linnaeus, 1767)	22=20+X+y	------	non-specific	Ag NOR	Virkki and Denton 1987b
Alagoasa januaria Bechyné, 1955	22=20+X+Y	------	autosomal-proximal region and Y-interstitial and proximal region	Ag NOR	Virkki 1983
Diabrotica speciosa (Germar, 1824)	21=20+X0(Bs)	2	pair 9-distal region	Ag NOR	Schneider et al. 2002
Omophoita albicollis Fabricius, 1787	22=20+X+y	------	non-specific	Ag NOR	Virkki and Denton 1987b
Omophoita annularis Illiger, 1807	22=20+X+Y	------	autosomal-proximal region	Ag NOR	Virkki 1983
Omophoita cyanipennis Fabricius, 1798	22=20+X+y	------	non-specific	Ag NOR	Virkki and Denton 1987b
Omophoita octoguttata Fabricius, 1775	22=20+X+Y	------	autosomal-proximal region	Ag NOR	Virkki 1983
Omophoita personata Illiger, 1807	22=20+X+Y	------	autosomal-proximal region	Ag NOR	Virkki 1983
Hispinae					
Botanochara angulata (Germar, 1824)	51=48+$X_p$neoXneoY_p	------	some bivalents	SC	Postiglioni et al. 1990
Chelymorpha variabilis Boheman, 1854	22=20+Xy_p	2	pair 5-distal region	Ag NOR and SC	Postiglioni and Brum-Zorrilla 1988; Postiglioni et al.1990, 1991
CURCULIONOIDEA					
Curculionidae					
Diaprepes abbreviatus (Linnaeus, 1764)	22=20+Xy_p	------	Xy_p and coincident with CH	Ag NOR	Virkki and Sepúlveda 1990; Virkki et al. 1990

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Resumen

Se presenta un análisis comparativo de cuatro especies Neotropicales de Elateridae y se muestra por primera vez, una revisión sobre el patrón de regiones organizadoras del nucléolo (NORs) en cromosomas de Coleoptera. La caracterización citogenética de *Conoderus malleatus* (Conoderini), *Pyrearinus candelarius*, *Pyrophorus divergens* y *Pyrophorus punctatissimus* (Pyrophorini) fue efectuada a través del estudio de células mitóticas y meióticas sometidas a tinción estándar (Giemsa) y diferencial (impregnación por plata y fluorocromos CMA_3 específicos para GC y DAPI específicos para AT). El análisis de células espermatogoniales mostró que el número diploide de *C. malleatus* es de 2n=17 mientras que en *Pyrearinus candelarius*, *Pyrophorus divergens* y *Pyrophorus punctatissimus* es de 2n=15. En estas especies, el sistema de determinación del sexo es del tipo X0. Se confirmó la morfología acrocéntrica de casi todos los cromosomas. El estudio de células meióticas de las cuatro especies mostró la ocurrencia de sinapsis total entre los autosomas, la presencia de un quiasma terminal o intersticial en la mayoría de los bivalentes y la conducta reduccional y segregación regular de todos los cromosomas. Aunque las tres especies de Pyrophorini mostraron varias características cariotípicas similares, hay una discrepancia que se observó en células en diplotene en relación al número de bivalentes con dos quiasmas. Se verificó que *Pyrearinus candelarius* sólo presenta un bivalente con dos quiasmas, *Pyrophorus divergens* dos bivalentes y *Pyrophorus punctatissimus* hasta cuatro bivalentes con dos quiasmas. Las células testiculares impregnadas con nitrato de plata evidenciaron dos NORs

terminales localizadas en el cuarto par autosómico de la especie de Conoderini y en el segundo par autosómico de las tres especies representativas de Pyrophorini. El empleo de la tinción con CMA_3/DA/DAPI en *Pyrearinus candelarius* y *Pyrophorus punctatissimus* mostró que las regiones marcadas por CMA_3 eran coincidentes con las NORs. En este trabajo se discute el mecanismo de diferenciación cariotípica que ha ocurrido entre las cuatro especies de Elateridae y otras especies próximas, así como la tendencia general de alteraciones en la localización de las NORs durante la evolución de los cromosomas de Coleoptera.

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Artigo III

Running title: Strategies of karyotype differentiation in Elateridae

Singular strategies of karyotype differentiation traced in two subfamilies of Elateridae (Coleoptera, Polyphaga: Agrypninae, Elaterinae)

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Resumo

A análise cromossômica de cinco espécies da família Elateridae, pertencentes as subfamílias Agrypninae e Elaterinae, bem como uma revisão dos dados citogenéticos previamente registrados para esta família, permitiram o estabelecimento das estratégias principais de diferenciação cariotípica que ocorreram neste grupo de espécies relacionadas. Em Agrypninae, as três espécies estudadas (*Conoderus fuscofasciatus*, *Conoderus rufidens*, e *Conoderus* sp.) mostraram o cariótipo 2n=16+X0 nos machos. Esta uniformidade cariotípica detectada nestas espécies de *Conoderus* também tem sido compartilhada com outras espécies deste mesmo gênero, diferindo consideravelmente da heterogeneidade cromossômica verificada na subfamília Agrypninae. O emprego da técnica de bandamento C em *Conoderus fuscofasciatus* e *Conoderus* sp. revelou heterocromatina constitutiva na região pericentromérica da maioria dos cromossomos. Em *Conoderus fuscofasciatus*, blocos adicionais na região terminal do braço longo de quase todos os cromossomos foram observados. Nos representantes de Elaterinae, o cariótipo $2n=18+Xy_p$ encontrado em *Pomachilius* sp.2 é similar aquele verificado na maioria das espécies de Coleoptera e também considerado como ancestral para a subordem Polyphaga, contrastando com a fórmula cromossômica 2n=18+X0 detectada em *Cardiorhinus rufilateris*, a qual tem sido comum entre as espécies de Elaterinae. Na subfamília Agrypninae, a diferenciação cromossômica freqüentemente tem envolvido grande redução do número diplóide e alteração dos tipos de sistema de determinação sexual, enquanto na subfamília Elaterinae, o cariótipo ancestral tem sido conservado ou alternativamente, tem sofrido algumas poucas mudanças, as quais foram causadas por fusões ou fissões autossômicas e/ou perda do cromossomo sexual y_p.

Palavras-chave: banda C, cromossomos, evolução, meiose, sistema de determinação sexual

Abstract

The chromosome analysis of five species of the family Elateridae, belonging to the subfamilies Agrypninae and Elaterinae, as well as an overview of the cytogenetic data previously recorded for this family, permitted the establishment of the main strategies of karyotypic differentiation that has occurred in these groups of related species. In Agrypninae, the three species studied (*Conoderus fuscofasciatus*, *Conoderus rufidens*, and *Conoderus* sp.) showed the karyotype 2n=16+X0 in males. This karyotypic uniformity detected in these species of *Conoderus* has also been shared with other species of the same genus, differing considerably from chromosomal heterogeneity verified in the subfamily Agrypninae. The use of the C-banding technique in *Conoderus fuscofasciatus* and *Conoderus* sp. revealed constitutive heterochromatin in the pericentromeric region of the majority of the chromosomes. In *Conoderus fuscofasciatus*, additional blocks in the long arm terminal region of almost all chromosomes were noticed. In the representatives of Elaterinae, the karyotype 2n=18+Xy$_p$ encountered in *Pomachilius* sp.2 was similar to that verified in the majority of the Coleoptera species and also considered as ancestral for the suborder Polyphaga, contrasting with the chromosomal formula 2n=18+X0 detected in *Cardiorhinus rufilateris*, which is common among the species of Elaterinae. In the subfamily Agrypninae, the chromosomal differentiation has frequently involved high reduction of the diploid number and alteration of the types of the sex determination system, while in the subfamily Elaterinae the ancestral karyotype has been conserved or alternatively, has suffered minimal changes, which were caused by autosomal fusions or fissions and/or loss of the y$_p$ sex chromosome.

