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EVOLUÇÃO CROMOSSÔMICA EM CASSIDINAE *SENSU LATO* (COLEOPTERA, POLYPHAGA, CHRYSOMELIDAE)

MILENA DE JULIO

Dissertação apresentada ao Instituto de Biociências do Campus de Rio Claro, Universidade Estadual Paulista, como parte dos requisitos para obtenção do título de Mestre em Ciências Biológicas (Biologia Celular e Molecular).

Fevereiro - 2009

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SUMÁRIO

1. RESUMO 1

2. ABSTRACT 4

3. INTRODUÇÃO 7

4. REVISÃO BIBLIOGRÁFICA 10

5. OBJETIVOS 15

6. MATERIAL E MÉTODOS 17

6.1. Material 17

6.2. Métodos 18

6.2.1. Sem a utilização de colchicina 18

6.2.2. Com a utilização de colchicina 18

6.2.3. Coloração convencional 19

6.2.4. Análises cromossômicas 19

7. RESULTADOS E DISCUSSÃO 20

8. CONSIDERAÇÕES GERAIS 55

9. REFERÊNCIAS BIBLIOGRÁFICAS 57

10. ANEXO 69

1. RESUMO

Das subfamílias de Chrysomelidae, Cassidinae *sensu lato* (*s.l.*), formada por 6.000 espécies e 43 tribos, possui aproximadamente 100 espécies analisadas citogeneticamente e a maioria delas apresentou 2n=18=16+Xy_p, o qual é menor que aquele considerado basal para os Polyphaga, 2n=20=18+Xy_p. No entanto, alguns grupos de espécies demonstraram manutenção do número diplóide basal e outros, aumento desse número; algumas espécies do último grupo também exibiram variação em relação ao tipo de sistema cromossômico sexual (SCS). Considerando a revisão taxonômica recentemente realizada para as espécies de Cassidinae *s.l.*, a existência de relação filogenética para algumas espécies dessa subfamília, a alta diversidade de espécies desse grupo registrada para a região Neotropical e o baixo número de espécies que tiveram seus cromossomos estudados, o presente trabalho teve como objetivo verificar os mecanismos envolvidos na evolução cariotípica dessa subfamília através do estudo de sete espécies da fauna brasileira e da revisão dos dados citogenéticos. Os espécimes foram coletados em Avaré e Rio Claro, SP, Nonoai, RS, e Ponta Grossa, PR. As preparações cromossômicas obtidas de embrião e testículos de machos adultos foram coradas com solução de Giemsa. As espécies *Agroiconota inedita* (2n=42=40+Xy_p), *Charidotella (sensu stricto) immaculata* (2n=22=20+Xy_p), *Charidotella (sensu stricto) sexpunctata* (2n=22=20+Xy_p), e *Stolas chalybaea* (2n=24=22+Xy_p) revelaram número diplóide maior do que aquele estabelecido como basal para os Polyphaga e cromossomos com dois braços. Os cariótipos de *Cteisella confusa, Deloyala cruciata,* e *Metriona elatior* mostraram a fórmula cromossômica 2n=18=16+Xy_p, considerada modal para os Cassidinae *s.l.*, e cromossomos com dois braços. As sete espécies apresentaram cromossomos sexuais facilmente identificáveis por serem os menores do complemento. A análise das células meióticas de todas as espécies mostrou paquítenos com um bloco heteropicnótico positivo, representativo da cromatina sexual; diplótenos, com um alto número de bivalentes com dois quiasmas e cromossomos sexuais em uma configuração típica em pára-quedas, e metáfases II que comprovaram a morfologia

cromossômica, o tipo de SCS, e a segregação regular de todos os cromossomos. Os dados citogenéticos de seis espécies das sete examinadas foram descritos pela primeira vez. Considerando os dados citogenéticos aqui obtidos e aqueles das espécies de Cassidinae *s.l*. já descritas, o aumento no número de autossomos observado em algumas espécies pode ser explicado por fissão cêntrica seguida por inversão pericêntrica. Por outro lado, a diminuição do número diplóide em outras espécies pode ter ocorrido por inversão pericêntrica seguida de fusão cêntrica. Em ambos os eventos, cromossomos com dois braços e o SCS do tipo Xy_p foram mantidos na maioria das espécies. A ocorrência de outros tipos de SCS, simples ou múltiplos, em certas espécies de Cassidinae *s.l*., parece ter sido conseqüência do desaparecimento do cromossomo $y_{p,}$ fissão cêntrica do $y_{p,}$ translocação entre cromossomo sexual e autossomo, fissão cêntrica do cromossomo X_p e translocação subseqüente com autossomo, formando SCS múltiplos mais complexos. Levando-se em conta os dados filogenéticos e citogenéticos das espécies de Cassidinae *s.l*., a diferenciação cariotípica nesse grupo parece ter ocorrido a partir do cariótipo basal dos Polyphaga por diminuição do número cromossômico e subseqüentemente por aumento desse número. Inversões pericêntricas, fusões e fissões cêntricas parecem ter sido os principais mecanismos que promoveram a evolução dos autossomos, enquanto na evolução dos cromossomos sexuais, os mecanismos envolvidos foram fissão cêntrica e/ou translocação cromossômica.

Palavras-chave: Bivalente; Evolução; Meiose; Quiasma; Sistema cromossômico sexual

2. ABSTRACT

Among the subfamilies of Chrysomelidae, Cassidinae *sensu lato* (*s.l.*), formed by 6 000 species and 43 tribes, possesses approximately 100 species cytogenetically analyzed and most of them presented 2n=18=16+Xy_p, which was smaller than 2n=20=18+Xy_p considered basal for Polyphaga. However, some groups of species presented maintenance of the basal diploid number and others showed increase of this number; certain species of the latter group also exhibited variation in the sex chromosome system (SCS). Considering the recent taxonomic revision accomplished for the Cassidinae *s.l*. species, the existence of phylogenetic relationship for some species of this subfamily, the high diversity of species of this group in the Neotropical region, and the low number of Cassidinae *s.l.* species karyotyped so far, the aim of the present work was to verify the main mechanisms involved in the karyotype evolution of this subfamily through the study of seven species of the Brazilian fauna and the overview of the cytogenetic data. The individuals were collected in Avaré and Rio Claro, SP, Nonoai, RS, and Ponta Grossa, PR. The chromosomal preparations obtained from embryo and testes of adult males were stained with Giemsa solution. The species *Agroiconota inedita* (2n=42=40+Xy_p), *Charidotella (sensu stricto) immaculata* (2n=22=20+Xy_p), *Charidotella (sensu stricto) sexpunctata* (2n=22=20+Xy_p), and *Stolas chalybaea* (2n=24=22+Xy_p) revealed diploid number higher than that established as basal for Polyphaga and biarmed chromosomes. The karyotype of *Cteisella confusa, Deloyala cruciata,* and *Metriona elatior* showed the chromosomal formulae 2n=18=16+Xy_p considered modal for Cassidinae *s.l*. and biarmed chromosome. The seven species exhibited easily identified sex chromosomes due to their smallest size in the complement. The analysis of meiotic cells of all the species showed pachytenes with a positively heteropycnotic block, corresponding to the sex chromatin; diplotenes, with a high number of bivalents with two chiasmata and sex chromosomes in a parachute configuration, and metaphases II that confirmed the chromosomal morphology, the type of SCS, and the regular segregation of all chromosomes. The number and morphology of the chromosomes, their

behavior during meiosis, and type of SCS were described for the first time for six species of the seven examined. Considering the cytogenetic data here obtained and those of Cassidinae *s.l*. species already described, the high autosome number observed in certain species could be explained through centric fission followed by pericentric inversion. On the other hand, the low diploid number in other species might have occurred by pericentric inversion followed by centric fusion. In both events, biarmed chromosomes and Xy_p type SCS were maintained in most species. The occurrence of other types of SCS, simple or multiple, in certain Cassidinae *s.l*. species, seems to have been consequence of the y_p chromosome loss, centric fission of the y_p chromosome, translocation between sex chromosome and autosome, centric fission of the X_p sex chromosome and subsequent translocation with autosome, giving rise to more complexes multiple SCS. Taking into account the phylogenetic and cytogenetic data of Cassidinae *s.l.* species, the karyotype differentiation of this group seems to have occurred from the basal karyotype of Polyphaga by decrease of the chromosome number and subsequent increase of this number. Pericentric inversion, centric fusion and fission seem to have been the main mechanisms that promoted the evolution of the autosomes. However, in the sex chromosome evolution, the mechanisms involved were centric fission and/or chromosomal translocation.

Keywords: Bivalent; Chiasma; Evolution; Meiosis; Sex chromosome system

3. INTRODUÇÃO

Coleoptera é a maior ordem dos insetos e contém aproximadamente 40% das espécies conhecidas da classe Insecta, sendo que mais de 357.899 já foram descritas taxonomicamente (COSTA, 2003).

A subordem Polyphaga inclui 90% das espécies de Coleoptera, distribuídas em 148 famílias, e possui a maior diversidade, tanto morfológica como biológica (COSTA, 2003). Dentro dessa subordem, encontra-se a família Chrysomelidae que corresponde a segunda maior quanto ao número de espécies de Coleoptera e é caracterizada por uma extensa variação cariotípica (VIRKKI, 1963; DE VAIO; POSTIGLIONI, 1974; STOLAR; BIDAU, 1997). De acordo com Seeno e Wilcox (1982), essa família inclui 35.000 espécies distribuídas em 16 subfamílias e segundo Chaboo (2007), nessa família estão agrupadas 37.000 espécies pertencentes a 19 subfamílias. Dessas subfamílias identificadas taxonomicamente, apenas 14 possuem espécies que foram analisadas sob o ponto de vista citogenético (PETITPIERRE et al.,1988). A maioria das espécies dessa família é considerada praga de plantas cultivadas, como, por exemplo, *Diabrotica speciosa* (Germar, 1984), que se alimenta de folhas do feijoeiro e soja (MARQUES; ÁVILA; POSTALIPARRA, 1999).

Dentro de Chrysomelidae, encontra-se a subfamília Cassidinae. Esse grupo apresenta um amplo histórico taxonômico quanto ao agrupamento de suas espécies, tendo sido considerado como duas subfamílias, Cassidinae e Hispinae (SEENO; WILCOX, 1982), como família, Cassidiformes, Cassidiadae, Cassididae, Hispidae (STEPHENS, 1829; WESTWOOD, 1920; CHEN, 1964), como superfamília, Cassidoidea (CHEN, 1973), como tribos de Clytrinae, Cassidini e Hispini (SUZUKI, 1988), e por fim com base em análises filogenéticas obtidas através de caracteres morfológicos (CHABOO, 2007) e moleculares (GÓMEZ-ZURITA et al., 2008), os representantes foram todos colocados em uma única subfamília, Cassidinae *sensu lato* (*s.l.*) que engloba as espécies dos antigos grupos Cassidinae e Hispinae, atualmente designados

como Cassidinae *sensu stricto* (*s.str*.) e Hispinae *sensu stricto* (*s.str*.) (CHABOO, 2007).

Os representantes de Cassidinae *s.l*. formam um grupo composto por 43 tribos, 324 gêneros e 6.000 espécies (CHABOO, 2007). Dessas espécies, apenas 101 têm registro sobre características cromossômicas.

As espécies dessa subfamília têm uma distribuição quase que global, embora haja uma maior diversidade nos trópicos, especialmente na América do Sul, sejam menos freqüentes em regiões temperadas da América do Norte e Austrália e mais abundantes em regiões temperadas da Eurásia (BOROWIEC, 2007). Essas espécies possuem um corpo com formato variável, podendo ser alongado e estreito ou curto e arredondado, algumas vezes, coberto por espinhos, uma coloração metálica, e algumas espécies são capazes de mudar rapidamente a sua cor quando perturbadas. O élitro é protuberante, semelhante ao casco de tartaruga, motivo pelo qual esses besouros são denominados “tortoise” e não são bons voadores. Muitas espécies são importantes pragas de grãos e algumas outras têm sido usadas para controle biológico de ervas daninhas (ALEGRE; PETITPIERRE, 1984; BOOTH; COX; MADGE, 1990).

Os coleópteros representantes de Cassidinae *s.l.* vivem em plantas das famílias Boraginaceae, Bignoniaceae, Asteraceae, Convolvulaceae, Solanaceae, além de associarem-se com outras plantas, provavelmente não hospedeiras (BUZZI, 1988; VIRKKI; SANTIAGO-BLAY; RILEY, 1992). As espécies *Charidotella (s.str.) sexpunctata* e *Deloyla guttata* são pragas de batata-doce (VIRKKI; SANTIAGO-BLAY; RILEY, 1992).

Citogeneticamente, os Cassidinae *s.l*. foram caracterizados como grupos portadores de um cariótipo altamente conservado, pois a maioria de suas espécies possui $2n=18=16+Xy_p$, com cromossomos meta-submetacêntricos (DE VAIO; POSTIGLIONI, 1974; BOROWIEC, 1999). Possivelmente, essa homogeneidade cariotípica deve estar relacionada com o baixo número de espécies analisadas. Assim, estudos cariológicos em um número maior de espécies seguramente ampliarão as informações quanto ao número e morfologia cromossômica, tipos de sistema cromossômico sexual, ocorrência de rearranjos cromossômicos, e conseqüentemente, auxiliarão no

entendimento da evolução cariotípica que poderia ter se processado nesse grupo.

4. REVISÃO BIBLIOGRÁFICA

O provável cariótipo basal para os Polyphaga, segundo Smith e Virkki (1978), é composto por 2n=20 cromossomos, com 9 pares de autossomos e 1 par de cromossomos sexuais que constitui o sistema cromossômico sexual do tipo Xy_p.

