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RESEARCH ARTICLE

CONSANGUINITY STUDY IN THE KNANAYA CATHOLIC CHRISTIAN COMMUNITY OF KOTTAYAM DISTRICT II. ROLE OF SOCIO-ECONOMIC CO-VARIABLES AND DEMOGRAPHIC DETERMINANTS

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SUMMARY The role of factors which influence the incidence of consanguinity in the endogamous Knanaya Catholic community of Kottayam were assessed. The major factors are, socio-economic correlates (education, occupation, family income) and demographic determinants (year of birth and marriage and spousal age at marriage). The low socio-economic classes by education registered the lowest consanguinity rate in low group while higher rates occur in medium and high classes. This implies that the converting negative association of consanguinity and literacy strongly prevalent in most known inbreeding population does not exist in the present Knanaya community. The data of demographic determinants in terms of occupation displayed a mixed trend characterised by incidence of low consanguinity rates both in the low and high class groups, while higher frequency in the middle class. The data displayed no declining trend in related consanguineous alliances in general in the younger generation population as in the case with all known population groups, and this shows that none of the demographic factors has been strongly operative resulting in a clear-cut time and temporal trend of consanguinity in the community.

Keywords: Consanguinity, Knanaya, Catholic, socio-economic, demographic factors.

INTRODUCTION

Human inbreeding or consanguinity is referred to as the phenomenon of marital alliance between spouses related to each other by common ancestry. Detrimental effects of inbreeding arise as a result of genetic homozygosis of deleterious recessive alleles often expressed as prereproductive mortality and morbid conditions of genetic predisposition (Bittles & Hussain 2000, Mathew 2017). The level of inbreeding and the magnitude of associated effects are dependent on a number of genetic and non-genetic factors. The genetic factors are primarily the nature, number and frequency of lethal/sub lethal recessive genes and duration of inbreeding, while the prominent non-genetic factors comprise socio-economic co-variables, demographic determinants, geographic factors etc. (Mathew 2018). Many castes and communities of Kerala region, belonging to diverse social classes and tribals, have been practising close kin alliances as part of their social customs, and studies carried out in them in relation to the influence of various non-genetic factors have yielded significant consanguineous related risk effects in varying degrees (Mathew 2018, Mathew & Jyothilekshmi 2017). The

Christian communities in Kerala surpassing denominational differences are averse to close kin marriages and prohibit alliances up to the second cousin degree spousal relationship by church ban. However, the Knanaya Christian of the State are a tightly endogamous community who make mate selection exclusively from within their own closed group which often leads to closer degree alliances inevitable and mean obligatory. The present study concerns the role of important non-genetic influencing factors such as socio-economic co-variables (education, occupation, family income) and demographic determinants (year of birth, year of marriage, spousal age at marriage) on the incidence and associated risk effects in the Knanaya Catholic Christians of the Kottayam district.

MATERIALS AND METHODS

The 'Knanaya' Christians are known to have existed in Kerala from the very early Christian era. They are a tightly endogamous community taking mates from their own community. There are two district denominations in the state, one belonging the Vijayaparam Catholic diocese confined to Kottayam district, which is fairly large in size and the other a smaller Jacobite faction, confined to a very few pocket areas in the state like Ranni (Pathanamthitta district), Chigavanam (Kottayam district) and a few families in the Thiruvanthapuram city. Consanguinity study was carried out in the Kottayam Knanaya group from a random sample of 565 families which constitute about 10%. Data of important socio-economic co-variables and demographic determinants were collected using a comprehensive questionnaire by door-to-door visits. The educational level was considered under three classes such as low (illiterate and up to primary school), medium (secondary, higher secondary and technical) and high (university level of arts, science, humanities and technical). Occupational level was grouped under three classes such as low (agricultural labourers, marginal farmers, skilled workers, class IV government and quasi government employees and trade service organisations), medium (school teachers, para professional, class III and II government employees) and high (professionals, executives, land lords, business personals and class I government employees). A fourth category, unemployed housewife, was also included in the case of women. The family income was grouped under four classes such as low (annual income below Rs.25000), lower middle (Rs.25000–50000), upper middle (Rs.50000–75000) and high (above Rs.75000). The age of spouses in terms of period of birth was considered under eight classes (25–30, 31–35, 36–40, 41–45, 46–50, 51–55, 56–60, 61–65, 66–70, 71 and above), and the year of marriage classified into six 10 year classes (1950-59, 1960-69, 1970-79, 1980-89, 1990-99 and above 2000), and spousal age at marriage under six classes (below 15, 15–20, 21–25, 26–30, 31–35, 36–40). Appropriate statistical methods were used to determine the significance levels of association.

OBSERVATIONS

Frequency of consanguinity by level of education was highest for the high education class in both men (47.1%) and women (51.1%) and least in the low class for both sexes (Table 1). Frequency by occupational status showed highest incidence in medium occupational class in man (41.4%) and in unemployed women (55.2%). Both men and women registered intermediate values in the high occupation class (Table 2). Consanguinity rate by family income showed highest incidence in the upper medium in consanguineous group (39.1%)

TABLE 1: Frequency of consanguinity by level of education of husband and wife in Knanayas of Kottayam district.

Spouse	Level of education	No. of marriages	Consanguinity (%)
Husband*	Low	12	13.8
	Middle	34	39.1
	High	41	47.1
	Total	565	15.4
Wife*	Low	11	12.6
	Middle	31	35.6
	High	45	51.7
	Total	565	15.4

Consanguinity effect: *Significant $p<0.05$.

TABLE 2: Frequency of consanguinity by occupation status of husband and wife in Knanayas of Kottayam district.

Spouses	Level of education	No. of marriages	Consanguinity (%)
Husband*	Unemployed	7	8.0
	Low	14	16.1
	Middle	36	41.4
	High	30	34.5
	Total	565	15.4
Wife*	Unemployed	48	55.2
	Low	5	5.7
	Middle	12	13.8
	High	22	25.3
	Total	565	15.4

Consanguinity effect: *Significant $p<0.05$.

TABLE 3: Frequency of consanguinity by family income of Knanayas of Kottayam district.

Level of family income	No. of marriages	Consanguinity (%)
LIG	3	3.4
LMIG	30	34.5
UMIG	34	39.1
HIG	20	23.0
Total	565	15.4

TABLE 4: Frequency of consanguinity by present age of husband and wife in Knanayas of Kottayam district.

Spouse	Age group (y)	No. of marriages	Consanguinity (%)
Husband	25 — 30	1	1.1
	31 — 35	2	2.3
	36 — 40	11	12.6
	41 — 45	9	10.3
	46 — 50	18	20.7
	51 — 55	15	17.2
	56 — 60	13	14.9
	61— 65	4	4.6
	above 65	14	16.1
	Total	565	15.4
Wife	25 — 30	7	8.0
	31 — 35	9	10.3
	36 — 40	17	19.5
	41 — 45	12	13.8
	46 — 50	14	16.1
	51 — 55	9	10.3
	56 — 60	6	6.9
	61— 65	6	6.9
	above 65	7	8.0
	Total	565	15.4

TABLE 5: Frequency of consanguinity by year of marriage in Knanayas of Kottayam district.

Year of marriage	Frequency	Percentage
Before 1964	3	3.4
1965 — 1974	11	12.6
1975 — 1984	8	9.2
1985 — 1994	24	27.6
1995 — 2007	30	34.5
2008 — 2017	11	12.6
Total	565	15.4

TABLE 6: Frequency of consanguinity by age at marriage of husband and wife in Knanayas of Kottayam district.