Key words: C-band, chromosome, evolution, meiosis, sex determination system

Introduction

The family Elateridae possesses approximately 9,300 known species (Costa, 2003) and less than 1% of these have been cytogenetically analysed (Smith and Virkki, 1978; Ferreira et al., 1984; Vidal, 1984; Virkki et al., 1984; Virkki and Denton, 1987; Yadav and Vyas, 1993, 1994; Rozek and Lachowska, 2001; Rozek et al., 2004; Schneider et al., 2006; Schneider et al., under submission). Of the Elateridae species whose chromosomes were studied, 72% showed a particular characteristic in relation to the chromosomal evolution, when they were compared with the majority of the coleopteran species, such that, in these Elateridae species, the karyotype differentiation seems to have occurred by reduction of the diploid number (Table 1), taking into account the karyotype $2n=18+Xy_p$, which has been proposed as ancestral for the suborder Polyphaga beetles. In the other Elateridae species, 20% exhibited a diploid number higher than 2n=20 and 8% retained the ancestral number. Despite the fact that the reduction in the chromosome number is predominant in the Elateridae, the strategies of chromosomal evolution seem to be different among the four subfamilies, which have representatives that have been subject to examination from a cytogenetic point of view.

The subfamily Agrypninae has 31 analysed species and reveals 11 distinct karyotypic formulae (Table 1), showing a broad range of diploid numbers from 2n=4 (Ferreira et al., 1984) to 2n=22 (Rozek et al., 2004) and sex determination system of the Xy_p, X0, Xy, neoXY, and X_1X_2Y types (Smith and Virkki, 1978; Virkki et al., 1984; Virkki and Denton, 1987; Yadav and Vyas, 1994; Rozek et al., 2004; Schneider et al., 2006; Schneider et al., under submission). In Agrypninae, a decrease in the chromosome number is noted in almost all species, and is certainly the consequence of some chromosomal rearrangements, such as loss of the y_p sex chromosome, fusion between autosomes, and fusions between autosomes and sex chromosomes. Only one representative of Agrypninae revealed diploid number higher than 2n=20 (Rozek et al., 2004), which was probably the result of chromosomal rearrangements of the fission type, involving only autosomes, without alteration in the ancestral sex determination system of the Xy_p type.

The subfamily Cardiophorinae possesses only 8 analysed species (Table 1) and distinct from the three other subfamilies, has not shown any representative with a diploid number lower than 2n=20 up to the present. In this subfamily, the number of chromosomes varies from 2n=20 (Smith, 1960) to 2n=22 (Smith, 1953, 1960) and the sex determination system is either the Xy_p or X0 type (Smith 1953, 1960; Yadav and Vyas, 1993). In Cardiophorinae, the main event of chromosomal derivation seems to be the fission of one autosomal pair. Moreover, the elimination of the y_p sex chromosome has occurred in some species.

The subfamily Denticollinae possesses 24 examined species and exhibits five karyotypic formulae (Table 1), in which the diploid numbers 2n=17, 2n=19, 2n=20, 2n=21, and 2n=22, and the Xy_p and X0 sex determination systems were detected (Smith and Virkki, 1978; Rozek and Lachowska, 2001). In this subfamily, various species retained the ancestral karyotype of the suborder Polyphaga and others showed karyotypic diversification as a result of a decrease or increase in the chromosomal number. The decrease in the diploid number seems to have arisen through the loss of the y_p chromosome or through additional fusions between autosomes, while the increase in the chromosomal number is probably derived from the fission of autosomes.

The subfamily Elaterinae, with 26 species studied is the second largest among the elaterids in number of representatives examined and shows six different karyotypes (Table 1). Considering the predominance of the karyotype 2n=18+X0 (Smith and Virkki, 1978; Yadav and Vyas, 1993) in this subfamily, it is possible to suggest that the principal mechanism of chromosomal evolution in this group was by loss of the y_p chromosome without alteration in the number of autosomal pairs. However, autosomal fission and fusion between autosomes and sex chromosomes have also occurred in some species of Elaterinae.

Due to the high karyotypic variability and the scarcity of cytogenetic data in the representatives of Elateridae, mainly from the Neotropical region, this work had the aim of cytogenetically characterizing five species belonging to the subfamily Agrypninae (*Conoderus fuscofasciatus*, *Conoderus rufidens*, and *Conoderus* sp.) and Elaterinae (*Cardiorhinus rufilateris* and *Pomachilius* sp.2).

For this cytogenetic characterization, the diploid/haploid number of chromosomes, the type of sex determination system, and the behaviour of the chromosomes during the meiosis were determined for all species. Additionally, the pattern of distribution of the constitutive heterochromatin was established in *Conoderus fuscofasciatus* and *Conoderus* sp.

Table 1. Cytogenetic data of Elateridae species. M=metacentric. Sm=submetacentric. A=acrocentric.