De acordo com Smith e Virkki (1978), o aumento do número cromossômico em algumas espécies e a predominância de cromossomos metacêntricos na maioria das espécies de Polyphaga parecem indicar que, de modo geral, a evolução cromossômica nesse grupo ocorreu por fissões cêntricas, seguidas por inversões pericêntricas ou por adição de material heterocromático constitutivo. Entretanto, a evolução cariotípica dos Cassidinae *s.l.* parece ter ocorrido principalmente por fusões autossômicas (SMITH; VIRKKI, 1978), considerando que a maioria das espécies dessa subfamília exibiu 2n menor que 20 cromossomos. O número diplóide e o tipo de sistema cromossômico sexual que prevalece em Cassidinae *s.l.* é $2n=18=16+Xy_p$, que foi proposto como fórmula modal para a subfamília (VIRKKI; SANTIAGO-BLAY; RILEY, 1992). Quando se compara essa fórmula com a considerada basal para Polyphaga, $2n=20=18+Xy_p$, verifica-se que existem apenas 11 espécies que conservaram as características basais dos Polyphaga, isto é, número diplóide, tipo de sistema cromossômico sexual, e morfologia cromossômica; porém, espécies que apresentam número diplóide abaixo de 2n=20 aparecem com uma maior freqüência, principalmente nas tribos Aspidimorphini, Basiprionotini, Cassidini, Chalepini, e Hispini. Na maioria dos casos em que as espécies possuem 2n menor que 20, o sistema cromossômico sexual do tipo Xy_p e a morfologia cromossômica metacêntrica foram mantidos. No entanto, espécies com número cromossômico maior que 2n=20 também foram encontradas, principalmente na tribo Stolaini; mas, em uma freqüência menor. Nesse último caso, o sistema cromossômico sexual pode ser mantido ou evolutivamente derivado, originando outros sistemas, do tipo simples ou múltiplo, e cromossomos telo-acrocêntricos podem ocorrer em número variável. Os maiores números cromossômicos de Cassidinae *s.l.* ocorrem em algumas

espécies do gênero *Botanochara* que podem apresentar até 2n=51 cromossomos e que geralmente inclui um tipo de sistema cromossômico sexual múltiplo e complexo (Tabela 1). Além disso, a morfologia da maioria dos autossomos é telo-acrocêntrica, sugerindo um possível processo de fissão cêntrica, seguido por inversão pericêntrica, e fissões adicionais em alguns autossomos (DE VAIO; POSTIGLIONI, 1974).

Alguns grupos de Cassidinae *s.l.* possuem certa uniformidade em relação ao número diplóide e ao tipo de sistema cromossômico sexual, como, por exemplo, a tribo Aspidimorphini (2n=18=16+Xy_p) e os gêneros *Chelymorpha* (2n=22=20+Xy_p) e *Chalepus* (2n=18=16+Xy_p). Por outro lado, em outros gêneros essas características cariotípicas são bastante variáveis, como em *Botanochara* (2n=27=24+neo$X_p$neoyy_p a 2n=51=48+$X_p$neoXneoY_p) e *Cassida* (2n=18=16+Xy_p a 2n=38=36+neoXY).

Entretanto, os números cromossômicos extremos nesse grupo são 2n=16=14+Xy_p em *Notosacantha maculipennis*, *Polyconia caroli*, *Platypria hystrix*, e outras espécies, e 2n=51=48+$X_p$neoXneoY_p em *Botanochara angulata* (DE VAIO; POSTIGLIONI, 1974; ALEGRE; PETITPIERRE, 1984; PETITPIERRE et al., 1988). Além do sistema cromossômico sexual do tipo Xy_p, em Cassidinae *s.l.*, outros tipos de sistemas também ocorrem, como Xy, X0, Xy_c, Xy_r, neoXY, Xyy_p, $X_p$neoXneoy_p, $X_p$neoXneoY_p, neo$X_p$neoyy_p, neoX_{p1}neoX_{p2}neoXneoY_p, neoX_{p1}neoX_{p2}neoXneoY e $X_{p1}X_{p2}$neoXneoY (DE VAIO; POSTIGLIONI, 1974; YADAV; PILLAI, 1975; SMITH; VIRKKI, 1978; PANZERA; MAZZELLA; DE VAIO, 1983; MAZZELLA; PANZERA, 1983; VIDAL, 1984; PETITPIERRE, 1985; DEY, 1986; POSTIGLIONI et al., 1990; VIRKKI; SANTIAGO-BLAY; RILEY, 1992; STOLAR; BIDAU, 1997).

O provável cariótipo ancestral de Chrysomelidae pode ter sido composto por 11 pares autossômicos metacêntricos e mais o X e o Y. A redução no número de autossomos de Cassidinae *s.l.* em relação às demais subfamílias de Chrysomelidae pode ter ocorrido por meio de fusões cêntricas (VIRKKI, 1984; PETITPIERRE et al., 1988).

Os estudos citogenéticos realizados em Cassidinae *s.l.* têm demonstrado que as espécies dessa subfamília são cromossomicamente mais conservadas que outras espécies relacionadas de Chrysomelidae, como aquelas de

Alticinae, Galerucinae e Chrysomelinae (ALEGRE; PETITPIERRE, 1984) que possuem número diplóide e sistema cromossômico sexual extremamente variáveis.

O sistema cromossômico sexual do tipo Xy_p é o mais freqüente entre os Coleoptera e está presente na maioria das famílias da subordem Polyphaga. Esse sistema é considerado ancestral para a ordem, pois ocorre em representantes de famílias basais, como Hydrophilidae e Scarabaeidae, e derivadas, como Lucanidae e Geotrupidae (SMITH; VIRKKI, 1978; KUKALOVÁ-PECK; LAWRENCE, 1993). Na subfamília Cassidinae *s.l.*, esse tipo de sistema é encontrado em aproximadamente 87% das espécies estudadas citogeneticamente.

O cromossomo sexual X nesse sistema é geralmente um metacêntrico de tamanho médio, o cromossomo y é um metacêntrico extremamente pequeno e o “p” representa a configuração assumida por esses cromossomos durante o processo de associação meiótica, a qual é semelhante a um pára-quedas. Sendo assim, o X representaria o pára-quedas, enquanto o y, a carga, e o espaço entre os cromossomos e as estruturas que conectam o X ao y pode ser preenchido por material nucleolar (STEVENS, 1906).

Existem diversos mecanismos que asseguram a associação e a segregação dos cromossomos Xy_p na meiose; porém, esses parecem variar dependendo da espécie considerada, podendo ocorrer por emparelhamento de segmentos terminais, como em algumas espécies de Chrysomelidae (SMITH, 1950; WHITE, 1973), através da ocorrência de material nucleolar, verificada em algumas espécies de Chrysomelidae e Tenebrionidae (STEVENS, 1906; JOHN; LEWIS, 1960), por associação não específica de regiões heterocromáticas constitutivas, detectada em algumas espécies de Chrysomelidae e Coccinellidae (DRETS et al. 1983; POSTIGLIONI; STOLL; BRUM-ZORRILA, 1991), ou, ainda, através da presença de material argentofílico não relacionado à região organizadora de nucléolo, encontrado em algumas espécies de Curculionidae (VIRKKI; MAZZELLA; DENTON, 1991).

O sistema Xy_c é muito raro, sendo encontrado em algumas famílias como Chrysomelidae, Cicindelidae e Curculionidae (SMITH; VIRKKI, 1978). Na subfamília Cassidinae *s.l.*, esse tipo de sistema sexual foi registrado somente

na espécie *Hilarocassis exclamationis*. Nesse tipo de sistema cromossômico sexual, a associação entre os cromossomos sexuais possivelmente ocorre através de uma extremidade do Y com as duas do X; o cromossomo X adquire assim a configuração de um V invertido. Essa configuração peculiar foi observada em uma espécie de Curculionidae por Takenouchi (1970), sem a presença de quiasma ou de nucléolo entre os cromossomos sexuais. Nas espécies das outras famílias, o tipo de associação entre os cromossomos sexuais não foi descrito.

O sistema Xy_r, observado em algumas espécies de Chrysomelidae, Coccinellidae, Meloidae, Scarabaeidae e Tenebrionidae, poderia estar relacionado com processos de heterocromatinização e inversões de regiões eucromáticas dos cromossomos sexuais pertencentes a um sistema neoXY. Nesse sistema, os cromossomos sexuais durante a meiose associam-se distalmente por um braço, através de um quiasma, formando uma haste (PIZA, 1958; WHITE, 1973; SMITH; VIRKKI, 1978; BISOI; PATNAIK, 1988; MARTINS, 1994). Este sistema foi encontrado em uma população de *Laccoptera (Laccopteroidea) nepalensis* (Cassidinae *s.l.*) do nordeste da Índia (DEY, 1986). No entanto, em uma outra população dessa mesma espécie, da região oeste da Índia, os sistemas encontrados foram o Xy_p e o Xyy_p (SHARMA; SOOD, 1978), mostrando que nessa espécie o sistema cromossômico sexual não se encontra estabelecido.

Outro sistema de determinação sexual encontrado em Cassidinae *s.l.* é o do tipo $X_p$neoXneoY_p, isto é, X_1X_2Y. Esse sistema foi também descrito em outras espécies de Coleoptera, como, por exemplo, naquelas pertencentes à Scolytidae, Tenebrionidae e Elateridae (DE VAIO; POSTIGLIONI, 1974; SMITH; VIRKKI, 1978; VIRKKI; FLORES; ESCUDERO, 1984). Dentro de Cassidinae *s.l.,* esse tipo de sistema somente foi encontrado em algumas espécies do gênero *Botanochara* (DE VAIO; POSTIGLIONI, 1974). O sistema $X_p$neoXneoY_p tem origem a partir do sistema Xy_p, através de uma translocação recíproca entre um segmento do cromossomo y_p e um autossomo com um braço totalmente heterocromático, originando o neo Y_p; o cromossomo X_p permanece intacto e o homólogo autossômico não fusionado é denominado neo X (DE VAIO; POSTIGLIONI, 1974; VIRKKI, 1980). Outros tipos de sistema

sexual múltiplo, como, neo$X_p$neoyy_p, neoX_{p1}neoX_{p2}neoXneoY_p, e $X_{p1}X_{p2}$eoXneoY também ocorrem nesse gênero e podem estar relacionados a translocações ou fusões entre cromossomos sexuais e autossomos (SMITH; VIRKKI, 1978; POSTIGLIONI, 1994).

5. OBJETIVOS

Considerando que atualmente existe uma proposta de filogenia, baseada em caracteres morfológicos, estabelecida para 92 espécies de Cassidinae *s.l.,* que a maior diversidade quanto ao número de espécies dessa subfamília ocorre na região Neotropical, que existem informações citogenéticas para apenas 2% das espécies de Cassidinae *s.l.* descritas taxonomicamente, e que das 101 espécies cromossomicamente já caracterizadas, apenas 10% pertencem à região Neotropical, esse trabalho teve como objetivo analisar citogeneticamente sete espécies da fauna brasileira e estabelecer os mecanismos envolvidos na evolução cromossômica das espécies pertencentes a essa subfamília. Para tal, foi necessário estudar os cromossomos mitóticos e meióticos com coloração convencional para:

a. verificar o número cromossômico diplóide e haplóide;

b. identificar o tipo de sistema cromossômico sexual;

c. descrever a morfologia cromossômica;

d. analisar o comportamento dos cromossomos, principalmente dos sexuais, durante a meiose;

e. comparar os resultados obtidos com aqueles descritos na literatura para espécies relacionadas, para tentar estabelecer a evolução cromossômica entre as espécies.

6. MATERIAL E MÉTODOS

6.1. MATERIAL

As sete espécies de Cassidinae *s.l.* estudadas nesse trabalho (Figura no anexo) foram *Agroiconota inedita* (1 embrião e 2 machos de Rio Claro, SP); *Charidotella (s.str.) immaculata* (9 machos de Rio Claro, SP e 1 de Avaré, SP); *Charidotella (s.str.) sexpunctata (*4 machos de Rio Claro, SP); *Cteisella confusa (*5 machos de Nonoai, RS e 1 de Ponta Grossa, PR); *Deloyala cruciata* (3 machos de Avaré, SP), *Metriona elatior (*4 machos de Rio Claro, SP), e *Stolas chalybaea* (3 machos de Nonoai, RS e 1 de Ponta Grossa, PR) (Tabela 2).

A identificação taxonômica das espécies foi feita pela MSc Flávia Rodrigues Fernandes e pela Dra. Cleide Costa, do Museu de Zoologia, da Universidade de São Paulo (USP), São Paulo, SP. Os exemplares analisados foram depositados na coleção de insetos do Museu acima mencionado.

6.2. MÉTODOS

As preparações citológicas, para o estudo de cromossomos mitóticos e meióticos, foram obtidas de embriões e gônadas de indivíduos adultos. De um total de 15 embriões que foram processados para obtenção de preparações cromossômicas, apenas 1 mostrou resultados satisfatórios quanto ao grau de condensação e espalhamento dos cromossomos, bem como quanto a quantidade de células com número de cromossomos completo. De todas as espécies analisadas, as preparações obtidas de ovário não mostraram células em divisão. Para a obtenção dessas preparações citológicas, utilizaram-se dois métodos descritos a seguir:

6.2.1. Sem a utilização de colchicina

As gônadas foram dissecadas em solução fisiológica para insetos (128,3 mM de NaCl, 16,7 mM de Na_2HPO_4 e 19,9 mM de KH_2PO_4), e colocadas em uma placa de Petri contendo solução hipotônica (água de torneira), durante 1 minuto e posteriormente, transferidas para uma placa de Petri com fixador Carnoy I (metanol e ácido acético – 3:1) durante 1 hora. Em seguida, essas

foram maceradas sobre uma lâmina, juntamente com uma gota de ácido acético 45 %. A lâmina foi seca em uma placa de metal à temperatura de 35° a 40° C. As preparações cromossômicas obtidas foram estocadas no freezer à –20° C. Para efetuar a coloração convencional com Giemsa, essas preparações foram retiradas do freezer e deixadas à temperatura ambiente por 24 horas.