Spouse	Age at marriage	No.of marriages	Consanguinity (%)
Husband	below 15	0	0
	21 — 25	14	16.1
	26 — 30	59	67.8
	31 — 35	12	13.8
	36 — 40	2	2.3
	Total	565	15.4
Wife	below 15	0	0.0
	16 — 20	25	28.7
	21 — 25	45	51.7
	26 — 30	16	18.4
	31 — 35	1	1.1
	Total	565	15.4

followed by low medium income class, while lowest for the low-income group (Table 3). Frequency by age of spouses showed highest (20.7%) in men of 46–50 y of age groups, and least in the youngest group of 25–30 y of in both husband and wife (Table 4). The frequency by year of marriage was highest (34.5%) for the year class 1995–2007 and least in the earliest period of before 1960 (Table 5). Rate of consanguinity by spousal age at marriage was highest (67.8%) for the 26–30 y of age categories in men and for the 21–25 y of age groups in women (Table 6). In both men and women, the least frequency was noticed in the below 15 y of age group followed by the highest age group.

DISCUSSION

There are many non-genetic influencing parameters which play a notable role in consanguineous unions, which include parental inbreeding, size of the mating unit, marital distance, traditional systems of mate selection based on social, cultural, ethnic, economic, demographic, linguistic, educational, religion and geographic variables (Mathew & Jyothi-lekshmi 2017). These may vary from region to region, population to population and caste to caste. Distribution of consanguinity rates across the world shows that very low rates are prevalent in population groups of socially developed and industrial countries, while relatively high rates in more traditional and rural areas and among the illiterate and poor societies (Bittles 1998). Moreover, the ethnic, religious and tribal traditions also play a positive role, both at regional and national levels. Of the various influencing factors, the important ones are the socio-economic co-variables which importantly comprise education, occupation and family income and demographic determinants which chiefly concern year of birth and year of marriage of spouses and spousal age at marriage. Among populations in which consanguinity is preferential, high rates of blood-related marriages have consistently reported in the rural and poor sections of the society. In most communities, the measured outcomes of consanguinity mostly occur among such groups, hence, failure to control for socio-economic differentials could lead to biased and exaggerated estimates of the effects of inbreeding (Bittles 1994).

Education

Most studies in the world over have indicated the social status, defined in terms of educational level of the members of the community as having strong influence on consanguinity rates. In almost all known studies, the literacy level is negatively correlated with the rate of consanguinity (Bittles 2012, Hussain & Bittles 1998, Mathew et al. 2006, Mathew & Jyothilekshmi 2017) such that lower in literacy level, higher the rate of consanguinity and vice versa. The role of education on consanguinity rate in the Catholic Knanaya of Kottayam district was assessed by considering three literacy classes such as low, medium and high. A general increasing trend was evident with increasing literacy level. The low education class registered lower consanguinity rates in both men and women (Table1),while notably higher rates in middle and higher education classes. Good section of the Knanayas of the district are having highly educated, and this reflected in their higher social status, irrespective of whether consanguineous or non-consanguineous. Earlier studies in diverse population groups of the state displayed a consistently declining trend of consanguinity associated with increasing literacy and was a strong negative

association existed between consanguinity rates and level of literacy, particularly evident in the tribal groups (Mathew et al. 2006).

Occupation

Most studies carried out earlier for determining the role of occupation on consanguinity rates have shown evidence suggestive that the practice of close-kin alliances goes beyond social and occupational boundaries (Bittles 2012, Mathew & Jyothilekshmi 2017, Shami et al. 1991). Multivariate analysis performed in certain Muslim nations demonstrated occupational status accounting heavily on the association between consanguinity level and social status and the finding revealed occupational status as a relevant indicator of social class for the purpose of controlling potential confounding variables (Khlat 1988). Positive association of consanguinity with level of occupation was reported in many population groups globally which was interpreted as due to decision of the family to keep occupationally well-placed youth attached to their family (Bittles & Hussain 2000). Almost similar association pattern was evident in the Kerala forward communities and the elite groups of backward classes (Mathew & Jyothilekshmi 2017); and in most cases the association was highly significant. In the present Knanaya group, the occupational status was considered under four levels of classes such as low, medium, high and unemployed. The unemployed women group was found to be associated with fairly high rate (55.2%) of consanguinity (Table 2). However, in men, the association was high in the high and medium occupational class although not significant. In this community most of the people are highly educated and fairly well occupied and economically sound so much so very little disparity evident between consanguineous and non-consanguineous families.

Family income

Financial income is considered as a major indicator of the social status level, and the world data amply correlate this. Consanguineous alliance has been felt as a feasible choice for many communities to avoid financial liabilities and uncertainties which often associated with dowry and bridal payments (Bittles 2001, Mathew et al. 2006). The association between consanguinity and family income in the present Knanaya group was clearly positive, in the high and low medium classes, the former showing an edge (Table 3). The level of consanguinity was least in the low-income group (3.4%). This is contrary to the type of association known in most world population groups including the general Kerala communities in which the low family income group consistently displayed the highest consanguinity rate. This was very much evident in the tribal groups of Kerala, a glaring exception of which is evident in the Christian Mala Arya tribal group in which the upper middle-income class registered the highest inbreeding rate just as the pattern in the present Knanaya.

Demographic determinants

Many studies globally have projected significant correlation of demographic factors with consanguinity, which yielded valid clues for interpreting the ascending and descending temporal trends of consanguinity (Bittles & Black 2010). Major demographic determinants are, year of birth of spouses, year of marriage and spousal age of marriage.

Year of birth: Analysis of age of spouses of both husband and wife with consanguinity rate in the Knanaya of Kottayam district was made by pooling the data of different 10-year classes of age (Table 4). It is found that the low rates occur in

both younger and older age groups in husbands and wives, with medium rates predominate in the middle aged couples in either sex. Great bulk of the known studies including the Kerala group of communities show consistent occurrence of low rates associated with younger age. This trend has been suggested as due to better awareness of the harmful effects of related marriages among the younger people, presumably due to their higher literacy. But this factor does not seem to have much operated in the present Knanaya.

Year of marriage: Assessment of consanguinity rate by year of marriage was performed by classifying the year of marriage under six 10-year classes, and the data showed lowest frequency (3.4%) during the earliest period. But mortality lower frequency (9.2 and 12.6%) was also found in marriages of middle and recent decades (Table 5). The data thus do not show any clear trend of association, because lower rates occur in the early, middle and recent decades. While high rates occur predominantly in the middle period.

Spousal age at marriage: Spousal age at marriage is demographically important as it determines the length of marital life and fertility span of couples. Most earlier studies have reported incidence of higher rates of close-kin alliances at the low age at marriage of spouses. The relationship between rate of consanguinity and age at marriage in the present community was assessed by classifying the age cohorts under five classes in either sex (Table 6). The studies, irrespective of social class difference revealed a mixed trend. In both the husband and wife categories, highest consanguinity rate was evident in the middle age at marriage group, which is highest (67.8%) in the 26–30 y class in men and 21–25 y (51.7%) class in women. In both sexes, no marriages occur in the below 15 y age class. In almost all known studies, there is consistent difference between consanguineous and non-consanguineous groups as regards the mean age at marriage in both sexes, and clear negative correlation existed almost universally (Bittles 2012).

Time and temporal trends

A number of factors are known to have played a significant role in effecting time and temporal trends in inbreeding populations. The major factors include spousal age, period of marriage, socio-economic co-variables, demographic determinants, religion etc., of which period of marriage and spousal age are major indicators. It has been noticed that the level of consanguinity is low in socio-economically advanced and elite communities and countries. Even in countries and communities in which consanguinity is widespread, its prevalence has greatly diminished in recent periods. However, in population groups in which consanguinity is preferential, high rates continue to occur. In almost all Kerala communities, a clear and consistent declining trend was obvious (Mathew & Jyothilekshmi 2017). Various factors operate resulting in the decline from the older to younger, and from the early to recent periods. In the present Knanayas, the prevailing consanguineous marriages, period-wise, do not indicate any clear-cut declining trend (Table 5). The consanguinity incidence in periods, prior to 1964 was only 3.4%, and clearly higher rates prevalent during the middle period (1980–2007), and thereafter, a steep fall in the rate. This is apparently suggestive that none of the demographic factors which reset in clear-cut time and temporal trend has been operative in the community.