Species	Chromosomal formula (2n males)	Chromosomal morphology	References
Agrypninae			
Agrypnini			
Adelocera colonicus (Candèze, 1881)	17=16+X0	------	Smith and Virkki, 1978
Adelocera modesta (Candèze, 1857)	17=16+X0	------	Smith and Virkki, 1978
Adelocera murina (Lineu, 1758)	$22=20+Xy_p$	------	Rozek et al., 2004
Adelocera rectangularis (Say, 1825)	17=16+X0	------	Smith, 1953
(?) *Adelocera* sp.	17=16+X0	------	Manna and Mandrira, 1972
Agrypnus fuscipes (Fabricius, 1775)	17=16+X0	16A+XA	Banerjee, 1959; Dasgupta, 1977
Agrypnus sp.	11=10+X0	6M+?	Kacker, 1963
Colaulon lezeleuci (Candèze, 1857)	17=16+X0	------	Virkki, 1962
Lacon profusa Candèze, 1857	14=12+neoXY	------	Smith and Virkki, 1978
Conoderini			
Conoderus dimidiatus Germar, 1839	17=16+X0	16A+XA	Schneider et al., 2006
Conoderus fuscofasciatus Eschscholtz, 1829	17=16+X0	16A+XA	This report
Conoderus malleatus (Germar, 1824)	17=16+X0	6Sm+10A+XA	Schneider et al., under submission
Conoderus pilatei (Candèze, 1859)	17=16+X0	------	Virkki, 1962
Conoderus rodriguezi (Candèze, 1881)	17=16+X0	------	Virkki, 1962
Conoderus rufidens (Fabricius, 1801)	17=16+X0	------	This report
Conoderus scalaris (Germar, 1824)	17=16+X0	16A+XA	Schneider et al., 2006
Conoderus stigmosus Germar, 1839	16=14+neoXY	2Sm+12A+neoXYA	Schneider et al., 2006
Conoderus ternarius Germar, 1839	17=16+X0	4M+12A+XA	Schneider et al., 2006
Conoderus sp.	17=16+X0	2M+4Sm+10A+XA	This report
Heteroderes lenis (Candèze, 1859)	17=16+X0	2M+14A+XA	Yadav and Vyas, 1994
Heteroderes macroderes Candèze, 1859	19=18+X0	12M+6A+XM	Agarwal, 1960, 1962
Heteroderes macroderes Candèze, 1859	17=16+X0	4M+12A+XA	Yadav and Vyas, 1994
Heteroderes modestus Candèze, 1859	17=16+X0	2M+14A+XA	Yadav and Vyas, 1994
Heteroderes sericeus Candèze, 1859	17=16+X0	2Sm+14A+XA	Yadav and Vyas, 1994
Monocrepidius sp. (=*Conoderus* sp.)	17=16+X0	------	Vidal, 1984
Hemirhipini			
Chalcolepidius silbermanni Chevrolat, 1835	12=10+Xy	10A+XyA	Virkki and Denton, 1987
Chalcolepidius zonatus Eschscholtz, 1829	4	2M+2A	Ferreira et al., 1984
Hemirrhipus lineatus (Olivier, 1790)	10=8+neoXY	------	Piza, 1958
Pyrophorini			
Pyrearinus candelarius (Germar, 1841)	15=14+X0	6Sm+8A+XSm	Schneider et al., under submission
Pyrophorus divergens Eschscholtz, 1829	15=14+X0	4Sm+10A+XSm	Schneider et al., under submission
Pyrophorus luminosus Illiger, 1807 (now in *Ignelater* Costa, 1975)	$17=14+X_1X_2Y$	------	Virkki, 1962; Virkki et al., 1984
Pyrophorus nyctophanus Germar, 1841 (=*P. phosporescens* Castelnau, 1840)	15=14+X0	2M+X?	Piza, 1960
Pyrophorus pellucens Eschscholtz, 1829 (=*P. phosporescens* Castelnau, 1840, *pars*)	15=14+X0	------	Smith, 1960
Pyrophorus punctatissimus Blanchard, 1843	15=14+X0	4Sm+10A+XSm	Schneider et al., under submission
Pyrophorus radians Champion, 1895 (now in *Deilelater* Costa, 1975)	11=10+X0	------	Virkki, 1962
Cardiophorinae			
Cardiophorus cardisce (Say, 1839)	$22=20+Xy_p$	------	Smith, 1960
Cardiophorus convexulus LeConte, 1853	$22=20+Xy_p$	------	Smith, 1953
Cardiophorus convexus (Say, 1823)	$20=18+Xy_p$	------	Smith, 1960
Cardiophorus fenestratus LeConte, 1859	$22=20+Xy_p$	------	Smith, 1953
Cardiophorus gagates Erichson, 1840	$22=20+Xy_p$	------	Smith, 1953
Cardiophorus haridwarensis Vats & Chauhan, 1991	21=20+X0	14M+6Sm+XM	Yadav and Vyas, 1993
Cardiophorus limbatus Candèze, 1860	21=20+X0	14M+6A+XM	Yadav and Vyas, 1993
Cardiophorus togatus Horn, 1871	$22=20+Xy_p$	------	Smith, 1953

Denticollinae			
Athoini			
Athous rufiventris Eschscholtz, 1822	19=18+X0	------	Smith and Virkki, 1978
Limonius aeger LeConte, 1853	$20=18+Xy_p$	------	Smith, 1950, 1953
Limonius griseus Beauvois, 1805	17=16+X0	------	Stevens, 1909
Prosternini			
Ctenicera aenea (Linnaeus, 1758)	19=18+X0	------	Smith, 1960
Ctenicera aeripennis aeripennis (Kirby, 1837)	19=18+X0	------	Smith, 1953
Ctenicera aeripennis destructor (Brown, 1935)	19=18+X0	------	Smith, 1953
Ctenicera appropinquans (Randall, 1838)	19=18+X0	------	Smith, 1953
Ctenicera appressa Randall, 1838	$22=20+Xy_p$	------	Smith, 1953
Ctenicera arata (LeConte, 1853)	17=16+X0	------	Smith, 1953
Ctenicera bombycina (Germar, 1843)	$22=20+Xy_p$	------	Smith and Virkki, 1978
Ctenicera hieroglyphica (Say, 1839)	21=20+X0	------	Smith, 1953
Ctenicera inflata (Say, 1825)	$20=18+Xy_p$	------	Smith, 1953
Ctenicera mediana (Germar, 1843)	$22=20+Xy_p$	------	Smith, 1953
Ctenicera nitidula (LeConte, 1853)	17=16+X0	------	Smith, 1953
Ctenicera ochreipennis LeConte, 1853	$22=20+Xy_p$	------	Smith, 1953
Ctenicera propola columbiana (Brown, 1936)	21=20+X0	------	Smith and Virkki, 1978
Ctenicera propola propola (LeConte, 1853)	21=20+X0	------	Smith, 1953
Ctenicera rufopleuralis (Fall, 1934)	17=16+X0	------	Smith, 1953
Ctenicera semimetallica (Walker, 1866)	19=18+X0	------	Smith, 1953
Ctenicera splendens (Ziegler, 1844)	19=18+X0	------	Smith, 1953
Ctenicera tarsalis (Melsheimer, 1846)	$20=18+Xy_p$	------	Smith, 1960
Ctenicera tessellata (Linnaeus, 1758)	$22=20+Xy_p$	------	Smith, 1960
Prosternon tesselatum (Linnaeus, 1758) = *Ctenicera tessellata* (Linnaeus, 1758)	22=20+Xy	20M/Sm+XA+y?	Rozek and Lachowska, 2001
Eanus estriatus (LeConte, 1853)	$20=18+Xy_p$	------	Smith, 1953
Eanus maculipennis LeConte, 1863	$20=18+Xy_p$	------	Smith, 1953
Elaterinae			
Agriotini			
Agriotella bigeminata (Randall, 1838)	19=18+X0	------	Smith, 1953
Agriotes lineatus (Linnaeus, 1767)	19=18+X0	------	Smith, 1960
Agriotes mancus (Say, 1823)	19=18+X0	10M+8A	Smith, 1956; Virkki, 1958a, b
Agriotes mancus (Say, 1823)	19=14+1IV+X	------	Smith and Virkki, 1978
Agriotes obscurus (Linnaeus, 1758)	19=18+X0	------	Smith, 1960
Agriotes sputator (Linnaeus, 1758)	$20=18+Xy_p$	------	Smith, 1960
Cardiorhinus rufilateris (Eschscholtz, 1822)	19=18+X0	------	This report
Pomachilius sp.2	$20=18+Xy_p$	6Sm+12A+XySm	This report
Ampedini			
Ampedina			
Ampedus apicatus (Say, 1839)	19=18+X0	------	Smith, 1953
Ampedus deletus (LeConte, 1853)	19=18+X0	------	Smith, 1953
Ampedus fusculus (LeConte, 1853)	19=18+X0	------	Smith, 1953
Ampedus luctuosus (LeConte, 1853)	19=18+X0	------	Smith, 1953
Ampedus melsheimeri (Leng, 1918)	19=18+X0	------	Smith, 1953
Ampedus pullus (Germar, 1844)	19=18+X0	------	Smith and Virkki, 1978
Ampedus sp., nr. *deletus* LeConte	19=18+X0	------	Smith, 1953
Ampedus sp., nr. *deletus* LeConte	21=20+X0	------	Smith, 1953
Ampedus sp., nr. *miniipennis* LeConte, 1853	19=18+X0	------	Smith, 1953
Dicrepidiina			
Dicrepidius politus Champion, 1894	23=22+X0	------	Virkki, 1962
Dicrepidius ramicornis Beauvois, 1805	23=22+X0	------	Virkki, 1962
Melanotina			
Melanotus fissilis (Say, 1839)	19=18+X0	------	Smith, 1960
Melanotus kamaunensis Vats & Chauhan, 1991	12=10+XY	10M+XYM	Yadav and Vyas, 1993
Melanotus leonardi (LeConte, 1853)	19=18+X0	------	Smith, 1953
Melanotus longicornis Candèze, 1860	19=18+X0	------	Manna and Mandrira, 1972
Melanotus oregonensis LeConte, 1853	19=18+X0	------	Smith and Virkki, 1978
Melanotus tenebrosus (Erichson, 1841)	19=18+X0	10M/Sm+8A+XA	Smith and Virkki, 1978; Yadav and Vyas, 1993
Melanotus trapezoideus LeConte, 1853	19=18+X0	------	Smith, 1953
Melanotus sp., nr. *communis* (Gyllenhal, 1817)	19=18+X0	------	Smith, 1953
Melanotus sp.	19=18+X0	------	Smith, 1960
Elaterini			
Elater sp.1	19=18+X0	------	Smith and Virkki, 1978
Elater sp.2	19=18+X0	------	Smith and Virkki, 1978