6.2.2. Com a utilização de colchicina

Os embriões e as gônadas foram dissecados em solução fisiológica para insetos e transferidos para um recipiente contendo solução de colchicina 0,05% (em solução fisiológica para insetos), durante 2 horas. Um volume de água de torneira semelhante àquele da solução de colchicina foi adicionado ao material, deixando-o em repouso durante 20 minutos. Após, adicionou-se 10 gotas de fixador Carnoy I (3 metanol : 1 ácido acético) ao material, deixando-o em repouso por 1 minuto e, em seguida, este foi transferido para fixador Carnoy I recém-preparado e deixado durante 1 hora. O material foi macerado sobre uma lâmina, juntamente com uma gota de ácido acético 45%. A lâmina foi seca em uma placa de metal à temperatura de 35° a 40° C. As preparações cromossômicas obtidas foram estocadas no freezer à –20° C. Para efetuar a coloração convencional com Giemsa, essas preparações foram retiradas do freezer e deixadas à temperatura ambiente por 24 horas.

6.2.3. Coloração convencional

As preparações cromossômicas foram coradas 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 15 minutos, e em seguida, foram lavadas em água destilada e secas ao ar.

6.2.4. Análises cromossômicas

O estudo dos cromossomos corados convencionalmente foi realizado em microscópio 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 Copex HDP 13 (AGFA), revelado em D72 (1:1 em água), por 6 minutos, à temperatura de 18º C. As ampliações fotográficas foram feitas em papel Kodabromide RC-F3 (Kodak) e reveladas em D72 (1:2 em água). Alternativamente, para registrar os resultados obtidos, foi utilizado um fotomicroscópio de captura de imagens Olympus BX51, com objetiva 100 de imersão, optovar 1.25 e filtro LBD, utilizando o software DP Controller. A morfologia dos cromossomos foi determinada de acordo com a nomenclatura proposta por Levan et al. (1964).

7. RESULTADOS E DISCUSSÃO

Os resultados obtidos através da análise citogenética de sete espécies de Cassidinae *s.l*. são apresentados na forma de um artigo científico.

Mechanisms of karyotype differentiation in Cassidinae *sensu lato* (Coleoptera, Polyphaga, Chrysomelidae) based on seven species of the Brazilian fauna and an overview of the cytogenetic data

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ABSTRACT

Among the subfamilies of Chrysomelidae, Cassidinae *sensu lato* (*s.l.*) includes 6 000 species distributed in 43 tribes. Approximately 100 of these species were cytogenetically analyzed and most of them presented 2n=18=16+Xy_p, which was smaller than 2n=20=18+Xy_p considered basal for Polyphaga. However, some groups of species presented maintenance of the basal diploid number and others showed increase of this number. Certain species of the last group also exhibited variation in the type of sex chromosome system (SCS). Considering the recent taxonomic revision accomplished for the Cassidinae *s.l.* species, the existence of phylogenetic relationship for some species of this subfamily, the high diversity of species of this group in the Neotropical region, and the low number of Cassidinae *s.l.* species karyotyped so far, the aim of the present work was to establish the main mechanisms involved in the karyotype evolution of this subfamily through the study of seven species of the Brazilian fauna and overview of the cytogenetic data. The individuals were collected in southeast and south of Brazil. The chromosomal preparations obtained from embryo and testes of adult males were stained with Giemsa solution. The species *Agroiconota inedita* (2n=42=40+Xy_p), *Charidotella (s.str.) immaculata* (2n=22=20+Xy_p), *Charidotella (s.str.) sexpunctata* (2n=22=20+Xy_p), and *Stolas chalybaea* (2n=24=22+Xy_p) revealed diploid number higher than that established as basal for Polyphaga and biarmed chromosomes. The karyotype of *Cteisella confusa, Deloyala cruciata,* and *Metriona elatior* showed the chromosomal formulae 2n=18=16+Xy_p considered modal for Cassidinae *s.l.* and biarmed chromosomes. The seven species exhibited easily identified sex chromosomes due to their size and/or morphology. The analysis of meiotic cells of all the species showed pachytenes with a positively heteropycnotic block probably corresponding to the sex chromosomes; diplotenes with a high number of bivalents with two chiasmata and sex chromosomes in a parachute configuration, and metaphases II that confirmed the chromosomal morphology, the type of SCS, and the regular segregation of all chromosomes. The data regarding to the number and morphology of the chromosomes, their behaviour during meiosis, and type of

SCS were inedited for the majority of the species. In relation to the all Cassidinae *s.l.* species that presented SCS of the Xy_p type, *A. inedita* was that with the highest diploid number. Furthermore, this work reported for the first time the cytogenetic information of representatives of *Cteisella* and *Metriona*. Taking into account the phylogenetic and cytogenetic data of Cassidinae *s.l*. species, the karyotype differentiation of this group seems to have occurred from the basal karyotype of Polyphaga by decrease of the chromosome number and subsequent increase of this number. Pericentric inversion, centric fusion and fission seem to have been the main mechanisms that promoted the evolution of the autosomes. However, in the sex chromosome evolution, the mechanisms involved were centric fission and/or chromosomal translocation.

Keywords: Bivalent; Chiasma; Evolution; Meiosis; Sex chromosome system

1. Introduction

Chrysomelidae is the second most numerous family within Coleoptera, including more than 35 000 described species that are distributed in 16 subfamilies (Seeno and Wilcox, 1982). Among these subfamilies, 14 have representatives that were cytogenetically analyzed, whose karyotype information were compiled by Petitpierre et al. (1988). Most of these subfamilies have very interesting cytogenetic characteristics. One of them refers to the chromosome number, which can be extremely diversified in Galerucinae, 2n♂=8=6+Xy to 2n♂=64=60+X_1+X_2Y+B, due to the variation in the number of autosomes and/or presence of supernumerary chromosomes, or higher than that considered basal for Polyphaga, 2n♂=20=18+Xy_p, in Cryptocephalinae. Other feature is related to the types of sex chromosome system (SCS), with preponderance of Xy_r system in Chryptocephalinae, X0 system in Chrysomelinae, X+y and diversified multiple systems in Galerucinae. These characteristics indicate that different trends of karyotypic differentiation occur in these subfamilies. Moreover, the knowledge of these particularities is extremely important to establish the mechanisms involved in the species karyotypic differentiation during the evolutionary process.

Among the subfamilies of Chrysomelidae, Cassidinae *sensu lato* (*s.l.*) comprises ca 6 000 species taxonomically described that are distributed in 43 tribes (Chaboo, 2007). According to this last author, Cassidinae *s.l.* includes species originally placed by Seeno and Wilcox (1982) in the subfamilies Cassidinae and Hispinae, currently designated as Cassidinae *sensu stricto* (*s.str*.) and Hispinae *sensu stricto (s.str*.), respectively. Thus, Cassidinae *s.l.* is constituted of Cassidinae *s.str*. and Hispinae *s.str*. Of the species of Cassidinae *s.l.* known, only 101 belonging to 13 tribes were investigated about the cytogenetic point of view. The karyotype data of cassidines mainly refer to the diploid number that varied from 2n♂=16 to 2n♂=51, and types of SCS that can be Xy_p, Xy, X0, Xy_c, Xy_r, neoXY, XY, Xyy_p, neoX_{p1}neoX_{p2}neoXneoY_p, and others multiples, existing few records about chromosomal morphology (Table 1). Nevertheless, the majority of the species of Cassidinae *s.l*. surprisingly showed a diploid number smaller than that considered basal for Polyphaga, and maintenance of the Xy_p system. These data indicate that probably the karyotype

differentiation in Cassidinae *s.l.* occurred mainly by alteration in the autosome number. On the other hand, all the karyotyped species of the tribe Stolaini of this subfamily (Table 1) showed a diploid number higher than the basal, 2n=20=18+Xy_p, and additionally, the species of *Botanochara* exhibited different types of multiple SCS. Certainly, the cytogenetic analysis of species that belong to different genera of Cassidinae *s.l.* will provide additional information concerning to the mechanisms involved in the karyotype evolution of related species.

Considering that the taxonomic position of the Cassidinae *s.l*. species was recently revised by Chaboo (2007) and Gómez-Zurita et al. (2008), there is a phylogenetic relationship for 92 species of this group, only 10% of the karyotyped species of this subfamily belonged to the Neotropical region, in spite of its high diversity in this region (Chaboo, 2007), and the lack of an evolutionary approach concerning to the variation of the chromosome number and morphology as well as types of SCS among their species, the aim of the present work was to establish the main mechanisms responsible for the chromosomal evolution of Cassidinae *s.l*. by means of analysis of seven species of the Brazilian fauna and overview of the cytogenetic data of this subfamily.

2. Material and methods

The Cassidinae *s.l.* species examined in this work were *Agroiconota inedita*, *Charidotella (s.str.) immaculata*, *Charidotella (s.str.) sexpunctata, Cteisella confusa*, *Deloyala cruciata*, *Metriona elatior,* and *Stolas chalybaea*. The number of individuals investigated and their respective collection sites are in the Table 2. The voucher individuals were deposited in the Museu de Zoologia, Universidade de São Paulo, USP, São Paulo, SP, Brazil. Cytological preparations of mitotic and meiotic chromosomes were obtained from embryo and testes of adult individuals, according to the technique described by Webb et al. (1978). For study of the chromosome behaviour during meiosis, some of the testes cytological preparations were made without colchicine treatment. The chromosomal preparations were conventionally stained with a 3% Giemsa solution (47 ml of distilled water, 1.5 ml of commercial Giemsa, and 1.5 ml of

phosphate buffer pH 6.8) for 15 minutes, at room temperature, and analyzed in a Zeiss light microscope. The mitotic and meiotic cells were photographed in an Olympus BX51 photomicroscope, using the software DP Controller, or Zeiss photomicroscope, using the Copex HDP 13 (AGFA) film. The chromosomal morphology was determined according to the nomenclature proposed by Levan et al. (1964).

3. Results

3.1. Tribe Cassidini

The study of mitotic metaphases of embryo and male *A. inedita* showed $2n=42=40+Xy_p$. The karyotype was formed by autosomal pairs that slightly decreased in size, with exception of the 20th pair that exhibited a remarkable small size (Fig. 1A). The chromosome pairs 2, 4, 6, 7, 9, 10, 12, 14, 15, 16, 17, and 18 were metacentric, pairs 1, 8, 13, and 19, submetacentric, and pairs 3, 5, and 11, subtelocentric. Due to its minute size, the morphology of the last autosomal pair was not identified. The metacentric X_p sex chromosome had a similar size regarding to the 17th and 18th pair, whereas the y_p chromosome, also metacentric, exhibited a slightly larger size than that of autosomal pair 20.

Pachytene cells exhibited autosomal bivalents typically paired and a positively heteropycnotic block, probably formed by the sex bivalent (Fig. 1B). Late prophase I and metaphase I cells showed $20II+Xy_p$ and the sex chromosomes associated in a typical parachute configuration. In most diplotene cells, up to three ring shaped bivalents with two terminal chiasmata were noticed. Other bivalents demonstrated one terminal or interstitial chiasma, showing a rod or cross shape, respectively (Fig. 1C). In spermatocytes I, the y_p sex chromosome presented negative heteropycnosis from diplotene to metaphase. Cells in metaphase II revealed the haploid complement $n=20+X_p$ (Fig. 1D) or $n=20+y_p$, confirming the regular chromosomal segregation in the precedent anaphase I.

The analysis of testicular cells of *Ch. (s.str.) immaculata* (Fig. 2A) and *Ch. (s.str.) sexpunctata* (Fig. 2B) revealed $2n=22=20+Xy_p$ in spermatogonial metaphases. In these species, all the autosome pairs were metacentric,

gradually decreasing in size. The X_p chromosome of *Ch. (s.str.) immaculata* was metacentric and similar in size to that of the last autosomal pair of the complement. The y_p chromosome was the smallest metacentric of the karyotype (Fig. 2A). The metacentric X_p chromosome of *Ch. (s.str.) sexpunctata* was smaller than the chromosome pair 10 and the y_p chromosome, whose morphology was not determined due to its extremely small size, showed negative heteropycnosis in some cells (Fig. 2B). One of the homologous of the 2nd pair of *Ch. (s.str.) immaculata* exhibited a secondary constriction in the short arm interstitial region (Fig. 2A).