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RESEARCH ARTICLE

ANTIBACTERIAL ACTIVITY OF THREE FERN TAXA

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SUMMARY Antibacterial activity of three fern taxa, such as *Adiantum latifolium* Lam., *Christella dentata* (Forssk.) Brownsey & Jermy and *Angiopteris evecta* (G. Forst.) Hoffm was investigated. The antimicrobial substances present in these species were extracted and subjected to antimicrobial assay against five human pathogenic bacteria, *Klebsiella pneumoniae, Salmonella typhimurium, Serratia marcescens*, *Shigella flexneri* and *Listeria monocytogenes*. The antibiotic susceptibility was analysed by disc diffusion method. The ethyl acetate and ethanol extracts of fern species showed notable antibacterial effect in varying degrees and extent, of which maximum action was evident against four pathogens such as *Serratia marcescens*, *K. pneumoniae, Salmonella typhimurium* and *L. monocytogenes,* while the aqueous extract failed to show any response.

Keywords: *Adiantum*, *Christella, Angiopteris,* antibacterial activity, disc diffusion.

INTRODUCTION

The pteridophytes, which comprise both eusporangiate and leptosporangiate are considered to have existed from Palaeozoic era. They have faced many stochastic disturbances which made them to adapt to different environmental conditions (Wallace et al. 1991). They are the second largest group of plants in the world flora and contributed greatly to plant diversity and as ecological indicators. The ferns constitute a group of about 12000 species, commonly known as cryptogamic embryophytic vascular plants reproducing via spores. They are endowed with a variety of economic and medicinal properties such as source of food, medicine, fodder, biofertilizer, ornamentals and also play a major role in bioremediation of contaminated soils due to ability to remove chemical pollutants from the environment. These attributes of the ferns may be a crucial factor for their evolutionary success which enabled them to survive for millions of years (Tanzin 2013). Various workers have made systematic study of fern extracts and have reported their effect against both gram positive and gram negative bacteria (Manickam & Irudayaraj 1992, Sharma & Vyas 1985). Medicinal and therapeutic properties of many fern species have been studied and analyzed by several workers in the recent past (Anto et al. 2015, Babu et al. 2012, Kumar et al. 2011, Sandhya et al. 2015), and they are reported to have in possession of various bioactive properties such as antioxidant, antitumor, antiHIV, antimicrobial, antiinflammatory and antiviral. The present study concerns the evaluation of the medicinal properties, in terms of the anti-

microbial activities of three species of ferns such as *Adiantum latifolium* Lam., *Christella dentata* (Forssk.) Brownsey & Jermy and *Angiopteris evecta* (G. Forst.) Hoffm. They are commonly grown as ornamental plants, and majority of them are also used in traditional medicines to cure various diseases like cough, fever, skin diseases, ulcer, epilepsy, leprosy, biliousness, inflammation, tumors of spleen, liver etc.

MATERIALS AND METHODS

Adiantum latifolium is a common weed growing in shady moist places having suberect to creeping habitat with lamina usually pinnate. *C. dentata* is a small fern found growing on the margins of rainforest and also in moist open areas. *Angiopteris evecta* is commonly known as the giant fern, king fern, oriental vessel fern, mule's foot fern and elephant fern. The plant grows in the deep shade of the forest floor, usually near a stream or within a drainage channel. Fresh whole plants of these ferns were collected from the Kallimel region of Mavelikkara, Alapphuza district and also from the Botanical Garden of St. Peter's College, Kolenchery, Ernakulam district, Kerala. The plants were identified using a standard flora (Manickam & Irudayaraj 1992).

The plant samples were shade dried for about three wks. The leaves were powdered and stored in air tight sterile containers and the active components were extracted by hot solvent extraction method using a soxhlet apparatus in which 20 g of powdered samples were taken in a cloth bag and inserted into the extraction tube. Extraction was done based on polarity, using different solvents such as petroleum ether, ethyl acetate, ethanol and double distilled water for 8 h. About 200 ml of each solvent was percolated through the soxhlet apparatus. The temperature was adjusted as per the boiling temperature of each solvent. The extracts were concentrated in a rotary evaporator at 40º C and stored in air tight bottles.

The bacterial strains were procured from the CSIR National Chemical Laboratory, Pune, which was maintained as primary culture stock in the Department of Botany, St. Peter's College, Kolenchery. The bacterial strains used for the study are, *Klebsiella pneumoniae* (NCIM-2883), *Shigella flexneri* (NCIM-5265), *Serratia marcescens* (NCIM-2919), *Salmonella typhimurium* (NCIM-2501) and *Listeria monocytogenes* (NCIM-5260). These are all disease causing human pathogenic gram negative bacteria except *L. monocytogenes* , which is gram positive.

Antibacterial analysis was performed using standard disc diffusion method (Bauer et al. 1966). The nutrient agar slants were prepared by taking 2.8 g of nutrient agar in 100 ml distilled water and 6 ml of hot media was poured into the test tubes and plugged with cotton. All the five bacterial strains were inoculated by streak method on to nutrient agar slants from the stock culture. These were then transferred on to nutrient broth, and the tubes were shaken well and kept for incubation at 30º C for 16 h.

The nutrient agar plates were prepared by taking 5.6 g of nutrient agar in 200 ml distilled water in a 500 ml conical flask. About 20 ml of the agar media was poured aseptically to the autoclaved petriplates which were kept undisturbed for solidification. The lawn plates were prepared for bacterial culture (log phase) from nutrient broth using sterilised swabs. The

medium and equipments were sterilized in autoclave at 151 lbs/inch² pressure for 15 min.

The sterile disc of Hi Media were dipped in 20 µl of the concentrated extract mixed with DMSO (0.05 µl), and then the discs were placed on the surface of inoculated plates. The solvents (20 µl), DMSO (2 µl) and AMP (10 µg) discs were taken as control. The plates were finally kept at 4º C for 30 min for better diffusion and kept in an inverted position within the incubator. The plates were incubated for 16 h at 30º C. The zone of inhibition was measured using Hi Media scale and expressed in mm. All the experiments were carried out in triplicate.

The results of antibacterial activities of the various extracts of the three plants tested against the bacterial strains were statistically evaluated using Chi-square test. The standard deviation and Chi-square test were calculated using the standard procedure.

OBSERVATIONS

The results of the antimicrobial activities of the various extracts of the three plants indicated that they exhibited moderate to high inhibitory effect against the bacterial strains tested.

Antibacterial activity of *Adiantum latifolium*

The results revealed different degrees of bacterial susceptibility responses; ethyl acetate and ethanol extract showed higher inhibitory action as compared to petroleum ether whereas the aqueous extract did not show any effect against the bacterial strains tested. The highest susceptibility was shown by *Salmonella typhimurium* against the three extracts (Table 1). Ethyl acetate extract was more effective against *K. pneumoniae*, than *L. monocytogenes* and *Shigella flexneri*. Lowest response was shown by *Serratia marcescens*. In the ethanol extract, more action was shown against *Serratia marcescens* and *L. monocytogenes*, moderately against *K. pneumoniae*

TABLE 1: Antibacterial activity of whole plant extracts of *Adiantum latifolium.*

Bacterial strains	Types of extracts Inhibition zone: mm, Values = mean ± s d (Chi-square value)				
	Petroleum ether	Ethyl acetate	Ethanol	Aqueous	Control
Klebsiella pneumoniae (NCIM - 2883)	2.33± 0.04 (12.49)	13.33 ± 2.8 (0.73)*	8.5 ± 0.5 (4.12)	-	16.83 ± 0.28
Shigella flexneri (NCIM -5265)	-	12.67 ± 1.5 (0.96)*	8.33 ± 2.3 (4.165)	-	16.66 ± 0.28
Listeria monocytogenes (NCIM-5260)	-	12.67 ± 0.58 (0.02)*	9 ± 1 (0.87)*	-	12.26 ± 0.31
Salmonella typhimurium (NCIM -2501)	5 ± 1.36 (3.27)	14.67 ± 0.58 (1.22)*	10 ± 1 (0.09)*	-	11 ± 0.3
Serratia marcescens (NCIM-2919)	-	12.3 ± 1.15 (0.00)*	9.3 ± 2.08 (0.00)*	-	-