Material and methods

The sample of male individuals analysed in this work was collected in Rio Claro (22°24'S, 47°33'W), São Paulo State, Brazil, and Ponta Grossa (25°05'S, 50°09'W), Paraná State, Brazil, and included: 1 specimen of *Cardiorhinus rufilateris*, 2 of *Conoderus rufidens*, and 3 of *Conoderus* sp., from Rio Claro, and 8 specimens of *Conoderus fuscofasciatus* and 2 of *Pomachilius* sp.2, from Rio Claro and Ponta Grossa. The vouchers were deposited in the entomological collection of the Departamento de Biologia, UNESP, Rio Claro, São Paulo State, Brazil.

Chromosomal preparations were obtained from the gonads of adult specimens. The gonads of alive individuals were removed in insect saline solution, placed in hypotonic solution (tap water) for 2 min, and fixed in Carnoy I (3 methanol: 1 acetic acid) for 30 min. To obtain metaphasic chromosomes, some gonads were initially immersed in 0.05% colchicine solution prepared with insect saline solution for 90 min. For slide preparations, the gonads were macerated in 40% acetic acid to obtain a cell suspension. This cell suspension was spread on the slides, which were dried on a metal plate at 40° C. The chromosomal preparations of all five species were stained with 3% Giemsa solution for 12 min. Additionally, some chromosomal preparations of *Conoderus fuscofasciatus* and *Conoderus* sp. were submitted to the standard/C-banding sequential staining, using 3% Giemsa solution, for 12 min and the C-banding technique described by Sumner (1972). Chromosomal morphology was determined according to the nomenclature proposed by Levan et al. (1964).

Results

Subfamily Agrypninae

The study of spermatogonial metaphases of *Conoderus fuscofasciatus* and *Conoderus* sp. demonstrated the diploid number 2n=17 and the sex determination system of the X0 type (Fig.1a, c). In *Conoderus fuscofasciatus*, all the autosomes and the X sex chromosome showed acrocentric morphology, while in *Conoderus* sp. pairs 1 and 3 were submetacentric, pair 4 was

metacentric, and pairs 2, 5, 6, 7, 8, and the X chromosome were acrocentric. The karyotypic analysis of these two species also revealed that the autosomes gradually decreased in size; the *Conoderus fuscofasciatus* X sex chromosome possessed intermediate size between the 5th and 6th autosomal pairs and the *Conoderus* sp. X chromosome was among the smallest elements of the karyotype (Fig. 1a, c).

Mitotic cells of both species were subjected to the Giemsa/C-banding sequential technique. In *Conoderus fuscofasciatus*, the constitutive heterochromatin appeared in the pericentromeric region of all autosomes, with the exception of pair 6, and in *Conoderus* sp., pericentromeric C bands were detected in the autosomal pairs 1, 3, 4, 7, and 8 (Fig. 1b, d). Additionally, in *Conoderus fuscofasciatus,* C band positive blocks were observed in the terminal region of the long arm of all autosomes, except pairs 6 and 8. In the X sex chromosome of *Conoderus fuscofasciatus*, constitutive heterochromatin was detected in the pericentromeric region and long arm terminal region; in the X chromosome of *Conoderus* sp., the C band was only noticed in the interstitial region of the long arm (Fig. 1b, d). Mitotic metaphase cells of *Conoderus rufidens* were not obtained in the sample examined; however, the number of chromosomes and the type of sex determination system in this species were established through the analysis of meiotic cells.

Late prophase I and metaphasic I spermatocytes of the three species of the subfamily Agrypninae showed 2n=8II+X0 (Fig. 2a-d). In some stages of the meiosis (pachytene, diplotene, diakinesis, and anaphase I), the X sex chromosome was highly condensed and positively heteropycnotic in relation to the autosomes. In pachytene spreads, the total synapsis between the autosomes was confirmed by the number of bivalents and double thickness of the chromosomal filaments (Fig. 2a). Diplotenic and diakinetic cells revealed the occurrence of only one interstitial or terminal chiasma in the autosomal bivalents (Fig. 2b-c). In the metaphasic I spermatocytes, all the autosomal bivalents exhibited chromosomal elements associated end to end, without evidence of the occurrence of chiasma (Fig. 2d). During the anaphase I, two cellular poles with different haploid numbers were verified, n=8+X and n=8,

confirming the reductional segregation of all chromosomes in the meiosis I (Fig. 2e-f).

Subfamily Elaterinae

The karyotypic study of *Pomachilius* sp.2 showed the diploid number 2n=20 and the sex determination system of the Xy_p type (Fig. 3a). The majority of the chromosomes had acrocentric morphology, with the exception of the 2nd, 3rd, and 9th autosomal pairs, as well as the X_p and y_p sex chromosomes, which were submetacentric. In relation to chromosome size, the autosomes could be classified into two categories: pair 1 was the largest of the complement and the others were medium/small elements that gradually decreased in size. The X_p sex chromosome exhibited similar size to that of the 9th autosomal pair and the y_p chromosome was an extremely small element.

Diakinesis and metaphase I cells of *Pomachilius* sp.2 demonstrated the meiotic formula $2n=9II+Xy_p$, with sex chromosomes associated in a typical parachute configuration (Fig. 3b). All metaphasic II cells revealed the same haploid number n=10; however, only some cells had a dot-like structure, which corresponded to the y_p sex chromosome. In the metaphases II cells, it was not possible to identify the X_p chromosome, because it did not demonstrate differential behaviour or pycnosis in relation to the autosomes (Fig. 3c-d).

In *Cardiorhinus rufilateris*, the cytogenetic analysis was only achieved in the meiotic testicular cells, which revealed the presence of 9 autosomal bivalents and 1 sexual univalent, making it possible to establish the chromosomal number 2n=19 and the X0 sex determination system for this species (Fig. 4). In the pachytene cells, the total synapsis between the autosomes was verified, and the X sex chromosome was easily recognized due to its high degree of condensation and positive heteropycnosis (Fig. 4a). Diplotene and diakinesis spermatocytes showed that the majority of the autosomal bivalents possessed only one interstitial or terminal chiasma; however, bivalents with two chiasmata were also observed (Fig. 4b-c). Metaphasic I cells exhibited autosomal bivalents and an X chromosome typically aligned on the equatorial plate (Fig. 4d).