Pachytene cells had 10 autosomal bivalents typically synapsed and one positively heteropycnotic block probably formed by Xy_p sex bivalent (Figs. 2C and H). Diplotene, diakinesis, and metaphase I cells demonstrated $10II+Xy_p$. In diplotene of *Ch. (s.str.) immaculata,* up to two autosomal bivalents in a ring configuration were observed, indicating the occurrence of two terminal or interstitial chiasmata; the other autosomal bivalents denoted a rod or cross shape, indicating the presence of one terminal or interstitial chiasma, respectively (Fig. 2D). Some of these cells allowed to confirm the metacentric morphology of the X_p and y_p sex chromosomes (Fig. 2E). Diplotene cells of *Ch. (s.str.) sexpunctata* exhibited up to five autosomal bivalents with two chiasmata and the others with only one terminal or interstitial chiasma (Fig. 2I). From diplotene to metaphase I, the Xy_p sex bivalent showed a typical parachute configuration. Both species revealed metaphases II with $n=10+X_p$ (Figs. 2F and J) or $n=10+y_p$ (Figs. 2G and K), indicating the regular anaphasic segregation of the chromosomes. The study of these cells also allowed to confirm the metacentric morphology of the autosomes and X_p chromosome of *Ch. (s.str.) immaculata* and *Ch. (s.str.) sexpunctata*. The y_p chromosome of this latter species showed negative heteropycnosis during meiosis I and II. Within the analyzed sample of *Ch. (s.str.) sexpunctata,* one individual showed variation regarding to the number of autosomal bivalents in 20 prophase I cells (Fig. 2L), but without alteration in the type of SCS, that is, $11II+Xy_p$. The morphology and size of the chromosomes could not be determined because mitotic metaphase and metaphase II cells were not found.

Spermatogonial cells of *Ct. confusa*, *D. cruciata,* and *M. elatior* revealed the chromosomal formulae $2n=18=16+Xy_p$ in mitotic metaphases. The karyotype of *Ct. confusa* (Fig. 3A) showed the metacentric pairs 1, 3, 5, 6, 7, and 8, and submetacentric pairs 2 and 4. In the karyotype of *D. cruciata* (Fig. 3B) and *M. elatior* (Fig. 3C), all the autosomes showed metacentric morphology. The X_p chromosome in *Ct. confusa* and *D. cruciata* was metacentric and in *M. elatior* (Fig. 3D) was subtelocentric. The morphology of the y_p chromosome of the first two species was not identified due to its extremely small size, whereas in *M. elatior* (Fig. 3D) this chromosome was metacentric. The autosomes in the three species gradually decreased in size and sex chromosomes were easily identified because they were the smallest of the complement. The y_p chromosome appeared negatively heteropycnotic in some cells of *Ct. confusa* and *M. elatior*. In mitotic cells, a secondary constriction in the long arm interstitial region of the 1st pair of *Ct. confusa* (Fig. 3A) and 4th pair of *M. elatior* (Fig. 3E) was observed.

Pachytene cells of the three species showed 8 autosomal bivalents typically associated and one positively heteropycnotic block related to the sex bivalent (Figs. 3F, J and N). Spermatocytes I in diplotene and metaphase exhibited $8II+Xy_p$ and sex bivalent in a parachute configuration. Furthermore, the y_p chromosome of *Ct. confusa* and *M. elatior* was negatively heteropycnotic in most meiotic cells (Figs. 3G and O). In diplotene of *Ct. confusa* (Fig. 3G), *D. cruciata* (Fig. 3K), and *M. elatior* (Fig. 3O), up to five, four, and six biquiasmatic autosomal bivalents were respectively observed. The other bivalents revealed one terminal or interstitial chiasma. Spermatocytes in metaphase II showed $n=8+X_p$ (Figs. 3H, L and P) or $n=8+y_p$ (Figs. 3I, 3M and 3Q), indicating regular anaphasic segregation of all chromosomes.

3.2. Tribe Stolaini

The study of spermatogonial metaphases of *S. chalybaea* revealed the chromosomal formulae $2n=24=22+Xy_p$ with all autosomes and y_p chromosome of the metacentric type, and submetacentric X_p chromosome (Fig. 4A). The autosomes gradually decreased in size and sex chromosomes were the smallest of the complement. Moreover, a prominent secondary constriction in

the long arm interstitial region of the 4th pair was observed in some mitotic metaphases (Fig. 4B).

In pachytene, 11 autosomal bivalents typically paired and a positively heteropycnotic block probably formed by the sex bivalent were found. The diplotene exhibited 11II+Xy_p and sex chromosomes associated in a parachute configuration. These cells also showed up to five autosomal bivalents with two terminal chiasmata and others with one terminal or interstitial chiasma (Fig. 4C). Cells in metaphase II revealed n=11+X_p (Fig. 4D) or n=11+y_p (Fig. 4E), confirming the regular anaphasic segregation of the chromosomes. From diplotene to metaphase II, the y_p sex chromosome was negatively heteropycnotic.

4. Discussion

Among the cytogenetically analyzed species of Cassidinae *s.l*. (Table 1), 45% showed 2n♂=18=16+Xy_p, mainly in the tribes Aspidimorphini, Basiprionitini, Cassidini, Physonotini, Anisoderini, Chalepini, and Sceloenoplini; 29% denoted a high variability and noticeable increase in the chromosome number, that is, 2n♂=22=20+Xy_p to 2n♂=51=48+$X_p$neoXneoY_p, in Cassidini, Goniocheniini, Stolaini, Leptispini, and Uroplatini; 11% revealed 2n♂=16=14+Xy_p, in Cassidini, Notosacanthini, Chalepini, and Hispini; 10% showed 2n♂=20=18+Xy_p, in Cassidini, Physonotini, and Uroplatini. The remaining 5% of the species exhibited 2n♂=18 without description or with variation in the type of SCS. In almost all the species, the chromosomes were predominantly meta-submetacentric, with exception of certain species of *Botanochara* (Stolaini), which had the majority of the chromosomes of the telo-acrocentric type.

The presence of 2n♂=20=18+Xy_p in representatives of Cassidinae *s.l.* indicates that the karyotype differentiation among the species of this group occurred from the basal karyotype proposed by Smith and Virkki (1978) for Polyphaga. Taking into account this proposal, chromosomal rearrangements of the pericentric inversion type followed by centric fusion seem to be responsible for the autosome number reduction of the Cassidinae *s.l.* species, giving rise to

2n=18 or 2n=16, with SCS of the Xy_p type and meta-submetacentric morphology of most chromosomes. On the other hand, chromosomal rearrangements of the centric fission type followed by pericentric inversion could explain the increase of the autosome number in some species of Cassidinae *s.l.,* originating chromosome numbers higher than 2n=20, with SCS of the Xy_p type and meta-submetacentric morphology of most chromosomes.

Despite the majority of the Cassidinae *s.l.* genera have few species cytogenetically characterized, some singularities concerning to the distribution of chromosome number were noticed. In *Aspidimorpha* and *Dactylispa*, all the species denoted diploid number smaller, in *Charidotella*, *Chelymorpha,* and *Stolas*, all the representatives exhibited chromosome number greater, whereas in *Chalepus* and *Gratiana*, the species showed either the same or smaller chromosome number, and in *Cassida*, the species revealed chromosome number which was the same, smaller or greater than 2n=20. In *Botanochara* species, besides the remarkable increase in the autosome number, there was a considerable variability in the SCS, occurring different types of multiple system. This information seems to indicate that closely related species of Cassidinae *s.l.* possess a similar pattern of chromosomal evolution. The cytogenetic analysis of a greater number of species per genus could confirm this supposition.

Considering the types of SCS in Cassidinae *s.l*., in addition to the Xy_p type that was recorded in the majority of its species, the neoXY, Xy, and XY types were also found. However, these last types occurred in an extremely low frequency among the species analyzed up to now. Other types, such as X0, Xyy_p, Xy_r, and Xy_c were observed; but, these represented intraspecific variations and most of them occurred as an intrapopulational variability with the Xy_p type. The Xy type as an established condition in some species also appeared as an intraspecific variation in relation to Xy_p in other representatives. These variations, involving different types of SCS and Xy_p type, reinforce the hypothesis that these different types of system had their origin from Xy_p system that was suggested as a basal condition for Polyphaga species by Smith and Virkki (1978). Moreover, different types of multiple SCS were described for *Botanochara* species (Table 1). However, some of them were not yet established in certain species, considering their intraspecific and inter and/or

intrapopulational variability. The main mechanisms involved in the origin of different types of SCS in relation to the Xy_p system in Cassidinae *s.l*. were disappearance of the y_p chromosome and/or translocation between X_p chromosome and autosome in the cases of simple SCS, and centric fission of the original sex chromosomes and/or translocation between sex chromosomes and autosomes in multiple SCS.

Among the Cassidinae *s.l.* species, the variability of the chromosome number occurs not only by chromosomal rearrangements of the centric fusion or fission type but also by the presence of supernumerary chromosomes. The occurrence of such chromosomes was not very frequent, having only been registered in three species, *Botanochara* sp. (Stolaini) with $2n=30=26+X_{p1}X_{p2}neoXneoY/2n=32=26+X_{p1}X_{p2}neoXneoY+2B$, *Cassida varians* (Cassidini) with $2n=18=16+Xy_p/2n=19=16+Xy_p+1B$, and *Laccoptera (Laccopteroidea) nepalensis* (Aspidimorphini) with $2n=18=16+Xy_r/2n=19=16+Xy_r+1B$ (Sharma and Sood, 1978; Panzera et al., 1983; Dey, 1986). In all these species, the supernumerary chromosome(s) behaved as univalent during meiosis and its metacentric morphology was only determined in *Cassida varians,* in which this chromosome was the largest of the complement. In the other two species, its size was similar to or smaller than that of the y_p chromosome. Most probably the origin of the B chromosome was from the regular set of the complement and according to Hewitt (1973), the presence of supernumerary is more tolerated by the species in the polysomic state if it arose from sex chromosomes. B chromosome can alter the chiasma frequency (Hewitt and John, 1967; Fontana and Vickery, 1975; Hewitt, 1975; Westerman and Dempsey, 1977), the expression of certain genes (Cabrero et al., 1986), the segregation of regular chromosomes during anaphase (Stephens and Bregman, 1972), and the processes or characters associated with vigour, fertility, and fecundity (Camacho et al., 2000). Its effects upon their carriers may have a different biological significance, that is, the supernumerary chromosomes can cause negative influence or beneficial effects or even be near neutral on host fitness (Camacho et al., 2000). Thus, it can affect the species evolutionary process or can have minor evolutionary significance. In the

Cassidinae *s.l.* species, there is no information concerning to the origin, frequency, and effects of the supernumerary.

Among the Cassidinae *s.l*. species cytogenetically characterized so far that showed the Xy_p type SCS (Table 1), *A. inedita* with 2n=42=40+Xy_p exhibited the highest diploid number. Additionally, besides the occurrence of meta-submetacentric chromosomes, subtelocentrics were also observed. In this species, the karyotype differentiation must have occurred by chromosomal rearrangements of the centric fission type followed by pericentric inversion and/or addition of constitutive heterochromatic material, involving all autosomes; but these events were not sufficient to reach 2n=42, taking into account the modal chromosome number for Cassidinae *s.l.* species, 2n=18=16+Xy_p, and additional centric fission in two more autosomal pairs followed or not by pericentric inversion probably have occurred. The chromosome number found in *A. inedita* differed of that observed by Petitpierre et al. (1988) and Virkki et al. (1992) in *Agroiconota propinqua* that showed 2n=38=36+Xy_p.

In *Ct. confusa*, *D. cruciata,* and *M. elatior*, the chromosome number, the type of SCS, and the meta-submetacentric autosomal morphology, that is, 2n=18=16+Xy_p were in agreement with the characteristics described for most Cassidinae *s.l.* species. The karyotype evolution of this group of species in relation to the basal for Polyphaga, 2n=20=18+Xy_p, involved two autosomal pairs, which underwent chromosomal rearrangements of the pericentric inversion type followed by centric fusion. Cytogenetic data of the genera *Cteisella* and *Metriona* representatives were described for the first time in this work. The karyotype of *D. cruciata* was the same to that found by Smith (1960) for *Deloyala guttata*. Nevertheless, this latter species exhibited intraspecific and intrapopulational variation regarding to the diploid number (2n=20=18+Xy_p) in the individuals examined by Virkki et al. (1992) and type of SCS (2n=18=16+Xy) in the sample analyzed by Nowlin (1906).

In *Ch. (s.str.) immaculata*, *Ch. (s.str.) sexpunctata*, and *S. chalybaea*, in spite of the chromosome number being higher than that considered modal for Cassidinae *s.l.*, 2n=22=20+Xy_p, 2n=22=20+Xy_p, and 2n=24=22+Xy_p, respectively, the metacentric morphology of the autosomes and the Xy_p type

SCS were conserved. The karyotype evolution in this group of species occurred by centric fission of two autosomal pairs in the species of *Charidotella* and three autosomal pairs in *S. chalybaea* followed by pericentric inversion and/or addition of constitutive heterochromatic material. Although the small sample of analyzed cells in the individual, the $2n=22=20+Xy_p/2n=24=22+Xy_p$ intrapopulational variation observed in *Ch. (s.str.) sexpunctata* could be consequence of chromosomal rearrangement of the centric fission type of one autosomal pair, but presence of one pair of supernumerary chromosomes can not be excluded. Intraspecific variation in the autosome number, without alteration of the type of SCS, is not a rare event among the cassidines since it has been reported in nine species.

The data obtained in the majority of the males of *Ch. (s.str.) sexpunctata* were coincident with those previously described by Nowlin (1906), Smith (1960), and Virkki et al. (1992), regarding to the diploid number and type of SCS. Nevertheless, the metacentric morphology of the autosomes and X_p sex chromosome was described for the first time in the present work. The diploid number found in *Ch. (s.str.) immaculata* and in most individuals of *Ch. (s.str.) sexpunctata* differed of that reported for *Charidotella (s.str.) quadrisignata*, $2n=24=22+Xy_p$, by Virkki et al. (1992).

In *S. chalybaea,* $2n=24=22+Xy_p$, the diploid number and type of SCS were in agreement with those described by Vidal (1984) for *Stolas festiva*. However, *S. chalybaea* revealed a diploid number that was different of that reported by Mazzella and Panzera (1983) and Panzera et al. (1983) for *Stolas lacordairei,* $2n=30=28+Xy_p$. Moreover, *S. chalybaea* exhibited a submetacentric X_p chromosome and *Stolas lacordairei* showed a metacentric X_p chromosome.