*significant ($p<0.05$)

TABLE 2: Antibacterial activity of whole plant extract of *Christella dentata.*

Bacterial strains	Types of extracts Inhibition zone: mm, Values = mean ± s d (Chi-square value)				
	Petroleum ether	Ethyl acetate	Ethanol	Aqueous	Control
Klebsiella pneumoniae (NCIM -2883)	2 ± 0.46 (13.1)	14.3 ± 1.15 (0.38)*	14.67 ± 1.52 (0.28)*	7 ± 1 (5.74)	16.83 ± 0.28
Shigella flexneri (NCIM -5265)	-	13 ± 0 (0.80)*	13.3 ± 1.15 (0.68)*	2 ± 0.46 (12.90)	16.66 ± 0.28
Listeria monocytogenes (NCIM -5260)	-	12.3 ± 0.58 (0.01)*	12 ± 1 (0.01)*	-	12.26 ± 0.31
Salmonella typhimurium (NCIM -2501)	-	13.3 ± 0.58 (0.48)*	12.67 ± 0.58 (0.25)*	4.67 ± 0.04 (3.64)*	11 ± 0.3
Serratia marcescens (NCIM -2919)	-	9.75 ± 0.5 (0)*	13.67 ± 0.58 (0)*	-	-

*Significant ($p<0.05$).

TABLE 3: Antibacterial activity of fronds extract of *Angiopteris evecta.*

Bacterial strains	Types of extracts Inhibition zone: mm, Values = mean ± s d (Chi-square value)				
	Petroleum ether	Ethyl acetate	Ethanol	Aqueous	Control
Klebsiella pneumoniae (NCIM -2883)	-	12 ± 1 (1.39)*	8 ± 2 (4.63)	7.3 ± 0.58 (5.39)	16.83 ± 0.28
Shigella flexneri (NCIM -5265)	2.3 ± 0.04 (12.38)	12.67 ± 0.58 (0.96)*	5.48 ± 1.93 (7.50)	-	16.66 ± 0.28
Listeria monocytogenes (NCIM -5260)	7.3 ± 0.58 (2.0)*	13.3 ± 1.15 (0.09)*	7 ± 6.08 (2.26)*	4.67 ± 1.04 (4.70)	12.26 ± 0.31
Salmonella typhimurium (NCIM -2501)	-	12.83 ± 0.76 (0.30)*	9.3 ± 2.082 (0.26)*	-	11 ± 0.3
Serratia marcescens (NCIM -2919)	4.67 ± 1.04 (0)*	15.5 ± 0.5 (0)*	14 ± 2.64 (0)*	7.3 ± 0.58 (0)*	-

*Significant ($p<0.05$).

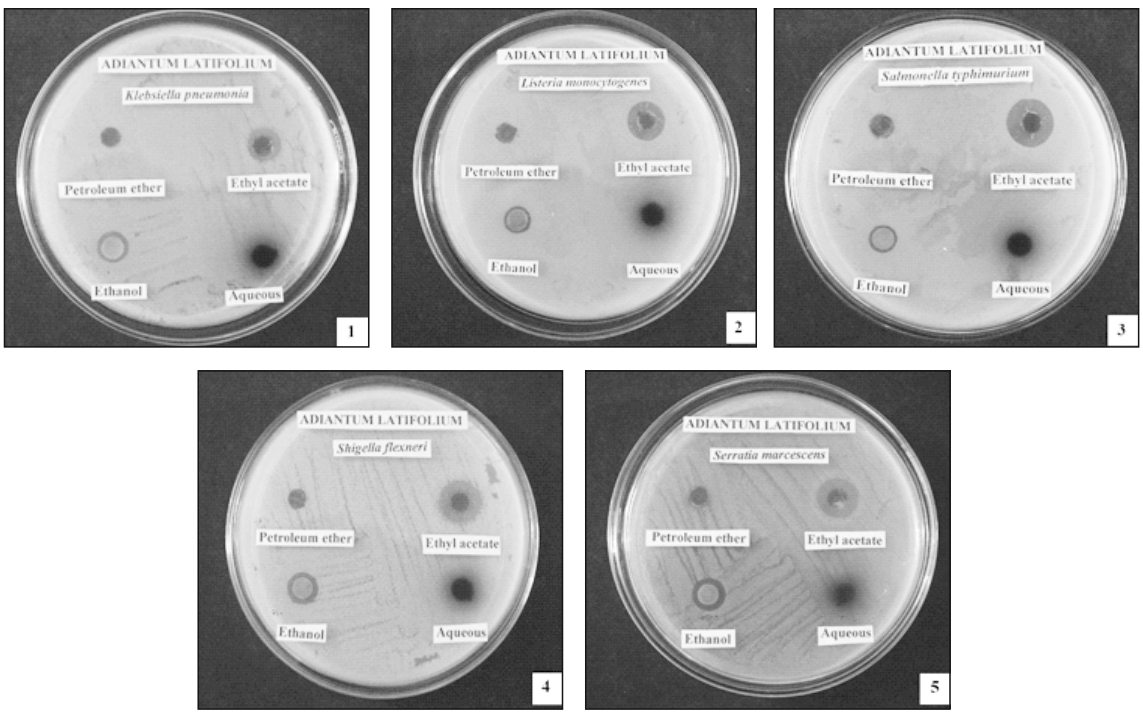
and *Shigella flexneri*. But in the petroleum ether, moderate action was shown against *K. pneumoniae* whereas all the others were found to be resistant (Figs 1–5).

Antibacterial activity of *C. dentata*

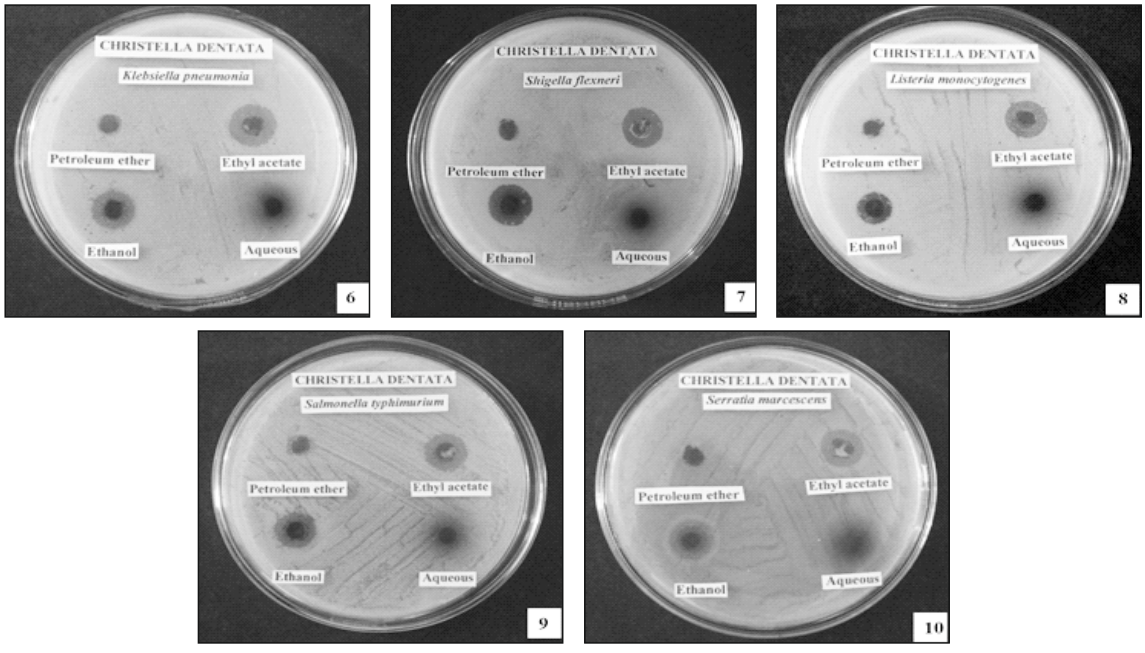
C. dentata showed a gradation in the inhibitory response and pronounced action was shown by ethyl acetate and ethanol extracts. The most significant inhibition was against *K. pneumoniae* (Table 2). Ethanol extract was fully effective against *Serratia marcescens*, followed by *Shigella flexneri*, and moderate effect was seen against *Salmonella typhimurium,* and *L. monocytogenes*. The ethyl acetate extract showed higher inhibition against *Salmonella typhimurium* (Figs 6–10). In case of aqueous extract, a moderate to low inhibitory action was shown against *Salmonella typhimurium* and *Shigella flexneri*. However, petroleum ether was effective against only *K. pneumoniae*.