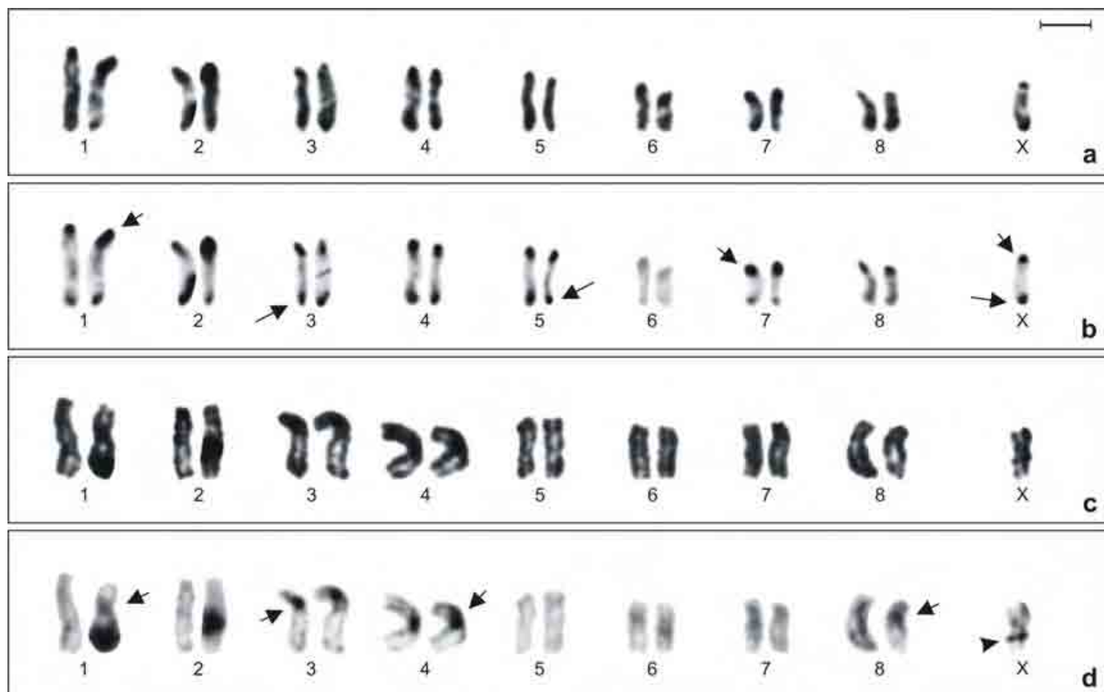

Figure 1. Karyotypes of male specimens of *Conoderus fuscofasciatus* (**a**-**b**) and *Conoderus* sp. (**c**-**d**) sequentially stained with Giemsa (**a**, **c**) and C-banded (**b**, **d**), exhibiting 2n=17+X0 and the acrocentric morphology of the majority of the chromosomes. Note in (**b**), the constitutive heterochromatin in the pericentromeric (small arrow) and terminal region (large arrow) of almost all chromosomes and in (**d**), the C band positive blocks in the pericentromeric region (small arrow) of some autosomal pairs and in the long arm interstitial region (arrowhead) of the X chromosome. Scale bar=5μm.

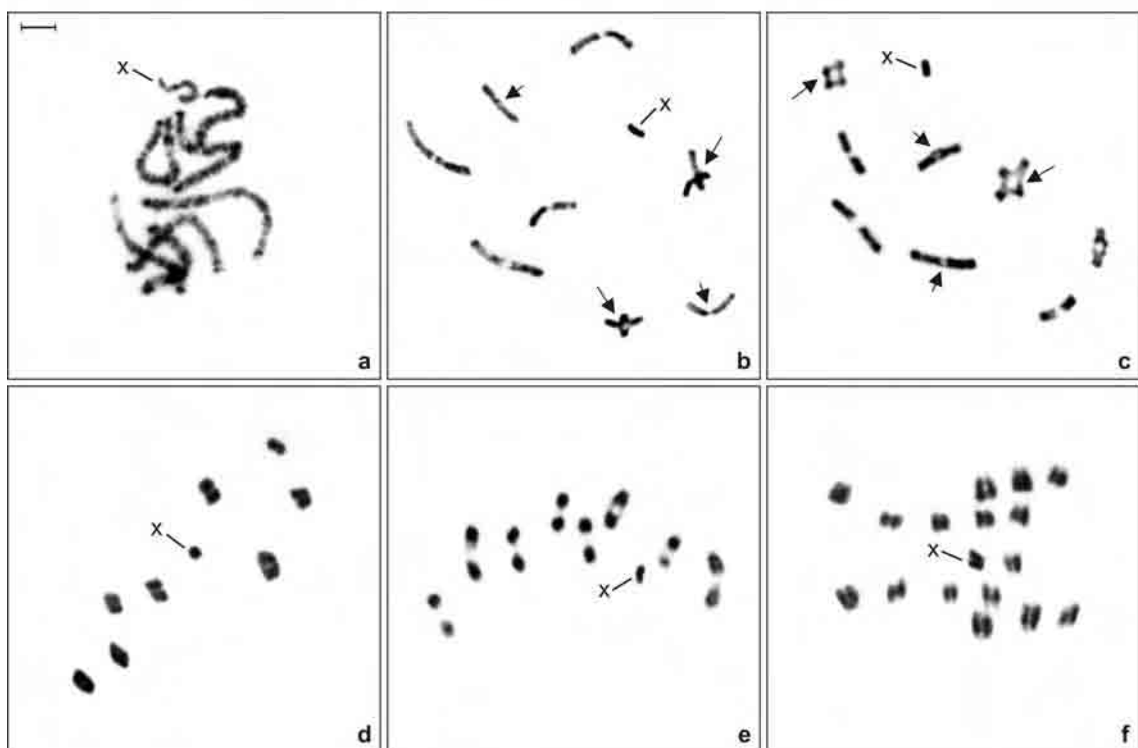

Figure 2. Meiotic testicular cells of *Conoderus fuscofasciatus* (**a**-**c**), *Conoderus rufidens* (**d**), and *Conoderus* sp. (**e**-**f**) stained with Giemsa. (**a**) Pachytene, 2n=8II+X0. (**b**-**c**) Diplotene and diakinesis, respectively, 2n=8II+X0, showing one interstitial (large arrow) or terminal (small arrow) chiasma in the autosomal bivalents. (**d**) Metaphase I, with 2n=8II+X0. (**e**) Early anaphase I, exhibiting the initial segregation of the homologous chromosomes. (**f**) Late anaphase I, with n=8 and n=8+X in the upper and lower cellular poles, respectively. Scale bar=5μm.

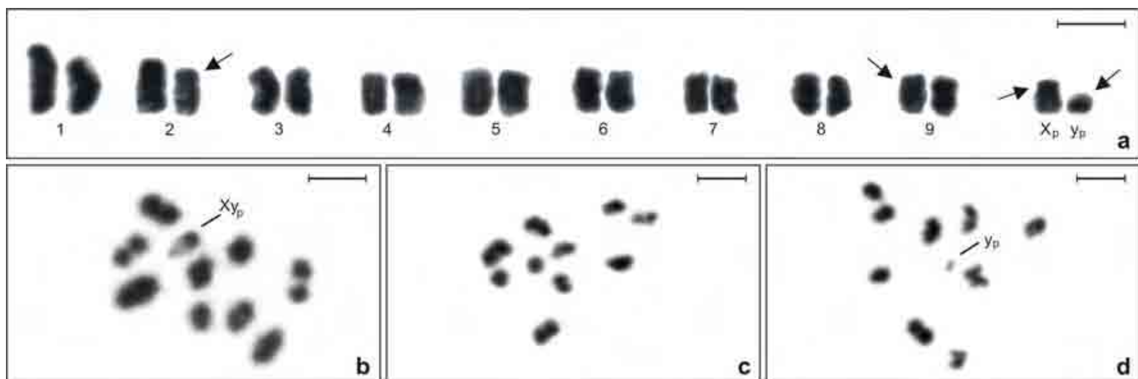

Figure 3. Testicular cells of *Pomachilius* sp.2 stained with Giemsa. (**a**) Karyotype, with 2n=18+Xy_p. The arrows indicate the submetacentric chromosomes. (**b**) Metaphase **I**, with 2n=9**II**+Xy_p. (**c**-**d**) Metaphases **II**, with n=9+X_p and n=9+y_p, respectively. Scale bar=5μm.