The X_p chromosome of almost all the species studied in this work also revealed the metacentric morphology, except the subtelocentric X_p chromosome of *M. elatior* and the submetacentric of *S. chalybaea*. Discrepant submetacentric morphology of the X_p chromosome was also found in other Cassidinae *s.l*. species (Table 1), such as *Aspidimorpha (s.str.) dorsata* and *Conchyloctenia (s.str.) nigrovittata* (Aspidimorphini) both with 2n=16=14+Xy (Yadav et al., 1995), and *Chelymorpha varians* (Stolaini) with $2n=22=20+Xy_p$ (De Vaio and Postiglioni, 1974). The occurrence of chromosomal

rearrangements of the pericentric inversion type and/or alteration in the quantity of constitutive heterochromatin could have originated X_p chromosome with morphology different of that metacentric.

The metacentric morphology of the y_p chromosome of *A. inedita*, *Ch. (s.str.) immaculata*, *M. elatior,* and *S. chalybaea* was in accordance with that of *Stolas lacordairei* (Stolaini) (Mazzella and Panzera, 1983; Panzera et al., 1983), *Cassida circumdata* (Yadav and Pillai, 1975), *Cassida varians* (Cassidini) (Sharma and Sood, 1978, 1979), and *Chelymorpha indigesta* (Stolaini) (De Vaio and Postiglioni, 1974). It is worth to emphasize that the majority of the Cassidinae *s.l.* species that possessed some information about chromosomal morphology, in general, this did not include a y_p chromosome characterization, probably due to its extremely small size.

The seven analyzed species showed a karyotype with chromosomes that gradually decreased in size, as those described for some species of Cassidini, *Cassida deflorata, Cassida vibex* (Petitpierre, 1977), and *Chirida* sp. (Yadav and Pillai, 1975), and Hispini, *Dicladispa occator, Dicladispa testacea, Hispa atra,* and *Polyconia caroli* (Alegre and Petitpierre, 1984). Concerning to other Cassidinae *s.l.* species, there is no data about this aspect. In relation to the size of the X_p chromosome of all species investigated in this work, it was smaller or slightly greater than the last autosomal pair. This characteristic was in agreement with that found by Sharma and Sood (1979) for *Cassida varians,* Yadav et al*.* (1995) for *Cassida vibex* (Cassidini), Alegre and Petitpierre (1984) for *Dicladispa occator*, *Dicladispa testacea*, *Hispa atra*, and *Polyconia caroli* (Hispini), and Yadav and Pillai (1974) for *Uroplata girardi* (Uroplatini). However, the X_p chromosome size was variable among the Cassidinae *s.l.* species, that is, this was similar to that of pair 2 in *Episticnina viridemaculata* (Basiprionotini) (Yadav et al., 1995), pair 3 in *Aspidimorpha dorsata* (Yadav et al., 1995) and *Aspidimorpha indica* (Aspidimorphini) (Dua and Kacker, 1977), pairs 3 and 4 in *Cassida circumdata* (Yadav et al., 1995)*,* pair 4 in *Cassida deflorata* (Cassidini) (Petitpierre, 1977), pair 6 in *Aspidimorpha miliaris* (Aspidimorphini) (Dua and Kacker, 1977), and it was the largest of the complement in *Cassida vibex* (Cassidini) (Petitpierre, 1977). Thus, in Cassidinae s.l., there is not a pattern regarding to the X_p chromosome size and this variability could occur among

species of the same genus and could be consequence of alteration in the quantity of constitutive heterochromatin in this chromosome. In all the Cassidinae *s.l*. species that showed SCS of the Xy_p type, the y_p chromosome was tiny. For the other Cassidinae *s.l.* species, besides those above mentioned, there was no information about the chromosome size.

In the karyotype of *A. inedita, Ch. (s. str.) sexpunctata*, and *D. cruciata,* the presence of a secondary constriction was not detected. On the other hand, in one of the elements of pair 2 of *Ch. (s.str.) immaculata*, in pair 1 of *Ct. confusa,* and in pair 4 of *M. elatior* and *S. chalybaea*, a prominent constriction was observed and this could be related to the nucleolar organizer region, as that registered in pair 5 of one other Cassidinae *s.l*. species, *Chelymorpha varians* (Stolaini) (Postiglioni et al., 1990), or correspond to a region with highly repeated base sequences.

In most analyzed species, the cytogenetic characteristics that stood out in diplotene phases were the presence of a high number of bivalents with two chiasmata and the negative heteropycnosis of the y_p chromosome. The high number of biquiasmatic bivalents seems to represent a singular characteristic among the Cassidinae *s.l*. species, taking into account that this characteristic was also found in species that belong to different tribes, such as *Chalepus sanguinicollis* (Chalepini) with $2n=18=16+Xy_p$, *Chelymorpha cribraria* (Stolaini) with $2n=22=20+Xy_p$, and *Octotoma scabripennis* (Uroplatini) with $2n=20=18+Xy_p$ that showed up to three biquiasmatic bivalents (Yadav and Pillai, 1974; Virkki et al., 1992). On the other hand, this feature is not exclusive of the Cassidinae *s.l*. species, having also been found in some Elateridae (Schneider et al., 2006, 2007) and Lampyridae species (Dias et al., 2007). However, according to Smith and Virkki (1978), the majority of the Coleoptera species revealed bivalents with just one chiasma. In relation to the negative heteropycnosis of the y_p chromosome, this characteristic was also noticed in mitotic metaphases and metaphases I of *Stolas lacordairei* (Stolaini) with $2n=30=28+Xy_p$ (Mazzella and Panzera, 1983). Negatively heteropycnotic y_p chromosome was recorded in some Coleoptera species that belong to different groups, such as *Nodostoma haroldi* of the subfamily Eumolpinae (Gill et al., 1987) and *Astylus variegatus* of the family Melyridae (Schneider et al., 2007).

However, in certain species of subfamilies phylogenetically related to Cassidinae *s.l.* (Gómez-Zurita et al., 2008), such as Eumolpinae and Galerucinae, and others of Curculionidae and Tenebrionidae, the y_p chromosome showed to be positively heteropycnotic (Smith, 1952; Virkki, 1964). This heteropycnotic variability probably is related to the chromatin nature that constitutes the y_p chromosome.

Even though the Cassidinae *s.l.* cytogenetic information are scarce, the insertion of the available karyotype data in the phylogenetic relationship proposed by Chaboo (2007) (Fig. 5) indicates that the chromosomal evolution occurred by decrease of the diploid number with conservation of the Xy_p type SCS and subsequent increase of this number with SCS that can be or not gradually converted from Xy_p into X0, neoXY, Xyy_p, $neoX_pneoyy_p$, $X_pneoXneoy_p$, or other multiple types. This hypothesis was based on the fact that diploid number smaller than 2n=20 was recorded in certain phylogenetically basal species, such as *Estigmena chinensis* (Anisoderini) and *Sceloenopla mantecada* (Sceloenoplini). Additionally, clades that possess greater number of representatives cytogenetically analyzed, such as (*Baliosus* (*Anoplites* (*Microrhopala* + *Octotoma*))), the basal species exhibited diploid number smaller than those derived.

Considering the phylogenetic and cytogenetic data of Cassidinae *s.l.* species, the karyotype differentiation in this group probably occurred by decrease and subsequent increase of the autosome number and appearance of different types of SCS. The mechanisms that caused such differentiation were not the same for the autosomes and sex chromosomes. Pericentric inversion followed by centric fusion, and later, centric fission followed by pericentric inversion seem to have been the main mechanisms that promoted the evolution of the autosomes. On the other hand, in the sex chromosomes evolution, the mechanisms involved were centric fission followed by translocation with autosome or just translocation between one of the sex chromosomes and autosome.

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Figures legends

Fig. 1. Embryo (A) and testicular germ line cells (B-D) of *Agroiconota inedita* conventionally stained with Giemsa. (A) Male mitotic karyotype with 2n=42=40+Xy_p. (B) Pachytene, exhibiting a positively heteropycnotic chromatin block, probably corresponding to the Xy_p sex chromosomes (empty arrow). (C) Diplotene with 20II+$Xy_{p,}$ showing autosomal bivalents with two chiasmata (large arrow) or one chiasma (small arrow). (D) Metaphase II with n=20+X_p. Scale bar=10 µm.

Fig. 2. Male germ cells of *Charidotella (s.str.) immaculata* (A and C-G) and *Charidotella (s.str.) sexpunctata* (B and H-L), conventionally stained with Giemsa. (A and B) Spermatogonial karyotypes with 2n=22=20+Xy_p, showing in (A) a secondary constriction in the short arm of one of the 2nd pair homologous (arrowhead). (C and H) Pachytenes with a positively heteropycnotic block, probably corresponding to the sex chromosomes. (D, E and I) Diplotenes with 10II+Xy_p, showing autosomal bivalents with two chiasmata (large arrow) or one chiasma (small arrow). Note in (E) the metacentric morphology of the sex chromosomes. (F and J) Metaphases II with n=10+$X_{p.}$ (G and K) Metaphases II with n=10+$y_{p.}$ (L) Diplotene cell of an individual with 11II+Xy_p, bearing an additional autosomal bivalent. Scale bar=10 µm.

Fig. 3. Gonadal cells of male *Cteisella confusa* (A and F-I), *Deloyala cruciata* (B and J-M), and *Metriona elatior* (C-E and M-Q) conventionally stained with Giemsa. (A-C) Mitotic karyotypes with 2n=18=16+Xy_p, exhibiting in (A) a secondary constriction in the 1st pair long arm (arrowhead). (D) Sex pair, emphasizing the subtelocentric and metacentric morphology of the X_p and y_p chromosomes, respectively. (E) The 4th autosomal pair, exhibiting a secondary constriction in the long arm (arrowhead). (F, J and N) Pachytenes with one positively heteropycnotic block that probably was related to the sex bivalent. (G, K and O) Diplotenes with 8II+Xy_p, showing autosomal bivalents with two chiasmata (large arrow) or one chiasma (small arrow). (H, L and P) Metaphases

II with n=8+$X_{p.}$ (I, M and Q) Metaphases II with n=8+y_p. Scale bars: (A-C and F-Q)=10 µm; (D and E)=5 µm.

Fig. 4. Male germ cells of *Stolas chalybaea*, conventionally stained with Giemsa. (A) Mitotic karyotype with 2n=24=22+$Xy_{p.}$ (B) The 4th autosomal pair, exhibiting a secondary constriction in the long arm (arrowhead). (C) Diplotene with 11II+Xy_p, showing some autosomal bivalents with two chiasmata (large arrow) and the others, with one interstitial or terminal chiasma (small arrows); note the sex chromosomes associated in a parachute configuration. (D) Metaphase II with n=11+$X_{p.}$ (E) Metaphase II with n=11+y_p. Scale bar=5 µm.

Fig. 5. Phylogenetic relationship of the subfamily Cassidinae sensu lato (modified from Chaboo, 2007) and karyotype information of the species or other representatives of the same genus (*) cytogenetically characterized (see Table 1). Group names in black are monophyletic and those in gray are paraphyletic.

Table 1 – Species of Cassidinae *sensu lato* (Chaboo, 2007) cytogenetically analyzed, with their respective different designation of that considered currently valid (DD), diploid number (2n) and types of sex chromosome system (SCS) in males, chromosomal morphology (CM), and provenance (P), not determined chromosomal morphology (?).

