Development of SCAR marker for sex determination in dioecious betelvine (*Piper betle* L.)

G. N. Khadke[1,*], K. Hima Bindu[1] and K. V. Ravishankar[2]

[1]Section of Medicinal Crops, and
[2]Division of Biotechnology, Indian Institute of Horticultural Research, Bangalore 560 089, India

Betelvine (*Piper betle* L.) is an economically important plant whose fresh leaves are masticatory and also widely used in traditional medicine. It is a dioecious plant belonging to the family Piperaceae. Development of sex-specific markers greatly facilitates both betelvine breeding and selection programmes. In the present study, 35 inter-simple sequence repeats (ISSR) DNA markers are used in 20 germplasm lines of betelvine. Out of the 35 tested primers, three primers showed co-segregation with gender. Two primers, viz. ISSR-10 and UBC-852 produced male specific bands of size 459 bp and 1250 bp respectively. ISSR-23 amplified a female-specific 636 bp fragment. These primers were validated in the individuals of the bulks and showed a consistent sex-specific expression. A sequence characterized amplified region (SCAR) was developed from the primer ISSR-23, which amplified fragments from the genomes of females, but not the male ones. This new SCAR marker will be valuable in determining the sex type of betelvine plants; furthermore it will help in designing efficient breeding programmes.

Keywords: Betelvine, dioecious, inter-simple sequence repeats, sequence characterized amplified region, sex.

BETELVINE (*Piper betle* L.) is an economically important cash crop valued for its fresh leaves in many parts of the country. In the Asiatic region, betelvine ranks second to coffee and tea in terms of daily consumption[1]. The plant belongs to the Piperaceae family, which consists about 10 genera and over 1,000 species of herbs, shrubs and climbers[2]. The most probable place of the origin of betelvine is Eastern Malaysia[3]. The green, heart shaped leaves of this plant are masticatory and popularly known as *paan* in India. Fresh leaves are chewed with condiments such as arecanut, *kattha*, clove, cardamom, fennel and candied rose. The leaves are also widely used in the Indian system of medicine and health traditions[4]. Several attributes of the leaves such as 'digestive', 'carminative', 'stimulant', 'antiseptic' and 'antifungal' activities have been described. A phenolic compound, hydroxyl-chevicol from the leaf was reported to possess anticarcinogenic property[3,5]. Roots are known for female contraceptive effects[6]. They have a positive stimulatory influence on intestinal digestive enzymes, especially lipase, amylase and disaccharides[7]. Anti-inflammatory properties of the ecotypes of this plant have been studied and three purified chemicals namely chavibetol, chavibetol acetate and chavicol were identified[8].

Betelvine is a dioecious crop and both male and female clones are cultivated based on local preference. Sex determination in this crop is not readily possible as flowering in both the sexes is observed in specific regions of the country (Northeast and Western Ghats). Gender-based differences were reported in *P. betle* with respect to many traits such as leaf shape, amount of chlorophyll, essential-oil composition and total phenol and thiocyanate content[2]. Unfortunately, little is known about the sex determination system of Piperaceae and has not been studied in *P. betle*. Classifying the germplasm and collection of new germplasm needs knowledge of the sex of the plant, which is a time-consuming process. A report on flowering of male and female vines and fruit setting in Bangalore conditions, India[9] opened up the possibility of hybridization in betelvine. Hybridization work carried out under the All-India Coordinated Research Project (AICRP) at Indian Institute of Horticultural Research (IIHR) has generated many segregating F1 populations. Sex identification in this population is possible only upon flowering which takes more than 2 years. Identifying the gender at an early stage aid in genetic improvement programme.

Molecular tools were employed in dioecious taxa for early identification of sex and understanding the developmental and evolutionary pathways of sexual dimorphism. A preferred approach is to find molecular markers tightly linked to sex. Such sex-linked markers have been reported in white Campion (*S. latifolia*)[10], Asparagus[11], Kiwi plant[12], Pistachio[13], Hemp[14], Hop[15], Grapevine[16] and the Basket Willow[17]. Among the molecular markers, random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR) markers have been used often to identify and determine sex.