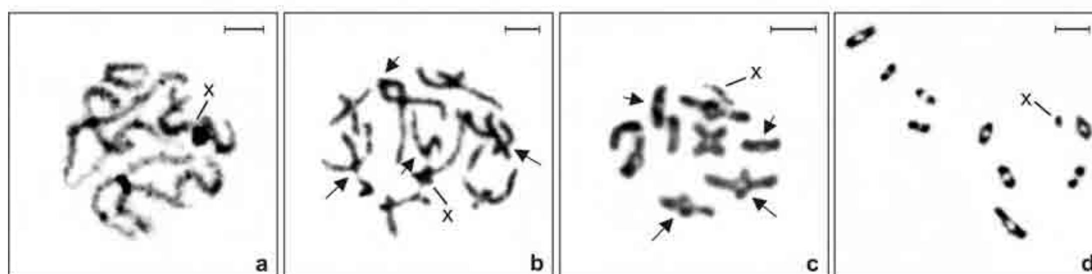

Figure 4. Spermatocytes of *Cardiorhinus rufilateris* stained with Giemsa, revealing the meiotic formula 2n=9**II**+X0. (**a**) Pachytene. (**b**-**c**) Diplotene and diakinesis, respectively, showing the majority of the autosomal bivalents with one interstitial (large arrow) or terminal (small arrow) chiasma. (**d**) Metaphase **I**. Scale bar=5μm.

Discussion

The chromosomal characteristics concerning to the diploid number (2n=17) and type of sex determination system (X0/XX) detected in *Conoderus fuscofasciatus*, *Conoderus rufidens*, and *Conoderus* sp. are shared with all eight representatives of the same genus described in the literature (Virkki, 1962; Vidal, 1984; Schneider et al., 2006; Schneider et al., under submission), except *Conoderus stigmosus* (2n=14+neoXY), and also with other species that belong to the tribe Conoderini reported by Agarwal (1960, 1962) and Yadav and Vyas (1994). The acrocentric morphology verified in the majority of the autosomal pairs and invariably in the X sex chromosome, is the most frequent pattern for the Conoderini (Table 1), but it differs considerably from the metacentric pattern proposed by Smith and Virkki (1978) as the basic for the coleopteran.

Taking into account this information, it is possible to infer that the karyotype 2n=16+X0, with a majority of the acrocentric chromosomes, was the common origin for the Conoderini species and was probably derived from the ancestral chromosomic constitution of the Polyphaga, through rearrangements of the type fusion between autosomes, pericentric inversions, and loss of the y_p sex chromosome. Furthermore, the data obtained in the chromosomal analysis of the Conoderini representatives in the present work, also corroborate the hypothesis that the diploid number 2n=17 and the X0 sex determination system could represent the basic condition for the species of the tribe Conoderini and that discrepant karyotypic characteristics could indicate chromosomal differentiation (Schneider et al., under submission). It should be emphasized that the high conservatism of the 2n=16+X0 has only been observed in this tribe of the subfamily Agrypninae, contrasting with the karyotypic diversity detected in the tribes Agrypnini, Hemirhipini, and Pyrophorini (Table 1). However, this karyotypic conservatism of the Conoderini could be due to the study of 15 species included in only two distinct genera. Thus, the analysis of some representatives of other genera of the tribe Conoderini, such as *Aeolus* and *Deronocus*, will certainly supply data for a clearer understanding of the strategies of chromosomal evolution in this group of closely related species.

The karyotypic characteristics found in *Pomachilius* sp.2, the first representative of this genus studied from a cytogenetic point of view, were similar to those described by Smith (1960) for one other species of the subfamily Elaterinae, *Agriotes sputator*. Only these two mentioned Elaterinae species have retained the diploid number 2n=20 and the sex determination system of the Xy_p type (Table 1), which are considered as ancestral for the suborder Polyphaga (Smith and Virkki, 1978). The unique difference detected in *Pomachilius* sp.2, in relation to the ancestral karyotype, referred to the morphology of some autosomal pairs, which were acrocentric and could have originated by pericentric inversions or deletion of constitutive heterochromatic material on the short arm.

The cytogenetic study of a species belonging to the *Cardiorhinus* genus was accomplished for the first time in this work and revealed a common chromosomal number and type of sex determination system among the representatives of the subfamily Elaterinae (Table 1). Similar to the 22 species of this subfamily already examined (Smith, 1953, 1956, 1960; Virkki, 1962; Manna e Mandrira, 1972; Smith and Virkki, 1978; Yadav and Vyas, 1993), the karyotype 2n=18+X0 of *Cardiorhinus rufilateris* certainly did not originate by alteration in the number of autosomal pairs, but rather by the elimination of the y_p sex chromosome. This event has been frequently registered in representatives of three other subfamilies of Elateridae (Table 1), as well as in many species of coleopteran, such that the sex determination system of the X0 type is the second most common among beetles (Smith and Virkki, 1978). The loss of the y sex chromosome can occur by its progressive heterochromatinization, giving rise to a genetically inert chromosome (Smith and Virkki, 1978), or by transference of the y chromosome genetic material to the autosomes (John and Shaw, 1967). Alternatively, Steinemann and Steinemann (1998) proposed that the accumulation of transposable elements, especially retrotransposons, could contribute to the switch from euchromatin into heterochromatin and, consequently, determine y chromosome degeneration.

A cytogenetic data overview of the subfamily Elaterinae (Table 1) showed that among the species of the three tribes that have already been analyzed, the ancestral karyotype 2n=18+Xy_p has been encountered only in representatives of the tribe Agriotini. This karyotype is probably the basic type for this tribe, taking into account that it certainly did not originate "de novo" during the chromosomal evolution of this group. In contrast, the 2n=18+Xy_p could not be the basic karyotype for the tribes Ampedini and Elaterini, considering that these chromosomal characteristics were not detected in any of the 21 species examined so far.

In the five species studied in this work, the meiotic behaviour of the all chromosomes, concerning to the synapsis, chiasmata, heteropycnosis, and type of segregation, did not differ from that described for the species of the family Elateridae (Stevens, 1909; Smith, 1956; Piza, 1958, 1960; Virkki, 1958a, b, 1962; Banerjee, 1959; Agarwal, 1962; Kacker, 1963; Ferreira et al., 1984; Virkki et al., 1984; Virkki and Denton, 1987; Rozek and Lachowska, 2001; Yadav and Vyas, 1993, 1994; Rozek et al., 2004; Schneider et al., 2006; Schneider et al., under submission). These results revealed that, despite the karyotypic diversity encountered among the elaterids, the pattern of meiotic behaviour of the chromosomes has been conserved in these species.