Species	DD	2n and SCS	CM	P	References
Family Chrysomelidae					
Subfamily Cassidinae *sensu stricto*					
Tribe Aspidimorphini					
Aspidimorpha (s.str.) difformis (Motschulsky, 1860)	*Aspidomorpha difformis*	18	----------	Japan	Takenouchi and Shiitsu (1972)
Aspidimorpha (s.str.) dorsata (Fabricius, 1787)	*Aspidomorpha dorsata*	16=14+Xy	12M+2A+XSM	India	Yadav et al. (1995)
A. (s.str.) dorsata	*A. dorsata*	18=16+Xy_p	----------	India	Yadav et al. (1987)
Aspidimorpha (s.str.) furcata (Thunberg, 1789)	*Aspidomorpha furcata*	18=16+Xy_p	10M-SM+6A+X(?)+y(?)	India	Dasgupta (1973) and Manna and Lahiri (1972)
Aspidimorpha (s.str.) indica Boheman, 1854	*Aspidomorpha indica*	18=16+Xy_p	----------	India	Dua and Kacker (1975, 1976)
A. indica	*A. indica*	18=16+Xy	16M+XM	India	Dua and Kacker (1977)
Aspidimorpha (s.str.) miliaris (Fabricius,1775)	*Aspidomorpha miliaris*	18=16+Xy_p	----------	India	Manna and Lahiri (1972)
A. (s.str.) miliaris	*A. miliaris*	18=16+Xy	10M+6SM+XM	India	Dua and Kacker (1975, 1976, 1977)
*Conchyloctenia nigrovittata (*Boheman, 1854)	----------	18=16+Xy_p	16M+XM+y(?)	India	Gill et al. (1987)
C. nigrovittata	*Aspidomorpha nigrovittata*	16=14+Xy	14M+XSM	India	Yadav et al. (1995)
Laccoptera (Laccopteroidea) nepalensis Boheman, 1855	*Laccoptera 4-maculata*	18=16+Xy_p	----------	----------	Smith and Virkki (1978)
L. (Laccopteroidea) nepalensis	*Laccoptera quadrimaculata*	18=16+Xy_p/19=16+Xyy_p	----------	India (West)	Sharma and Sood (1978)
L. (Laccopteroidea) nepalensis	*L.quadrimaculata*	18=16+Xy_r/19=16+Xy_r+1B	16M-SM+XM-SM+y(?)	India (Northeast)	Dey (1986)
L.(Laccopteroidea) nepalensis	*L.quadrimaculata*	18=16+Xy_p	10M+6SM+XM+y(?)	India	Gill et al. (1987)
L.(Laccopteroidea) nepalensis	*L.quadrimaculata*	18=16+Xy_p	----------	India	Yadav et al. (1987) and Kacker (1976)
L.(Laccopteroidea) nepalensis	*L.quadrimaculata*	18=16+Xy	----------	India	Dua and Kacker (1975)
Tribe Basiprionotini					
Basiprionota decemmaculata (Boheman, 1850)	----------	18=16+Xy_p	----------	----------	Petitpierre et al. (1988)
B. decemmaculata	*Prioptera decemmaculata*	18=16+Xy_p	----------	----------	Sharma and Sood (1978)
Craspedonta leayana (Latreille,1807)	----------	18=16+Xy_p	----------	----------	Petitpierre et al. (1988)
C. leayana	*Calopepla leayana*	18=16+Xy_p	----------	----------	Yadav (1971)
Epistictina viridimaculata (Boheman, 1850)	*Epistictina vividimaculata*	18=16+Xy	----------	India	Yadav et al. (1995)
Tribe Cassidini					
Agroiconota inedita (Boheman, 1855)	----------	42=40+Xy_p	24M+8SM+6ST+2(?)+XM+yM	Brazil	Present work
Agroiconota propinqua (Boheman, 1885)	----------	38=36+Xy_p	8M+30(?)	Puerto Rico	Virkki et al. (1992)
A. propinqua	*Coptocycla propinqua*	38=36+Xy_p	----------	----------	Petitpierre et al. (1988)
Cassida algirica Lucas, 1849	----------	18	----------	Spain	Petitpierre et al. (1998)
Cassida azurea Fabricius, 1801	----------	18=16+Xy_p	----------	France	Petitpierre et al. (1998)
Cassida bergeali Bordy, 1995	----------	18=16+Xy_p	----------	France	Petitpierre et al. (1998)
Cassida circumdata Herbst, 1799	----------	18=16+Xy_p/19=16+Xyy_p	14M+2SM+XM+yM	----------	Yadav (1973) and Yadav and Pillai (1975)
C. circumdata	----------	18=16+Xy_p	----------	----------	Manna and Lahiri (1972)
C. circumdata	----------	18=16+Xy_p	10M+2SM+4A+XM+y(?)	India	Yadav et al. (1995)
Cassida deflorata Suffrian, 1844	----------	18=16+Xy_p	----------	Spain	Petitpierre (1977) and Petitpierre et al. (1998)
Cassida enervis Boheman, 1862	----------	18=16+Xy_p/19=16+Xyy_p	----------	----------	Sharma and Sood (1978)

C. enervis	----------	18=16+Xy_p	10M+6SM+XM(?)+y(?)	India	Yadav et al. (1995)
Cassida flaveola Thunberg, 1794	----------	18=16+Xy_p	----------	Spain	Petitpierre (1977)
Cassida fuscorufa Motschulsky, 1866	----------	18=16+Xy_p	----------	Japan	Takenouchi and Shiitsu (1972)
Cassida hemisphaerica Herbst, 1799	----------	20=18+Xy_p	----------	France	Petitpierre et al. (1998)
Cassida hexastigma Suffrian, 1844	----------	18=16+Xy_p	----------	France	Petitpierre et al. (1998)
Cassida inquinata Brullé, 1832	----------	18=16+Xy_p	----------	----------	Petitpierre (1985)
Cassida leucanthemi Bordy, 1995	----------	18=16+Xy_p	----------	France	Petitpierre et al. (1998)
Cassida margaritacea Schaller, 1783	----------	30=28+Xy_p	----------	Spain	Petitpierre (1977)
Cassida nebulosa Linnaeus, 1758	----------	18=16+Xy_p	----------	Japan	Takenouchi and Shiitsu (1972)
Cassida pannonica Suffrian, 1844	----------	20=18+Xy_p	----------	Austria	Petitpierre et al. (1998)
Cassida panseri Weise, 1907	----------	18=16+Xy_p	----------	France	Petitpierre et al. (1998)
Cassida prasina Illiger, 1798	----------	18=16+Xy_p	----------	----------	Petitpierre (1985)
Cassida pusilla Waltl, 1839	----------	18=16+Xy_p	----------	----------	Petitpierre (1985)
Cassida rubiginosa Müller, 1776	----------	18=16+Xy_p	----------	Japan/France	Takenouchi and Shiitsu (1972) and Petitpierre et al. (1998)
Cassida rufovirens Suffrian, 1844	----------	18=16+Xy_p	----------	France	Petitpierre et al. (1998)
Cassida sanguinolenta Müller, 1776	----------	18=16+Xy_p	----------	France	Petitpierre et al. (1998)
Cassida sanguinosa Suffrian, 1844	----------	18=16+Xy_p	----------	France	Petitpierre et al. (1998)
Cassida sp.	----------	20=18+Xy_p	----------	----------	Sharma and Sood (1978)
Cassida subreticulata Suffrian, 1844	----------	30=28+Xy_p	----------	France	Petitpierre et al. (1998)
Cassida varians Herbst, 1799	*Cassida syrtica*	18=16+Xy_p/19=16+Xy_p+1B	6M+10SM+XM+yM+BM	----------	Sharma and Sood (1978, 1979)
Cassida vibex Linnaeus, 1767	----------	20=18+Xy_p/21=18+Xyy_p	8M+8SM+2A+XM+y(?)	Spain/Indian	Petitpierre (1977) and Yadav et al. (1995)
Cassida viridis Linnaeus, 1758	----------	24=22+Xy_p	----------	Spain	Geitler (1940), Petitpierre (1985) and Petitpierre et al. (1998)
C. viridis	----------	30=28+Xy_p	----------	Spain	Petitpierre (1977, 1988) and Petitpierre et al. (1998)
Cassida vittata Villers, 1789	----------	20=18+Xy_p	----------	----------	Petitpierre (1985)
Charidotella immaculata (Olivier,1790)	----------	22=20+Xy_p	20M+XM+yM	Brazil	Present work
Charidotella (s. str.) quadrisignata (Boheman, 1855)	----------	24=22+Xy_p	----------	Puerto Rico	Virkki et al. (1992)
Charidotella (s. str.) sexpunctata (Fabricius, 1781)	----------	22=20+Xy_p	----------	Puerto Rico	Virkki et al. (1992)
C. (s. str.) sexpunctata	*Coptocycla aurichalcea*	22	Majority M-SM	----------	Nowlin (1906)
C. (s. str.) sexpunctata	*Metriona bicolor*	22=20+Xy_p	----------	----------	Smith (1960)
C. (s. str.) sexpunctata		22=20+Xy_p/24=22+Xy_p	20M+XM+y(?) n	Brazil	Present work
Chiridopsis bipunctata (Linnaeus, 1767)	*Chirida bipunctata*	18=16+Xy_p	7M-SM+10A+y(?)	India	Dasgupta (1973)
C. bipunctata	*Chiridopsis promiscua*	18=16+Xy_p	----------	India	Bisoi and Patnaik (1990)
Chirida sp.	----------	36=34+Xy_p/37=34+Xyy_p	24M+10SM+XM+y(?)	----------	Yadav and Pillai (1974, 1975)
Coptocycla (Coptocyclella) adamantina (Germar, 1824)	----------	18=16+Xy_p	----------	Argentina	Vidal (1984)
Cteisella confusa (Boheman, 1855)	----------	18=16+Xy_p	12M+4SM+XM+y(?)	Brazil	Present work
Deloyala cruciata (Linnaeus,1758)	----------	18=16+Xy_p	16M+XM+y(?)	Brazil	Present work
Deloyala guttata (Olivier, 1790)	----------	18=16+Xy_p	----------	Canada	Smith (1960)
D. guttata	----------	20=18+Xy_p	18M	Puerto Rico	Virkki et al. (1992)
D. guttata	----------	18=16+Xy	----------	----------	Nowlin (1906)
D. guttata	*Coptocycla guttata*	18	Majority M-SM	----------	Nowlin (1906)
Glyphocassis trilineata (Hope, 1831)	----------	18=16+Xy_p	14M+2ST+XM+y(?)	----------	Yadav (1973) and Yadav and Pillai (1975)
G. trilineata	----------	16=14+Xy_p	----------	India	Yadav et al. (1987)
Gratiana lutescens (Boheman, 1854)	*Nuzonia lutescens*	18=16+Xy_p	----------	----------	Vidal and Henestrosa (1985) apud Dey (1986)
Gratiana pallidula (Boheman, 1854)	*Cassida pallidula*	18=16+Xy_p	----------	USA	Smith and Virkki (1978)
G. pallidula	*Nuzonia pallidula*	18=16+Xy_p	----------	----------	Petitpierre et al. (1988)
Gratiana spadicea (Klug, 1829)	----------	20=18+Xy_p	----------	----------	Postiglioni et al. (1990)
Helocassis clavata (Fabricius, 1798)	*Plagiometriona clavata*	18=16+Xy_p	----------	----------	Smith (1960)
H. clavata	*P. clavata*	18=16+Xy	----------	----------	Smith and Virkki (1978)

Hypocassida subferruginea (Schrank, 1776)	*Cassida subferruginea*	38=36+neoXY	----------	----------	Petitpierre (1985)
Metriona elatior Klug, 1829	----------	18=16+Xy_p	16M+XST+yM	Brazil	Present work
Oocassida pudibunda (Boheman, 1856)	----------	38=36+Xy_p	----------	----------	Sharma and Sood (1978) and Sood (1978)
*Oxylepus deflexicollis (*Boheman, 1862)	----------	18=16+Xy_p	----------	Spain	Petitpierre et al. (1998)
Rhytidocassis indicola (Duvivier, 1892)	*Cassida indicola*	18=16+Xy_p	14M+2SM+XM+y(?)	----------	Yadav (1973) and Yadav and Pillai (1975)
Tribe Goniocheniini					
Chlamydocassis (s.str.) metallica (Klug, 1829)	*Clamydocassis metallica*	32=30+Xy_p	----------	Argentina	Vidal (1984)
Tribe Notosacanthini					
*Notosacantha maculipennis (*Boheman, 1856)	----------	16=14+Xy_p	----------	----------	Petitpierre et al. (1988)
N. maculipennis	*Hoplionota maculipennis*	16=14+Xy_p	----------	----------	Sood (1978)
Tribe Physonotini					
Eurypedus thoni Barber, 1946	*Eurypedus oblonga*	20=18+Xy_p	----------	Argentina	Vidal (1984)
Eurypepla jamaicensis (Linnaeus, 1758)	*Physonota (Eurypepla) jamaicensis*	18=16+Xy_p	----------	Puerto Rico	Virkki et al. (1992)
Tribe Stolaini					
Botanochara angulata (Germar, 1824)	----------	51=48+$X_p$neoXneoY_p	4M+4SM+40A+ X_pSM+neoXM+neoY_pM	----------	De Vaio and Postiglioni (1974) and Postiglioni et al. (1990)
Botanochara bonariensis (Boheman., 1850)	----------	47=44+$X_p$neoXneoy_p	10M+34T	Argentina	Stolar and Bidau (1997)
B. bonariensis	----------	27=24+neo$X_p$neoyy_p	24M-SM	Argentina	Stolar and Bidau (1997)
B. bonariensis	----------	41=38+neo$X_p$neoyy_p	14M-SM+24T	Argentina	Stolar and Bidau (1997)
B. bonariensis	----------	44=40+neoX_{p1}neoX_{p2}neoXneoY_p	----------	----------	Postiglioni et al. (1990)
B. bonariensis	----------	44=40+$X_{p1}X_{p2}$neoXneoY	4M+8SM+28T+X_1T+X_2T+neoXSM+neoYSM	Uruguay	Panzera et al. (1983)
B. bonariensis	----------	44=40+$X_{p1}X_{p2}$neoXneoY	4M+2SM+2A+32T+X_{p1}T+X_{p2}T+neoXSM+neoYSM	Uruguay	Mazzella and Panzera (1983)
Botanochara duodecimverrucata (Boheman, 1850)	----------	44=40+neoX_{p1}neoX_{p2}neoXneoY_p	----------	Uruguay	Postiglioni et al. (1990)
B. duodecimverrucata	*Botanochara 12-verrucata*	31=30+X0	----------	Argentina	Vidal (1984)
Botanochara sp.	----------	44=40+neoX_{p1}neoX_{p2}neoXneoY_p	----------	Uruguay	Postiglioni et al. (1990)
Botanochara sp.	----------	30=26+$X_{p1}X_{p2}$neoXneoY/ 32=26+$X_{p1}X_{p2}$neoXneoY+2B	20M+2SM+4T+X_{p1}T+X_{p2}T+neoXM+neoYM	Uruguay	Panzera et al. (1983)
Botanochara sp.	----------	30=26+$X_{p1}X_{p2}$neoXneoY	14M+6SM+2A+4T+neoXSM+neoYSM	Uruguay	Mazzella and Panzera (1983)
Chelymorpha cassidea (Fabricius, 1775)	----------	22=20+Xy_p	----------	----------	Stevens (1906)
Chelymorpha cribraria (Fabricius, 1775)	*Chelymorpha cinctipennis*	22=20+Xy_p	----------	Argentina	Vidal (1984)
C. cribraria	*Chelymorpha multipunctata*	22=20+Xy_p	12 or 14M	Puerto Rico	Virkki et al. (1992)
Chelymorpha indigesta (Boheman, 1854)	----------	22=20+Xy_p	14M+6SM+XM+yM	Uruguay	De Vaio and Postiglioni (1974)
Chelymorpha nigricollis Boheman, 1854	----------	22=20+Xy_p	----------	----------	Vidal (1984)
Chelymorpha varians (Blanchard, 1851)	*Chelymorpha variabilis*	22=20+Xy_p	----------	Uruguay	Postiglioni et al. (1990)
C. varians	*C. variabilis*	22=20+Xy_p	18M+XSM+y(?)	----------	De Vaio and Postiglioni (1974) and Postiglioni et al. (1991)
Hilarocassis exclamationis (Linnaeus, 1767)	----------	34=32+Xy_c	----------	Puerto Rico	Virkki et al. (1992)
H. exclamationis	*Mesomphalia exclamationis*	34=32+Xy_p	----------	----------	Dey (1986)
H. exclamationis	*Stolas exclamationis*	34=32+Xy_p	----------	----------	Petitpierre et al. (1988)
Stolas chalybaea (Germar,1824)	----------	24=22+Xy_p	22M+XSM+yM	Brazil	Present work
Stolas festiva (Klug, 1829)	----------	24=22+Xy_p	----------	Argentina	Vidal (1984)
Stolas lacordairei (Boheman, 1850)	----------	30=28+Xy_p	----------	Argentina	Vidal (1984)
S. lacordairei	----------	30=28+Xy_p	24M+2ST+1ST/1M+XM+yM	Uruguay	Panzera et al. (1983)
S. lacordairei	----------	30=28+Xy_p	24M+2ST+1A/1SM+XM+yM	Uruguay	Mazzella and Panzera (1983)