Antibacterial activity of *Angiopteris evecta*

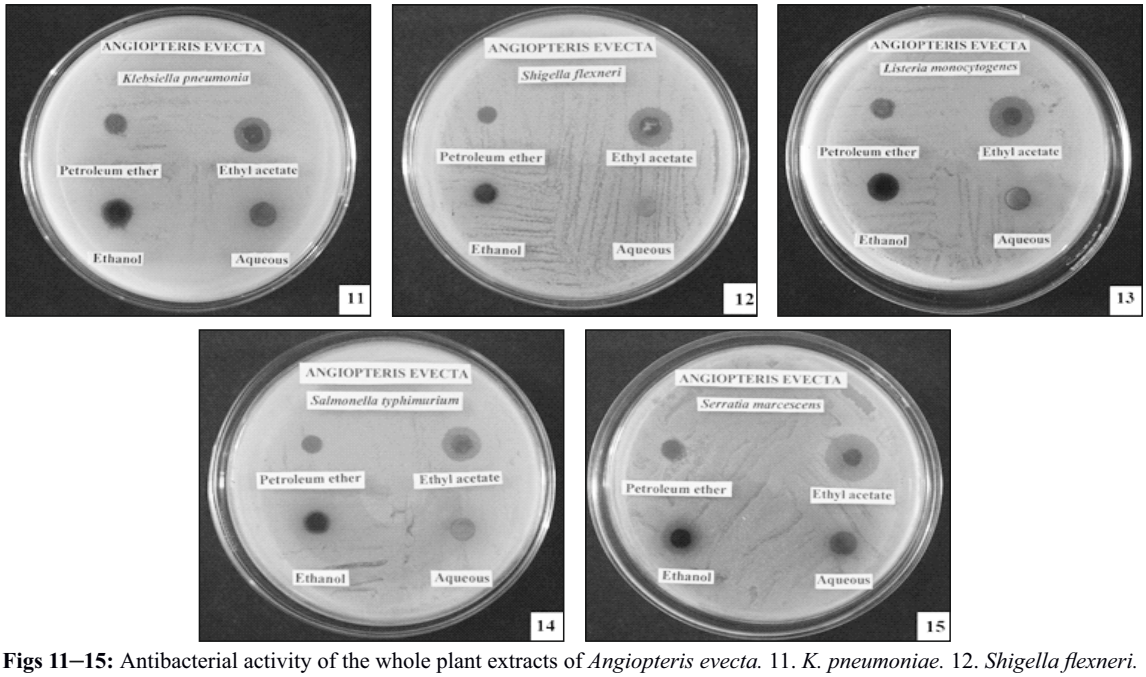
The inhibitory effect of ethyl acetate and ethanol was higher than that of petroleum ether and aqueous extract. Highest action was observed against *Serratia marcescens* by the three extracts (Table 3) and *L. monocytogenes* in case of petroleum ether extract (Figs 11–15). Ethyl acetate extract showed higher action against *L. monocytogenes*, moderately against *Salmonella typhimurium* and *Shigella flexneri* and least



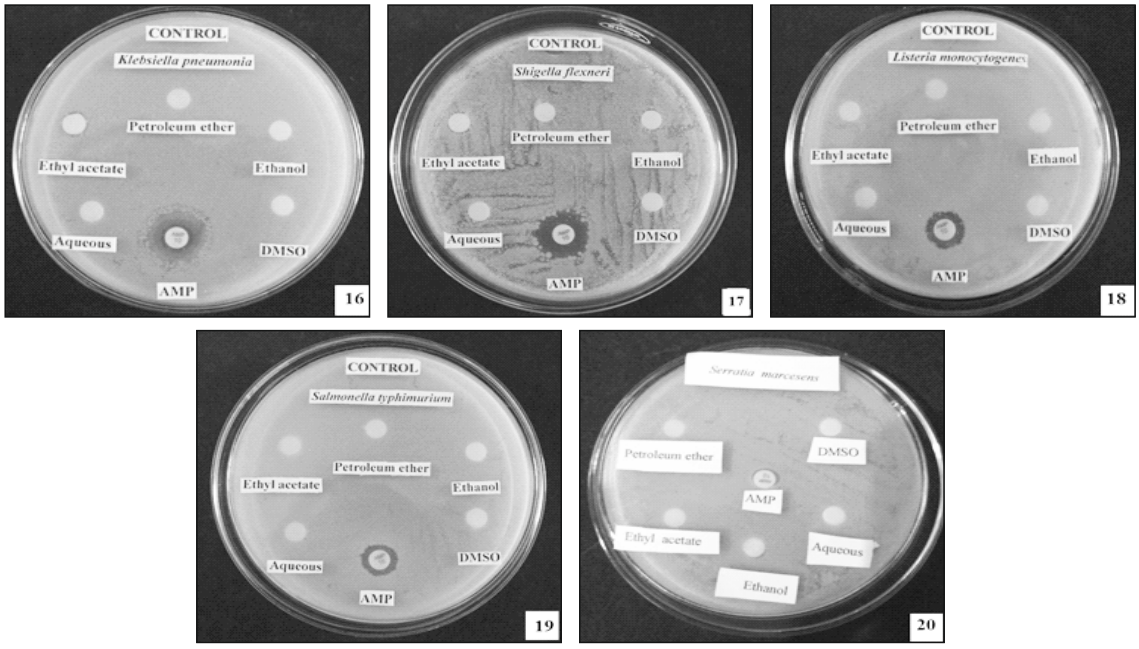
Figs 1–5: Antibacterial activity of whole plant extracts of *Adiantum latifolium.* 1. *K. pneumoniae.* 2. *Shigella flexneri.* 3. *L. monocytogenes.* 4. *Salmonella typhimurium.* 5. *Serratia marcescens.*



Figs 6–10: Antibacterial activity of the fronds extracts of *Christella dentata.* 6. *K. pneumoniae.* 7. *Shigella flexneri.* 8. *L. monocytogenes.* 9. *Salmonella typhimurium.*10. *Serratia marcescens.*



Figs 11–15: Antibacterial activity of the whole plant extracts of *Angiopteris evecta.* 11. *K. pneumoniae.* 12. *Shigella flexneri.* 13. *L. monocytogenes.* 14. *Salmonella typhimurium.* 15. *Serratia marcescens.*



Figs 16–20: Control. 16. *K. pneumoniae.* 17. *Shigella flexneri.* 18. *L. monocytogenes.* 19. *Salmonella typhimurium.* 20. *Serratia marcescens.*

against *K. pneumoniae*. In the ethanol extract, higher action was shown against *Salmonella typhimurium*, moderately against *K. pneumoniae* and *L. monocytogenes* and least against *Shigella flexneri*. Inhibition by petroleum ether extract was average against *Serratia marcescens* and *Shigella flexneri*, others showed resistance whereas aqueous extract was effective against *K. pneu-moniae* and *L. monocytogenes* but others were not inhibited at all. The solvents, petroleum ether, ethyl acetate, ethanol, distilled water and DMSO, taken as negative control showed no inhibitory effect whereas antibiotic AMP (10 µg) discs, taken as positive control showed inhibitory effect against all tested bacteria except *Serratia marcescens* (Figs 16–20).