Unfortunately, in *Pomachilus* sp.2, the mode of meiotic pairing of the X_p and y_p sex chromosomes could not be established; however, according to some literature reports, the Xy_p association in the parachute configuration could be attributed to the occurrence of chiasmata between the sex chromosomes (Smith, 1951), to non-specific association of constitutive heterochromatic regions (Drets et al., 1983), to nucleolar association (John and Lewis, 1960), or to argyrophilous material (Virkki et al., 1990). In *Pomachilius* sp.2, the employment of C-banding and silver nitrate impregnation techniques can be useful in understanding the mode of association of the Xy_p sex bivalent during the meiosis.

The chromosomes of *Conoderus fuscofasciatus* and *Conoderus* sp. and four other previously described species of the same genus previously described (Schneider et al., 2006), revealed a common pattern of constitutive

heterochromatin distribution in the pericentromeric region of the majority of the chromosomes when subjected to the C-banding technique; however, differences in the number of chromosomes with pericentromeric and telomeric C band, as well as in relation to the presence of constitutive heterochromatin in the interstitial region of the sex chromosomes, were observed in all these species. Therefore, these closely related species of *Conoderus* genus, which possess similar karyotypes, seem to have a species-specific pattern of C band distribution. The interspecific variations of the C band positive regions could be the result of small duplications or deletions of heterochromatic material or, as proposed by Schweizer and Loidl (1987), due to the dispersion of heterochromatin between equidistant sites of non-homologous chromosomes.

A general examination of the chromosomal data that were compiled from the family Elateridae (Table 1) and those obtained in this work, revealed that among the four subfamilies that have been studied, Agrypninae seems to be cytogenetically very interesting, in view of the fact that the employment of only standard staining techniques in the majority of the species, has already shown a high heterogeneity of chromosomal numbers and types of sex determination system. Special attention should be given to the tribe Hemirhipini, which exhibited distinct and highly differentiated karyotypes in only three species studied. In the subfamilies Cardiophorinae, Denticollinae, and Elaterinae, the study of a large number of representatives will probably also reveal karyotypic diversity; however, in these three subfamilies, the application of some techniques that detect specific chromosomal regions will definitely be useful for differentiating species with similar karyotypes and establishing the strategies of chromosomal evolution.

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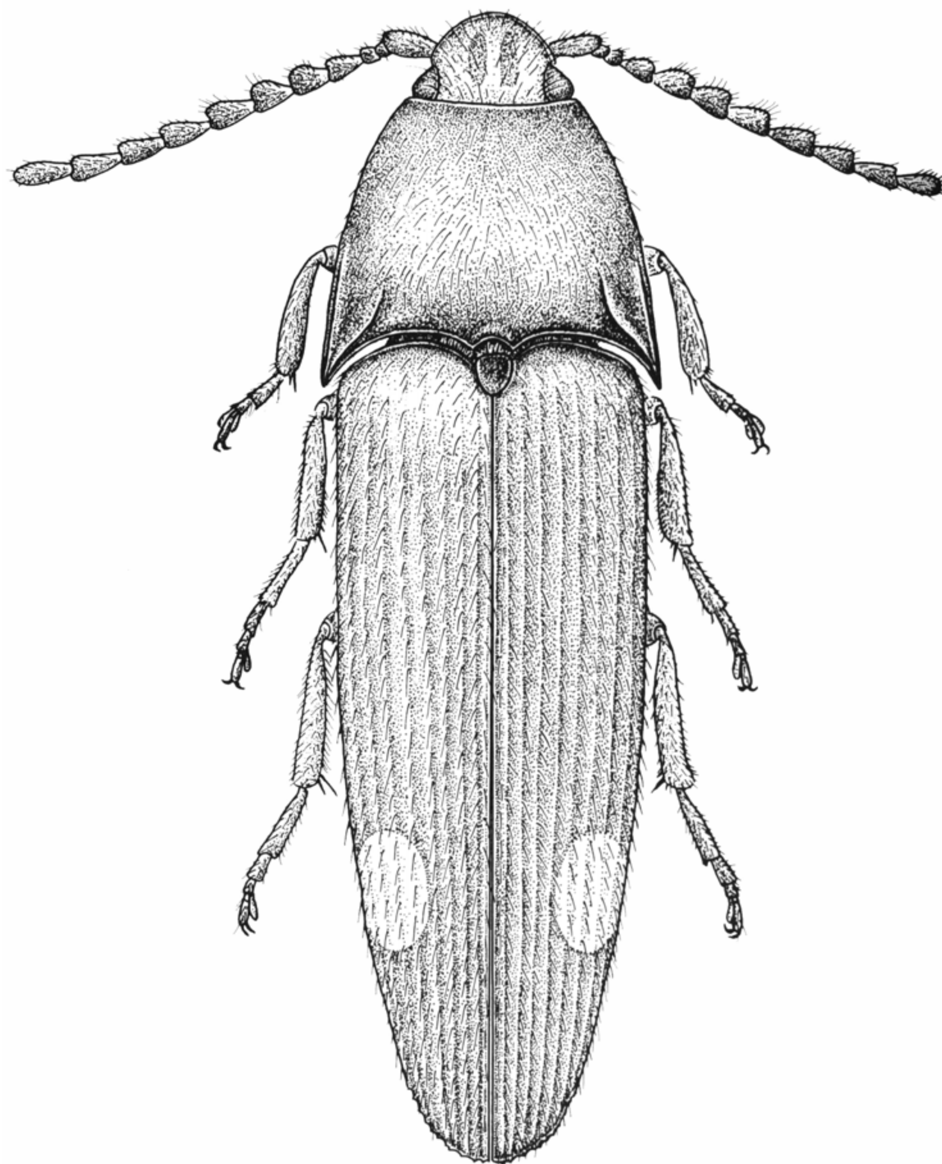


Physorhinus distigma

6. CONSIDERAÇÕES FINAIS

O estudo citogenético realizado nas 13 espécies da família Elateridae evidenciou que as principais estratégias empregadas na diferenciação cariotípica destes elaterídeos são semelhantes àquelas descritas para as outras 81 espécies pertencentes a este mesmo táxon (Smith e Virkki, 1978; Yadav e Vyas, 1993, 1994), bem como para a maioria dos representantes da ordem Coleoptera. Os eventos de diferenciação cromossômica dos Elateridae incluíram principalmente, a redução do número de cromossomos, a qual foi verificada em aproximadamente 72% das espécies da família. Em 31% destas espécies, a redução do número diplóide envolveu apenas a perda do cromossomo sexual y_p, em 33%, o desaparecimento do cromossomo y_p foi concomitante com a fusão entre autossomos e, em 8% ocorreu a fusão entre autossomos e cromossomo sexual. O aumento do número de cromossomos foi menos comum entre os Elateridae, sendo observado em cerca de 20% dos representantes desta família. Este aumento do número cromossômico teve origem por fissão de autossomos (11% das espécies), a qual, em alguns casos, também foi acompanhada pela perda do cromossomo sexual y_p (9% das espécies). Vale a pena ressaltar que, além destes últimos eventos mencionados serem menos freqüentes nos “click beetles”, o aumento do número de cromossomos neste grupo, envolveu, no máximo, dois pares de autossomos, ou seja, é muito pequena a divergência entre os cariótipos de

Elateridae originados por fissão autossômica e aquele cariótipo proposto como básico para os Polyphaga. Na família Elateridae, a manutenção do cariótipo $2n=18+Xy_p$ foi detectada em apenas 8% dos representantes estudados.