Subfamily Hispinae *sensu stricto*					
Tribe Anisoderini					
Anisodera excavata (Baly, 1858)	*Lesiochila excavata*	18=16+neoXY	----------	India	Dey (1986)
Estigmena chinensis (Hope)	----------	18=16+Xy_p	----------	----------	Yadav (1971) and Yadav (1974) apud Yadav and Pillai (1974)
Tribe Chalepini					
Baliosus californicus (Horn, 1883)	----------	18=16+Xy_p	----------	USA	Smith (1960)
Chalepus dorsalis Thunberg, 1805	----------	16=14+Xy_p	----------	----------	Stevens (1906)
Chalepus inaequalis (Weber, 1801)	*Anoplitis inaequalis*	18=16+Xy_p	----------	----------	Smith (1950)
Chalepus sanguinicollis (Linnaeus, 1771)	----------	18=16+Xy_p	----------	Puerto Rico	Virkki et al. (1992)
Chalepus sp.	----------	18=16+Xy_p	----------	----------	Smith (1960)
Odontota dorsalis (Thunberg, 1805)	*Xenochalepus dorsalis*	16=14+Xy_p	----------	----------	Stevens (1906)
Tribe Hispini					
Dactylispa atkinsoni (Gestro)	----------	16=14+Xy_p	----------	India	Kacker (1976)
Dactylispa brevispinosa (Chapuis, 1877)	----------	16=14+Xy_p	----------	----------	Sharma and Sood (1978)
Dactylispa humeralis (Weise, 1905)	----------	16=14+Xy_p	----------	----------	Sharma and Sood (1978) and Sood (1978)
Dicladispa occator (Brullé, 1838)	*Dactylispa occator*	16=14+Xy_p	16M	Spain	Alegre and Petitpierre (1984)
Dicladispa testacea (Linnaeus, 1767)	*Dactylispa testacea*	16=14+Xy_p	16M	Spain	Alegre and Petitpierre (1984)
Hispa armigera (Olivier, 1808)	*Dicladispa armigera*	12=10+Xy/18=16+Xy *	18A	Indian	Saha and Manna (1971) and Saha (1973)
Hispa atra Linnaeus, 1767	----------	16=14+Xy_p	16M	Spain	Alegre and Petitpierre (1984)
Platypria hystrix Fabricius	----------	16=14+Xy_p	----------	----------	Petitpierre et al. (1988)
Polyconia caroli (Leprieur, 1883)	----------	16=14+Xy_p	16M	Spain	Alegre and Petitpierre (1984)
Tribe Leptispini					
Leptispa filicornis (Germar, 1842)	----------	32=30+XY	Majority M, the others were A	Spain	Petitpierre and Sanchez-Font (1997)
Tribe Sceloenoplini					
Sceloenopla mantecada (Sanderson)	----------	18=16+Xy_p	16M	Puerto Rico	Virkki et al. (1992)
Tribe Uroplatini					
Microrhopala vittata (Fabricius, 1798)	----------	32=30+Xy_p	----------	----------	Smith and Virkki (1978)
Octotoma scabripennis Guérin-Meneville, 1844	----------	20=18+Xy_p	12M+6SM+XM	Australia	Yadav and Pillai (1974)
Uroplata girardi Pic, 1834	----------	20=18+Xy_p	14M+4SM+XM	Australia	Yadav and Pillai (1974)
Uroplata nigritarsis (Weise)	----------	20=18+Xy_p	Majority M	Uruguay	Petitpierre and Sanchez-Font (1997)
Not determined species	*Agroiconota aurichalcea*	22=20+Xy	----------	----------	Smith and Virkki (1978)

M: metacentric; SM: submetacentric; ST: subtelocentric; T: telocentric; A: acrocentric; B: supernumerary chromosome; *: triploid number.

Table 2 – Species of Cassidinae *sensu lato* (Chaboo, 2007) of the Brazilian fauna with their respective number of individuals analyzed per sex and collection locality.

Species	**Number of individuals**	**Collection locality**
Tribe Cassidini		
Agroiconota inedita (Boheman, 1855)	1 embryo; 2 males	Rio Claro (22°24´S, 47°33´W), SP
Charidotella immaculata (Olivier, 1790)	9 males	Rio Claro (22°24´S, 47°33´W), SP
	1 male	Avaré (23°06´S, 48°56´W), SP
Charidotella (s.str.) sexpunctata (Fabricius, 1781)	4 males	Rio Claro (22°24´S, 47°33 W), SP
Cteisella confusa (Boheman, 1855)	5 males	Nonoai (27°21'S, 52°45'W), RS
	1 male	Ponta Grossa (25°05´S, 50°09 W), PR
Deloyala cruciata (Linnaeus, 1758)	3 males	Avaré (23°06´S, 48°56´W), SP
Metriona elatior (Klug, 1820)	4 males	Rio Claro (22°24´ S, 47°33´W), SP
Tribe Stolaini		
Stolas chalybaea (Germar, 1824)	3 males	Nonoai (27°21' S, 52°45'W), RS
	1 male	Ponta Grossa (25°05´S, 50°09´W), SP

PR: state of Paraná; RS: state of Rio Grande do Sul; SP: state of São Paulo.