Statistical analysis

Assuming the hypothesis that mean antibacterial activity of the various extracts of the plants indicated is significantly greater than corresponding positive controls. Also, the alternate hypothesis as vice versa. By applying Chi-square test, the hypothesis was tested at one degree of freedom with significance level of 5 percentage. The Chi-square test revealed that the antibacterial activity of ethyl acetate and ethanol extracts of all the three plants tested were significant. Whereas, that of the petroleum ether and aqueous extracts were not significant (Tables 1–3).

DISCUSSION

Systematic surveys of antimicrobial activity of ferns were made earlier by several workers (Anto et al. 2015, Babu et al. 2012, Kumar et al. 2011, Sandhya et al. 2015, Sen & Nandi 1951). It has been found that the fern extracts were effective against both gram positive and gram negative

bacteria (Nilanthi et al. 2015). The present study showed similar antimicrobial activity against the tested bacterial strains. The polar extracts of three ferns showed the presence of active polar compounds in ethyl acetate and ethanol extract rather than in petroleum ether and aqueous extract, suggesting its effective antibacterial activity (Thomas 2015). The work by Anto et al. (2015) reported the effect of solvent extracts of certain other ferns such as *Lygodium flexuosum, Ceratopteris thalictroides* and *Salvinia molesta* against *K. pneumoniae.* The present data shows 87% inhibitory effect of crude ethanol extract of the *C. dentata* against *K. pneumoniae*, as against that of standard inhibitory value, and this implies that this extract is a powerful constituent against *K. pneumoniae*. This gram negative bacterium, which in recent years has become a major pathogen in nosocomial infections, being a causative agent of the disease pneumonia, which is characterized by destructive changes in human lungs with inflammation, haemorrhage and cell death. This bacterium is frequently resistant to antibiotics and may also cause rheumatic diseases. The present findings indicate notable potential of the plant extract against *K. pneumoniae* infections. Earlier studies have documented *C. dentata* as having antibacterial properties against *Bacillus subtilis, Escherichia coli, Salmonella typhi* and *Staphylococcus aureus (*Kumar & Kaushik 2011). The present study also revealed the notable effect of *C. dentata* against *K. pneumoniae*, *Serratia marcescens* and *Shigella flexneri.*

Studies conducted by Babu et al. (2012) and Sandhya et al. (2015) showed efficient bactericidal activities of *Adiantum latifolium* against human pathogens, of which methanolic extract of *Adiantum latifolium* showed highest antibacterial activity against gram negative bacteria. In the present study, extracts of *Adiantum latifolium* also correlates earlier studies.

The significant antimicrobial activity of *Angiopteris evecta* observed in the present study reveals that it is a potential source to isolate and purify novel antimicrobial agents like broad spectrum of antibiotics as reported earlier by Rubin (1993).

Hence, an inference based on observed results, the maximum specific bacterial inhibition in the tested bacteria which is significant only in the case of ethyl acetate and ethanol fern extract can be drawn as:

K. pneumoniae = C. dentata > Angiopteris evecta > Adiantum latifolium

Shigella flexneri = C. dentata > Adiantum latifolium > A. evecta

L. monocytogenes = A. evecta > C. dentata > A. latifolium

Salmonella typhimurium = C. dentata > A. latifolium > A. evecta

Serratia marcescens = A. evecta > C. dentata > A. latifolium

In the present study, even though the plant extracts showed different degrees of antibacterial activity against tested strains, a promising effect was seen against *Serratia marcescens* where standard antibiotic ampicillin did not show any effect at all (Figs 15 , 20). It was also revealed that *C. dentata* was showing a higher antibacterial property as compared to *Angiopteris evecta* and *Adiantum latifolium.*

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RESEARCH ARTICLE

KARYOTYPIC STUDIES ON FIVE SPECIES OF *MACROSIPHUM* FROM HIMACHAL PRADESH

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SUMMARY Karyotypic investigations on five species belonging to aphid genus *Macrosiphum* infesting rose plants were carried out. These species are, *M. centranthi*, *M. euphorbiae*, *M. pachysiphon*, *M. rosae* and *Macrosiphum* sp. Out of these, the karyotype of *M. centranthi* infesting rose plant was a new report, the karyotype of *M. euphorbiae* infesting rose plant was reported for the first time from India and the karyotype of *M. pachysiphon* infesting rose plant was reported for the first time from Himachal Pradesh. The diploid chromosome number in these species ranged from 2n = 10 to 18. The chromosomes were holocentric. For karyotype analysis, lengths of chromosomes were measured at metaphase stage from at least ten best selected plates. Total complement length as well as relative lengths of chromosomes were calculated. Idiograms were constructed based on relative length data of chromosomes and karyotypes were prepared.

Keywords: Karyotypes, *Macrosiphum*, holocentric chromosomes, total complement length, idiograms.

INTRODUCTION

Aphids are phloem feeders and soft bodied insects that have worldwide distribution. At present, there are about 5012 (Cocuzza et al. 2015) described aphid species all over the world in about 510 presently accepted genera on about 87000 plant species belonging to 300 plant families (Blackman & Eastop 2000, 2015, Favret 2015). Of these aphid species, chromosome numbers of about 1039 species are known which comprise about 24% of the total number of aphid species (Gavrilov-Zimin et al. 2015). Aphids are important herbivores of both wild and cultivated plants and about 250 aphid species are considered serious pests of crops and cause great losses of yield (Remaudiére & Remaudiére 1997).

Rose is one of the most important ornamental plants which is known for its beauty, stability and long flowering time (Gavanji et al. 2012). It is infested by 55 aphid species (Blackman & Eastop 2000) of which 39 are previously recognized from India (Raychaudhuri 1983, Chakrabarti & Sarkar 2001). They are the most important pests of roses in Europe and western Asia (Mehrparvar et al. 2008). Since no consolidated work has been done on chromosomes of rose aphids from Himachal Pradesh it was considered worth to study the chromosomes of different aphid species infesting rose plants from this region. Such studies are useful in ascertaining the karyotypes which may be of great importance for solving taxonomical problems in the closely related species as well as in determining their adaptability in different environments.

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Genus *Macrosiphum* belongs to family Macrosiphini and includes 120 species. Several of the well known species alternate from *Rosa* to herbaceous secondary hosts. The present paper deals with the karyotypes of five species of *Macrosiphum* viz., *M. centranthi* Theobald, *M. euphorbiae* (Thomas), *M. pachysiphon* Hille Ris Lambers, *M. rosae* (L.) and *Macrosiphum* sp.

MATERIALS AND METHODS

For chromosomal studies, different species of *Macrosiphum* genus were collected from rose plants of different localities of Himachal Pradesh (30° to 33°N latitude and 75° to 79°E longitude). For each sample collected, information pertaining to month, year and place of collection were recorded (Table 1).

Somatic embryonic tissue was used to make chromosomal preparations. The embryos were taken out by puncturing the posterior end of the abdomen of an adult parthenogenetic female aphid. Only young embryos in which eye pigments were not visible, were used for chromosomal studies as in these embryos, most of the cells were in dividing stages and thus well spread mitotic plates could be obtained. The material was processed for preparation of chromosome studies as follows:

The embryos were pretreated in 0.7% sodium citrate solution for 30 min. The pretreated embryos were fixed in 1:3 acetic-ethanol for about 15 to 20 min at room temperature. After fixation, embryos were squashed on a glass slide in a drop of 45% acetic acid for 3 to 5 min and stained with 2% Giemsa for about 25 to 30 min followed by mounting in DPX.