Uma outra diferenciação cromossômica observada nas espécies de Elateridae examinadas e também em outros representantes desta família já descritos, foi a alteração da morfologia metacêntrica para acrocêntrica, a qual envolveu alguns pares autossômicos ou até mesmo, todos os cromossomos do complemento. Provavelmente, rearranjos do tipo inversão pericêntrica e/ou deleção de material heterocromático constitutivo foram os responsáveis pela mudança da morfologia metacêntrica (prevalecente na maioria dos coleópteros) para a acrocêntrica. É possível que a predominância de cromossomos acrocêntricos, com região pericentromérica e braço curto totalmente heterocromáticos, tal como verificado em algumas das espécies analisadas, poderia facilitar a ocorrência de fusões autossômicas em Elateridae, considerando que, segundo Sumner (2003), as fusões envolvendo cromossomos acrocêntricos são comumente observadas em diversos grupos, uma vez que estas implicam em um menor número de rearranjos que aquelas envolvendo cromossomos meta/submetacêntricos.

O emprego da técnica de obtenção de bandas C revelou que uma grande quantidade de heterocromatina constitutiva pode representar um padrão comum às espécies da família Elateridae, levando-se em conta que nos representantes analisados no presente trabalho e naqueles já descritos por Rozek e Lachowska (2001) e Rozek *et al.* (2004), os cromossomos evidenciaram regiões de bandas C muito proeminentes. Além disso, nos elaterídeos, a heterocromatina constitutiva, especialmente aquela adicional presente na região terminal dos braços dos cromossomos autossômicos e do cromossomo sexual X, e que em alguns casos foi heteromórfica, poderia estar envolvida na diferenciação cromossômica das espécies, bem como ter conseqüência na meiose, influenciando a variabilidade genética, uma vez que, de acordo com John (1990), os segmentos heterocromáticos podem alterar a distribuição e a freqüência de quiasmas.

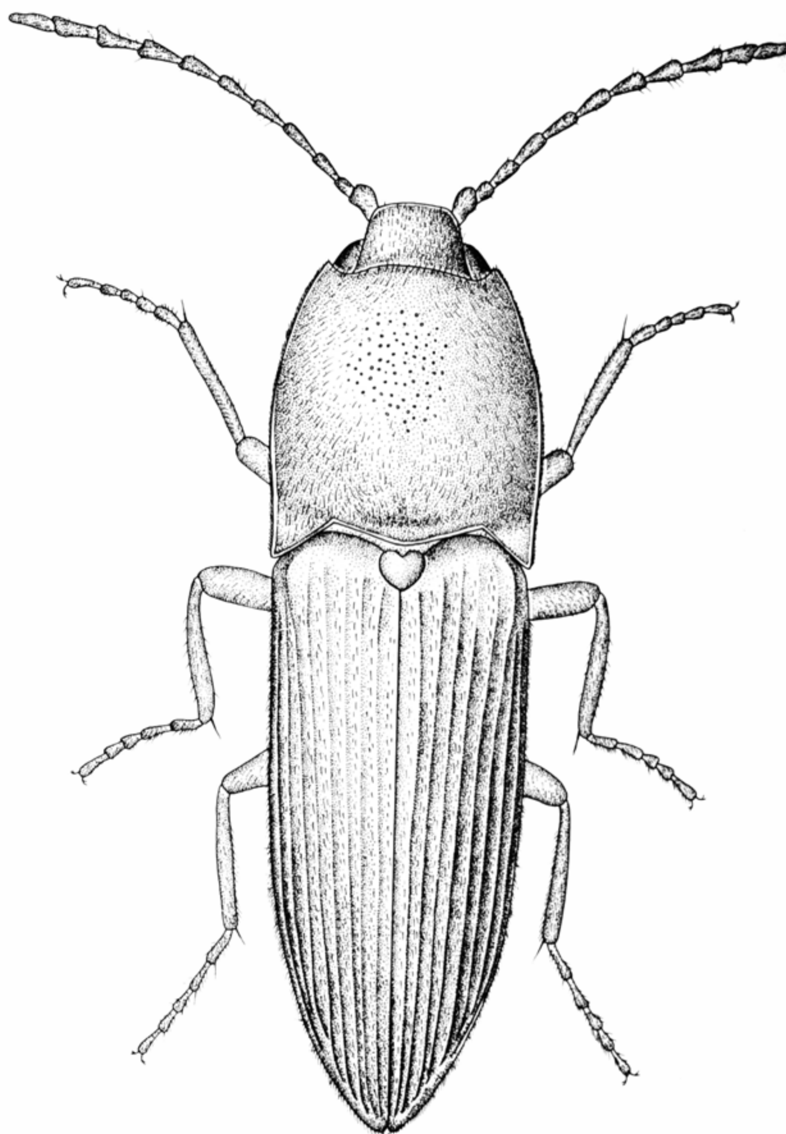
Nos Elateridae, as variações interespecíficas de número e distribuição das RONs não estão vinculadas as diferenças cariotípicas, considerando que na maioria dos elaterídeos analisados sob este aspecto, foram observadas 2 RONs localizadas na região terminal do braço curto do 2º par autossômico. Divergências em relação a este padrão foram verificadas em espécies com fórmulas cariotípicas similares e envolveram o número de RONs e/ou o par autossômico portador desta região. Porém, assim como detectado na maioria das espécies de Coleoptera, 2 RONs autossômicas também parece ser o padrão mais freqüente para os Elateridae.

O uso da coloração por fluorocromos base-específicos é interessante, pois além de revelar algumas particularidades da heterocromatina constitutiva, pode fornecer dados sobre a natureza química da eucromatina, tornando possível a inferência das possíveis funções de determinadas regiões coradas por fluorocromos, uma vez que os segmentos de DNA ricos em seqüências de base GC estão relacionados ao emparelhamento e recombinação gênica durante a meiose, a “housekeeping genes”, como aqueles das RONs, e são mais susceptíveis a quebras, enquanto as seqüências AT altamente repetidas estão vinculadas a organização estrutural do material genético e a eventos de início de replicação e transcrição (Sumner, 2003).

Em espécies da família Elateridae, o emprego da coloração por flurocromos base-específicos foi realizado pela primeira vez neste trabalho e evidenciou que, apesar da grande quantidade de heterocromatina constitutiva presente nos cromossomos destas espécies, poucas das regiões marcadas pela técnica de bandamento C estão compartimentalizadas em seqüências de base GC e nenhuma em seqüências AT. Adicionalmente, algumas regiões cromossômicas coradas brilhantemente pela Cromomicina A_3 foram coincidentes com as RONs, mostrando que o uso da coloração por fluorocromos em conjunto com a impregnação pelo íon prata pode ser útil para detectar as RONs nos elaterídeos, ao contrário do que tem sido observado em algumas espécies de besouros, principalmente aquelas pertencentes a superfamília Scarabaeoidea (Colomba *et al.*, 1996, 2000b; Vitturi *et al.*, 1999, 2003; Moura *et al.*, 2003; Bione *et al.*, 2005), nas quais o emprego de

fluorocromos base-específicos tem evidenciado a maioria das regiões heterocromáticas constitutivas, além das RONs.

Apesar de existirem aproximadamente 3.000 espécies de besouros cujos cromossomos foram de alguma maneira caracterizados, o aumento da representatividade de informações citogenéticas de espécies de Coleoptera, assim como de uma maior diversidade de famílias desta ordem, é ainda necessário para que seja possível traçar as principais estratégias de diferenciação cariotípica de grupos de espécies e sua relação com padrão de distribuição da heterocromatina constitutiva, das RONs, e das seqüências de bases GC e AT altamente repetidas.



Horistonotus sp.

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