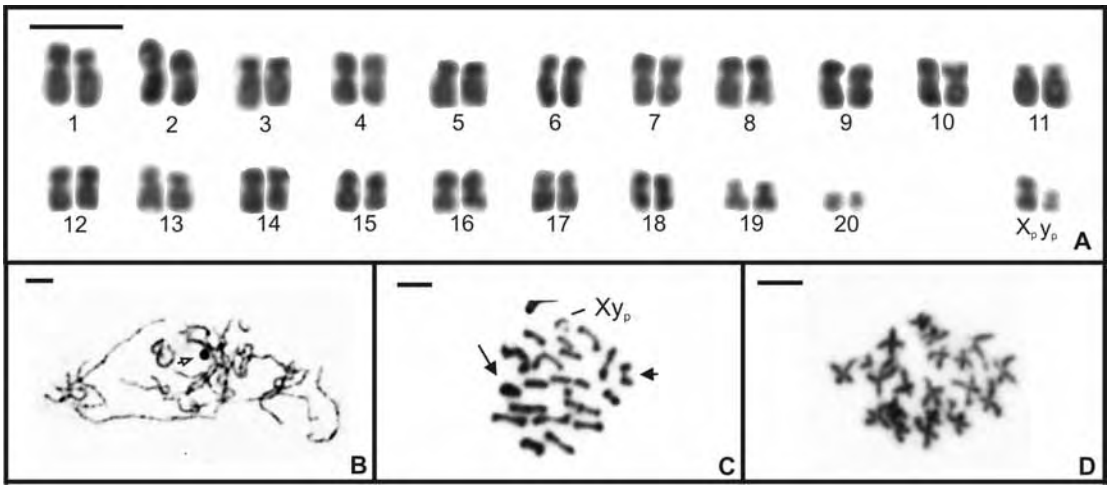

Fig.1

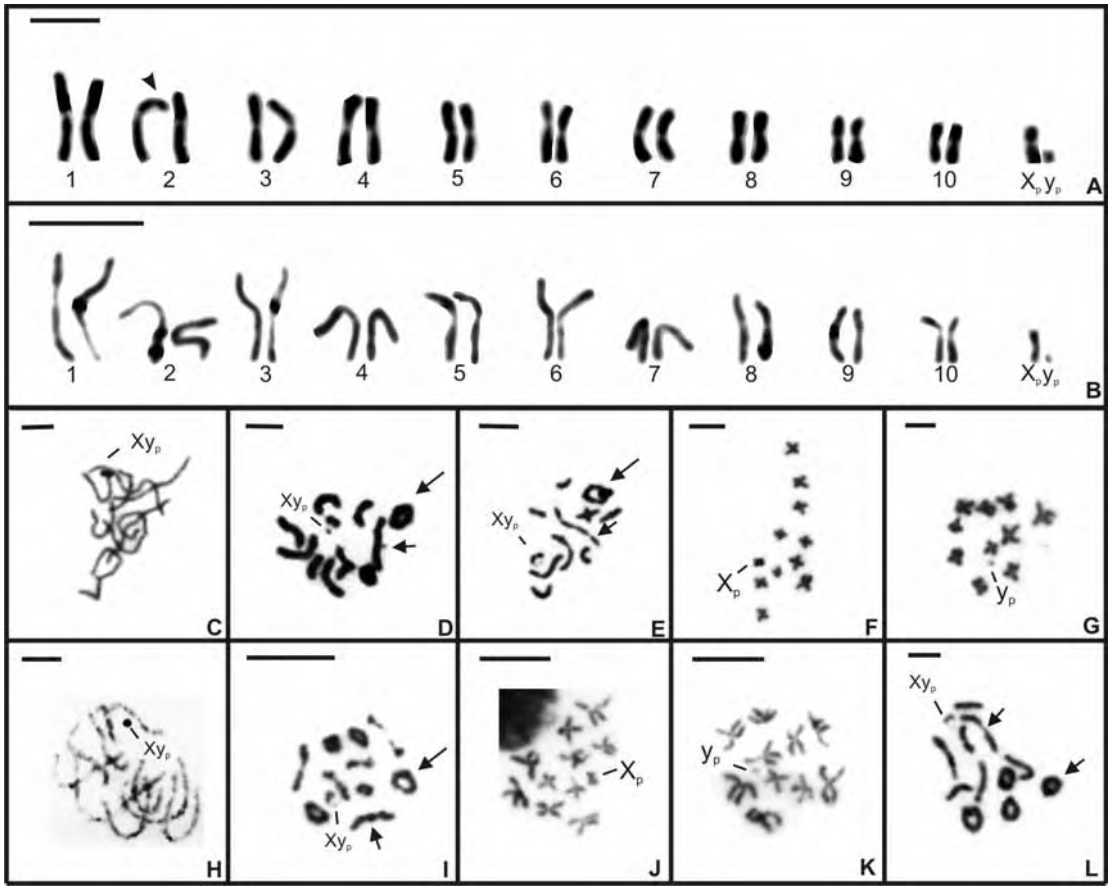

Fig.2

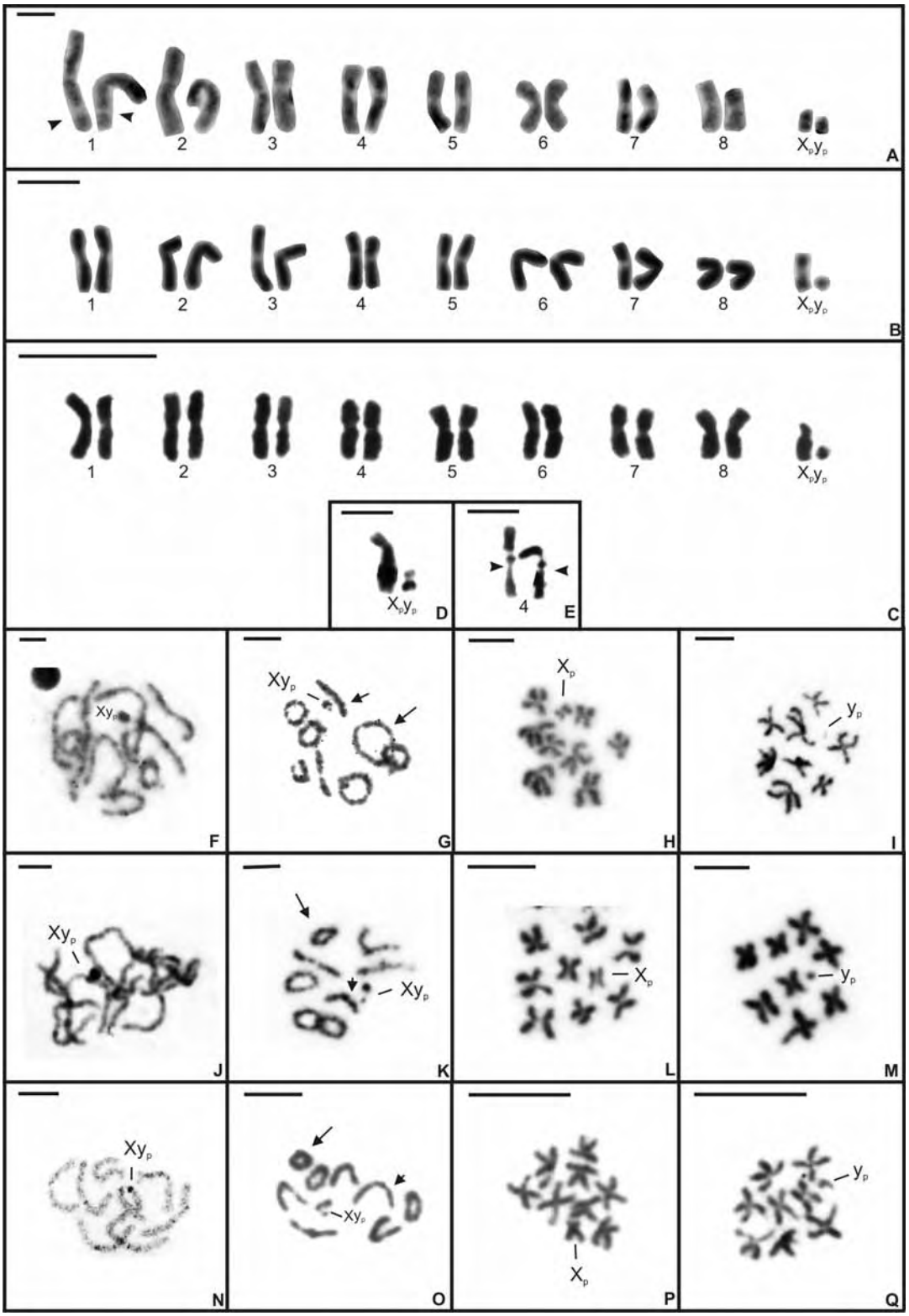

Fig.3

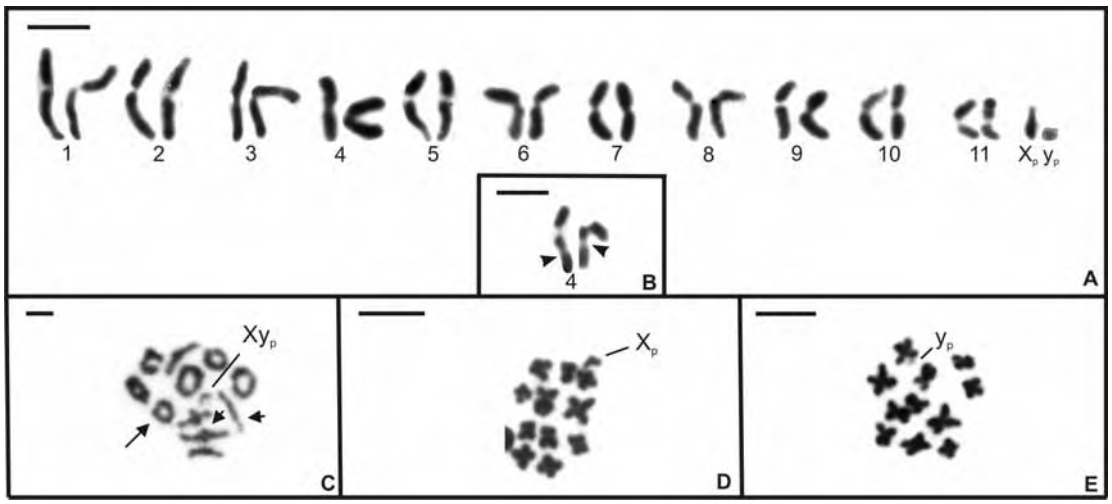

Fig.4

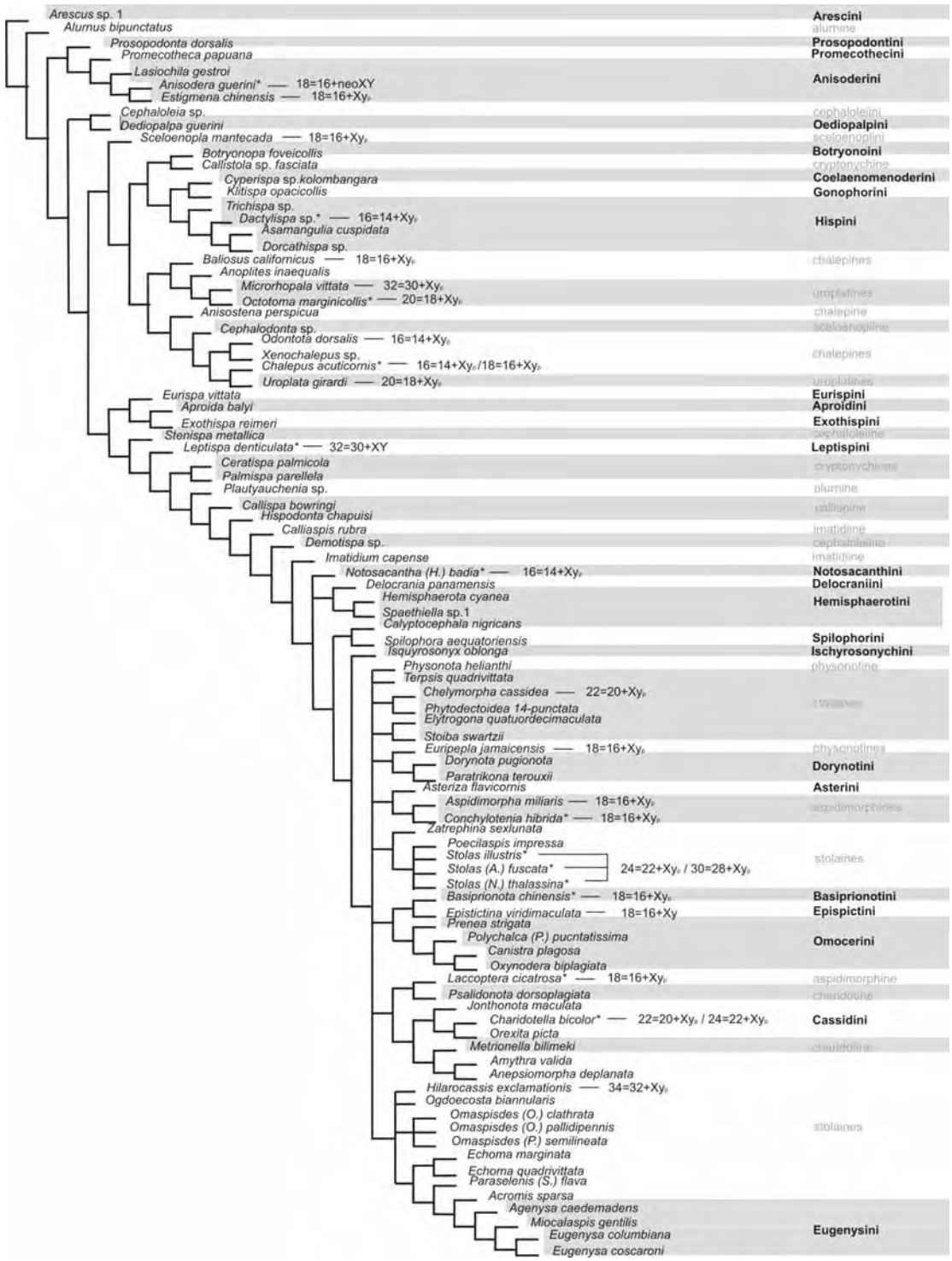
Arescus sp. 1
Alurnus bipunctatus
Prosopodonta dorsalis
Promecotheca papuana
Lasiochila gestroi
Anisodera guerini* — 18=16+neoXY
Estigmena chinensis — 18=16+Xyp
Cephaloleia sp.
Dediopalpa guerini
Sceloenopla mantecada — 18=16+Xyp
Botryonopa foveicollis
Callistola sp. fasciata
Cyperispa sp.kolombangara
Klitispa opacicollis
Trichispa sp.
Dactylispa sp.* — 16=14+Xyp
Asamangulia cuspidata
Dorcathispa sp.
Baliosus californicus — 18=16+Xyp
Anoplites inaequalis
Microrhopala vittata — 32=30+Xyp
Octotoma marginicollis* — 20=18+Xyp
Anisostena perspicua
Cephalodonta sp.
Odontota dorsalis — 16=14+Xyp
Xenochalepus sp.
Chalepus acuticornis* — 16=14+Xyp/18=16+Xyp
Uroplata girardi — 20=18+Xyp
Eurispa vittata
Aproida balyi
Exothispa reimeri
Stenispa metallica
Leptispa denticulata* — 32=30+XY
Ceratispa palmicola
Palmispa parellela
Plautyauchenia sp.
Callispa bowringi
Hispodonta chapuisi
Calliaspis rubra
Demotispa sp.
Imatidium capense
Notosacantha (H.) badia* — 16=14+Xyp
Delocrania panamensis
Hemisphaerota cyanea
Spaethiella sp.1
Calyptocephala nigricans
Spilophora aequatoriensis
Isquyrosonyx oblonga
Physonota helianthi
Terpsis quadrivittata
Chelymorpha cassidea — 22=20+Xyp
Phytodectoidea 14-punctata
Elytrogona quatuordecimaculata
Stoiba swartzii
Euripepla jamaicensis — 18=16+Xyp
Dorynota pugionota
Paratrikona terouxii
Asteriza flavicornis
Aspidimorpha miliaris — 18=16+Xyp
Conchylotenia hibrida* — 18=16+Xyp
Zatrephina sexlunata
Poecilaspis impressa
Stolas illustris*
Stolas (A.) fuscata*
Stolas (N.) thalassina*
24=22+Xyp / 30=28+Xyp
Basiprionota chinensis* — 18=16+Xyp
Epistictina viridimaculata — 18=16+Xy
Prenea strigata
Polychalca (P.) pucntatissima
Canistra plagosa
Oxynodera biplagiata
Laccoptera cicatrosa* — 18=16+Xyp
Psalidonota dorsoplagiata
Jonthonota maculata
Charidotella bicolor* — 22=20+Xyp / 24=22+Xyp
Orexita picta
Metrionella bilimeki
Amythra valida
Anepsiomorpha deplanata
Hilarocassis exclamationis — 34=32+Xyp
Ogdoecosta biannularis
Omaspisdes (O.) clathrata
Omaspisdes (O.) pallidipennis
Omaspisdes (P.) semilineata
Echoma marginata
Echoma quadrivittata
Paraselenis (S.) flava
Acromis sparsa
Agenysa caedemadens
Miocalaspis gentilis
Eugenysa columbiana
Eugenysa coscaroni
Arescini
alurnine
Prosopodontini
Promecothecini
Anisoderini
cephaloleiini
Oediopalpini
sceloenoplini
Botryonoini
cryptonychine
Coelaenomenoderini
Gonophorini
Hispini
chalepines
uroplatines
chalepine
sceloenopline
chalepines
uroplatines
Eurispini
Aproidini
Exothispini
cephaloleiine
Leptispini
cryptonychines
plumine
callispine
imatidiine
cephaloleiine
imatidiine
Notosacanthini
Delocraniini
Hemisphaerotini
Spilophorini
Ischyrosonychini
physonotine
cassines
physonotines
Dorynotini
Asterini
aspidimorphines
stolaines
Basiprionotini
Epispictini
Omocerini
aspidimorphine
chaerodine
Cassidini
chaerodine
stolaines
Eugenysini

Fig.5

8. CONSIDERAÇÕES GERAIS

Após a análise dos dados citogenéticos e da proposta de filogenia existentes para Cassidinae *s.l.,* foi possível constatar que:

- 87% das espécies apresentam SCS conservado do tipo Xy_p;
- 4 espécies das 7 examinadas mostraram constrição secundária em cromossomos de tamanho grande, como descrito na literatura para uma outra espécie de Cassidinae *s.l*., mostrando que essa pode ser uma característica conservada evolutivamente. Contudo, é necessária a análise de outras espécies para se confirmar essa proposição;
- Há um alto número de bivalentes com dois quiasmas, sendo o mínimo de um e o máximo de seis. Essa característica peculiar do grupo parece ser conservada evolutivamente;
- A análise dos cromossomos de Cassidinae *s.l*. com técnicas de marcação de regiões específicas, como aquelas de obtenção de Banda C e impregnação pelo íon prata, seguramente proporcionarão dados que também auxiliarão no entendimento sobre a evolução cariotípica desse grupo;
- As espécies que ocupam posição basal e derivada na relação filogenética de Chaboo (2007), pertencentes às tribos Arescini, Eugenysini, e Eurispini, parecem constituir amostras biológicas importantes para análise cariotípica, pois podem fornecer informações complementares sobre o processo evolutivo dos cromossomos nessa subfamília. Por outro lado, as espécies das tribos de Cassidinae *s.l.* determinadas como parafiléticas, são também interessantes do ponto de vista citogenético, pois os dados

cariotípicos talvez pudessem, adicionalmente, ser utilizados para estudo filogenético desses grupos não resolvidos.

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ANEXO

ANEXO - Indivíduos machos das espécies de Cassidinae *sensu lato* (Chaboo, 2007) da fauna brasileira estudadas nesse trabalho. (A) *Agroiconota inedita. (*B) *Charidotella (s. str.) immaculata. (*C) *Charidotella (s. str.) sexpunctata. (*D) *Cteisella confusa. (*E) *Deloyala cruciata. (*F) *Metriona elatior. (*G) *Stolas chalybaea.* Scale bar=30 mm.