The permanent slides were observed under research binocular microscope and photomicrographs were taken. Well spread metaphase plates were selected for chromosomal measurements. Actual lengths of chromosomes were measured using ocular micrometer. From actual lengths, the total complement length (TCL) was calculated for each species. From actual length data, the relative lengths of chromosomes were calculated. Chromosome pairs can be categorized into long, medium and short by comparing idiograms as well as by analysis of morphometric data (relative lengths of chromosomes) of individual chromosome pairs which showed considerable difference among them.

TABLE 1: Aphid species along with host plant, month and year and place of collection.

Species	Host plant	Month and year of collection	Place of collection
M. centranthi	*Rosa* sp.	July, 2017	Bagetu, Bilaspur (375 m^{asl})
M. euphorbiae	*Rosa* sp.	November, 2016	Dungrin, Hamirpur (1189 m^{asl})
M. pachysiphon	*Rosa* sp.	December, 2015	Rajgarh, Sirmaur (1555 m^{asl})
M . rosae	*Rosa* sp.	January, 2015	Rohru, Shimla (1154 m^{asl})
Macrosiphum sp.	*Rosa* sp.	December, 2016	Bhota, Hamirpur (738 m^{asl})

asl: above sea level.

TABLE 2: Actual and relative lengths of somatic chromosomes of aphid species.

Species	Total complement length (µm ± *SE)	Actual length		Relative length	
		L	S	L	S
M. centranthi	50.02 ± 0.20	5.97 ± 0.14	4.34 ± 0.24	11.63 ± 0.31	8.35 ± 0.21
M. euphorbiae	28.57 ± 1.08	4.86 ± 0.16	1.12 ± 0.09	17.14 ± 0.68	3.91 ± 0.27
M .pachysiphon	35.60 ± 0.19	3.88 ± 0.18	1.23 ± 0.11	11.04 ± 0.60	3.43 ± 0.22
M. rosae	35.40 ± 1.45	5.90 ± 0.19	1.46 ± 0.08	16.26 ± 0.56	4.13 ± 0.16
Macrosiphum sp.	38.41 ± 0.14	4.41 ± 0.13	3.25 ± 0.11	11.49 ± 0.10	8.47 ± 0.17

SE: Standard error about mean.

For identification of aphid species, keys developed by Blackman & Eastop (1984, 2000) were used.

OBSERVATIONS

Karyotypes of five species of *Macrosiphum* have been analysed. None of the species studied presented primary constrictions in the chromosomes.

M. centranthi

Medium-sized, yellowish green aphids were observed heavily infesting *Rosa* sp. (Figs 1, 2). This species has diploid chromosome number of 10 (Figs 13, 14). The length of chromosomes ranged from 4.34 µm to 5.97 µm. TCL was 50.02 µm. The relative length of chromosomes ranged from 8.35 to 11.63 (Table 2). The somatic complement consists of 5 pairs of chromosomes showing gradual decrease in their lengths (Fig. 15).

M. euphorbiae

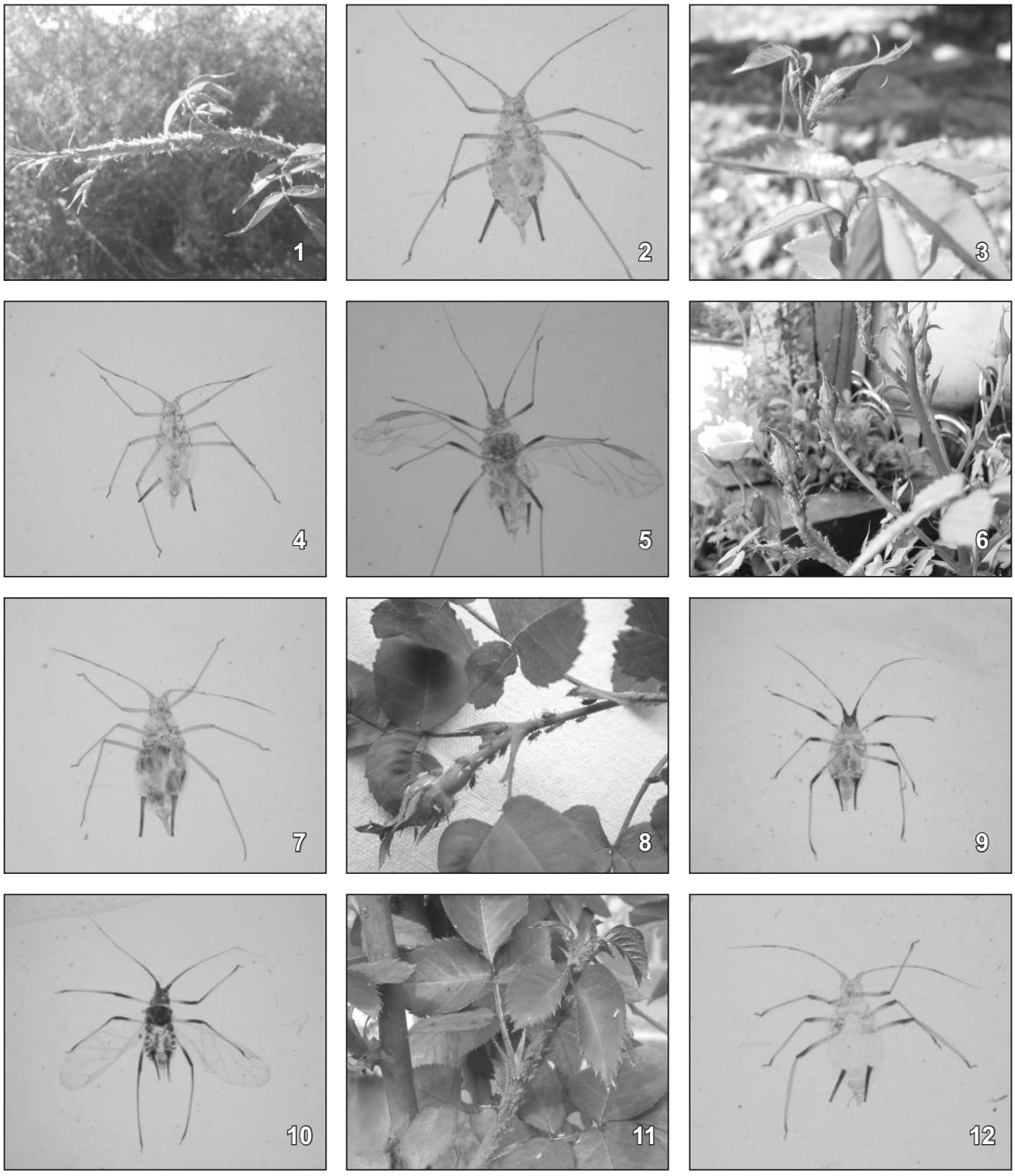
Medium-sized, shiny orange coloured aphids were observed infesting *Rosa* sp. (Figs 3, 4, 5). The diploid chromosome number was 10 (Figs 16, 17). The length of chromosomes ranged from 1.12 µm to 4.86 µm. TCL was 28.57 µm. The relative length of chromosomes ranged from 3.91 to 17.14 (Table 2). The somatic complement consists of gradually decreasing chromosomes in their lengths with 1 pair of long, 3 pairs of medium-sized and 1 pair of short chromosomes (Fig. 18).

M. pachysiphon

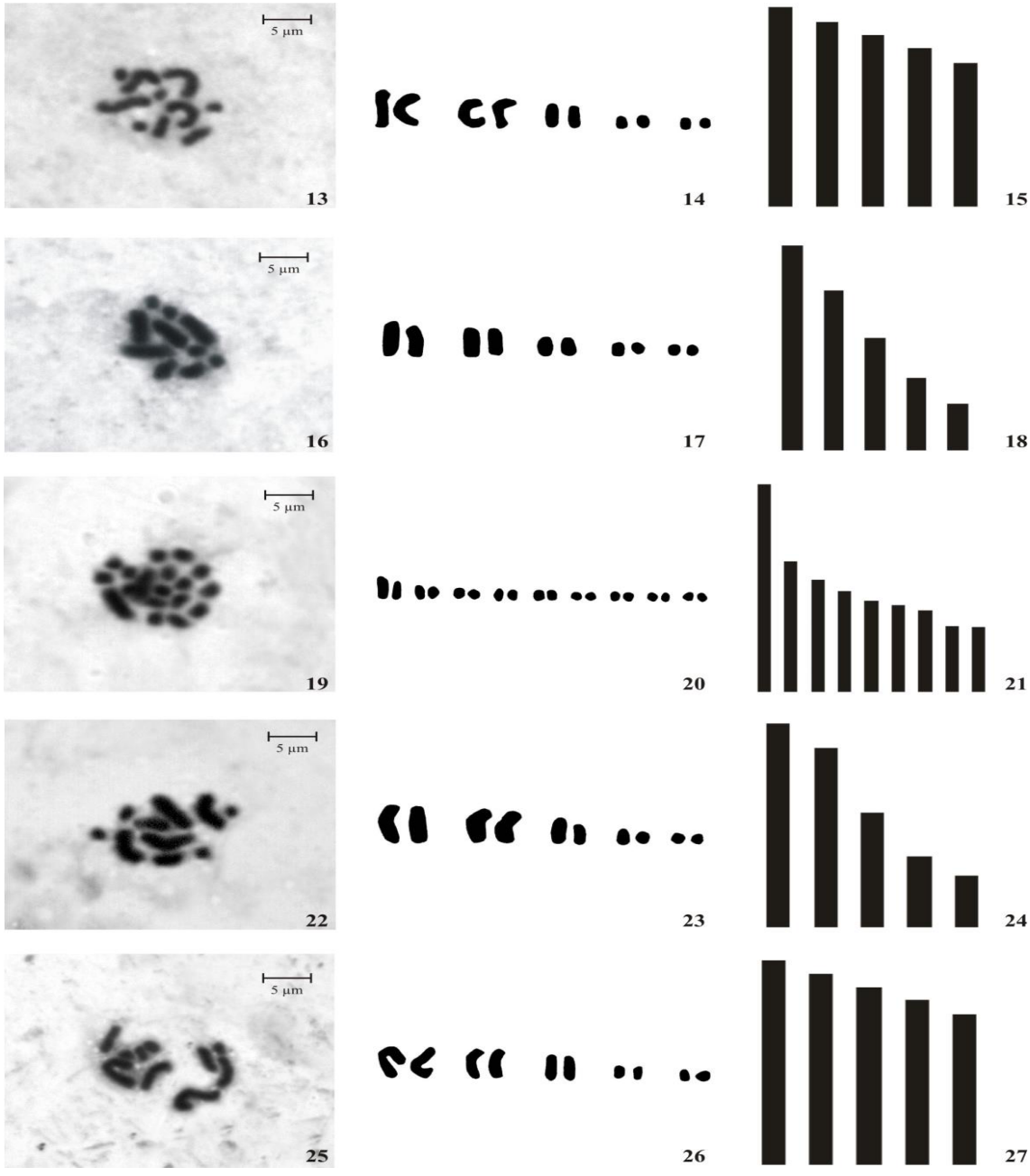
Large-sized, orange coloured aphids with black siphunculi were observed heavily infesting *Rosa* sp. (Figs 6, 7). It has the diploid chromosome number of 18 (Figs 19, 20). The length of chromosomes ranged from 1.23 µm to 3.88 µm. TCL was 35.60 µm. The relative length of chromosomes ranged from 3.43 to 11.04 (Table 2). The somatic complement includes first pair of comparatively longer chromosomes than the other pairs followed by 6 pairs showing gradual decrease in their lengths and 2 pairs of chromosomes almost of equal size (Fig. 21).

M. rosae

Large-sized, shiny reddish brown aphids with shiny head and siphunculi were observed infesting *Rosa* sp. (Figs 8–10). The diploid chromosome number was found to be 10 (Figs 22, 23). The length of chromosomes ranged from



Figs 1–12: Infestated parts and whole mounts (WM). 1, 2. *M. centranthi.* 1. Infestation. 2. Apterous WM. 3–5. *M. euphorbiae.* 3. Infestation. 4. Apterous WM. 5. Winged WM. 6, 7. *M. pachysiphon*. 6. Infestation. 7. Apterous WM. 8–10. *M. rosae.* 8. Infestation. 9. Apterous WM. 10. Winged WM. 11, 12. *Macrosiphum* sp. 11. Infestation. 12. Apterous WM.



Figs 13–27: Karyotypes of aphids. 13–15. *M. centranthii*. 13. Somatic chromosomes. 14. Karyotype. 15. Idiogram. 16–18. *M. euphorbiae.* 16. Somatic chromosomes. 17. Karyotype. 18. Idiogram. 19–21. *M. pachysiphon.* 19. Somatic chromosomes. 20. Karyotype. 21. Idiogram. 22–24. *M. rosae*. 22. Somatic chromosomes. 23. Karyotype. 24. Idiogram. 25–27. *Macrosiphum* sp. 25. Somatic chromosomes. 26. Karyotype. 27. Idiogram.

1.46 µm to 5.90 µm. TCL was 35.40 µm. The relative length of chromosomes ranged from 4.13 to 16.26 (Table 2). The somatic complement consists of 2 pairs of long, 2 pairs of medium-sized and 1 pair of short chromosomes (Fig. 24).

***Macrosiphum* sp.**

Medium-sized, light orange coloured aphids were observed heavily infesting *Rosa* sp. (Figs 11, 12). This species has diploid chromosome number of 10 (Figs 25, 26). The length of chromosomes ranged from 3.25 µm to 4.41 µm. TCL was 38.41 µm. The relative length of chromosomes ranged from 8.47 to 11.49 (Table 2). The somatic complement includes 5 pairs of chromosomes showing gradual decrease in their lengths (Fig. 27).

DISCUSSION

Of the five species of *Macrosiphum* studied here, in *M. centranthi*, *M. euphorbiae*, *M. rosae* and *Macrosiphum* sp., the diploid chromosome number is 2n = 10 whereas *M. pachysiphon* has 2n = 18.

M. centranthi with diploid chromosome number of 10 reported here is in conformity with earlier reports of Blackman & Eastop (1984) from aphids collected from different host plant. The same diploid chromosome number was reported earlier by Robinson & Chen (1969) in *M. rosae*, *M. pallidum*, *M. euphorbiae*, *M.geranii*, *M. hamiltoni*, *M. kickapoo* and *M. maintobans*.

The chromosome number of 2n = 10 reported in the present study for *M. euphorbiae* is in conformity with the earlier reports for this species by Monti et al. (2011) and Anupriya & Gautam (2017) from different host plants. Stevens (1905), Sun & Robinson (1966), Khuda-Bukhsh (1980) and Gautam & Kapoor (2002) also reported 2n = 10 in different species of this genus.

M. pachysiphon with diploid chromosome number of 18 reported here is in conformity with earlier reports of Kurl (1980) and Gautam & Kapoor (2002). Other species of *Macrosiphum* such as *M. clematifoliae*, *M. indicum* and *M. rosaeiformis* have the same diploid chromosome number i.e., 2n = 18 (Khuda-Bukhsh 1980, Kurl & Misra 1983, Blackman 1986).

The chromosome number of 2n = 10 reported here for *M. rosae* is in conformity with the earlier reports of various workers (Stevens 1905, Cognetti 1961, Khuda-Bukhsh 1980, Kar & Khuda-Bukhsh 1989, Samkaria et al. 2010, Devi & Gautam 2012).

Macrosiphum sp. has diploid chromosome number of 10 as reported by various workers in different species of *Macrosiphum* (Sun & Robinson 1966, Robinson & Chen 1969, Gut 1976, Blackman 1980, 1986, Gautam & Dhatwalia 2003, Monti et al. 2011).

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