Plant materials used in this study are shown in Table 1. They include 10 male and 10 female cultivars which were sampled from the field at Central Horticultural Experimental Station (CHES), Hirehalli, a substation of IIHR, Bangalore, which is maintained under AICRP on betelvine.

Genomic DNA was extracted using a modified cetyltrimethyl ammonium bromide (CTAB) protocol described by Doyle[18]. Frozen tissue (2–3 g) was ground with mortar and pestle in liquid nitrogen and homogenized in 12 ml of preheated (65°C) DNA extraction buffer containing 100 mM Tris-HCl (pH 8.0); 1.5 M NaCl; 20 mM EDTA (pH 8.0); CTAB (2% w/v) and β-mercaptoethanol (0.2% v/v) with addition of 50 mg polyvinyl pyrrolidone (PVP). The mixture of extraction buffer and β-mercaptoethanol was incubated for 45 min at 65°C with occasional mixing

*For correspondence. (e-mail: khadke921@hotmail.com)

Table 1. Betelvine germplasm with their geographical origin and sex form

Accession No.	Cultivar name	Geographical origin	Sex of plant
IIHRBV-01	Tellaku, Ponnur	Andhra Pradesh	Male
IIHRBV-04	Tellaku, Chinthalapudi	Andhra Pradesh	Male
IIHRBV-06	Kuljedu Cuddapah	Andhra Pradesh	Male
IIHRBV-08	Vasani Kapoori	Maharashtra	Male
IIHRBV-09	Shirpurkata	Maharashtra	Male
IIHRBV-10	Kapoori	Tamil Nadu	Male
IIHRBV-11	Kapoori Chinacheppalli	Andhra Pradesh	Male
IIHRBV-18	Yellow Leaf	Andhra Pradesh	Male
IIHRBV-20	Patchaikodi	Tamil Nadu	Male
IIHRBV-47	Swarna Kapoori	Andhra Pradesh	Male
IIHRBV-30	Ramtek Bangla	Maharashtra	Female
IIHRBV-31	Kalipathi	Maharashtra	Female
IIHRBV-32	Bangla Nagaram	Uttar Pradesh	Female
IIHRBV-33	Godi Bangla	Odisha	Female
IIHRBV-35	Bangla	Uttar Pradesh	Female
IIHRBV-42	Simurali Babna	West Bengal	Female
IIHRBV-43	Simurali Babna local	West Bengal	Female
IIHRBV-45	Khasi Pan	Assam	Female
IIHRBV-37	Sirugamani-1	Tamil Nadu	Female
IIHRBV-105	Vellaikodi-2	Tamil Nadu	Female

and gentle swirling. DNA was extracted with an equal volume of chloroform : isoamyl alcohol (24 : 1 v/v) precipitated with equal volume of cold isopropanol. The precipitated DNA was centrifuged and washed with 70% ethanol for 5 min, dried at room temperature and resuspended in 200 µl Tris-EDTA pH 8.0 (1 M Tris and 0.5 M EDTA). The genomic DNA was treated with RNAase to avoid contamination. It was further purified using chloroform : isoamyl alcohol (24 : 1) and re-precipitated with cool isopropanol. The DNA was re-suspended in 200 µl Tris-EDTA buffer and quantification was carried out by using Hoefer's Dyna quant (Pharmacia Biotech, USA) and diluted in autoclaved double distilled water to 25 ng/µl.

Two bulk DNA samples (male and female) were prepared by pooling an equal amount of DNA from 10 individual male and female cultivars (Table 1). These bulks were amplified with 35 ISSR primers. A DNA marker present in either the male bulk sample or female bulk sample was considered for further screening with bulk segregate analysis (BSA). Sex specificity of the marker was tested through BSA using all 20 individuals. Putative sex-linked markers which differentiated the male and female bulks as well as the individuals of each sex are used for developing the SCAR marker.

Out of the 90 ISSR primers used in the initial screening, 35 primers (Table 2) were selected for BSA in betelvine. This included UBC series primers obtained from Bioserve, India and ISSR primers reported in *Piper longum*[19] and primers showed sex linkage in betelvine[20]. Polymerase chain reaction (PCR) amplification for the ISSR was performed in 25 µl reaction volume, containing 10× PCR buffer (10 mM tris-HCl pH 8.0), 1.5 mM $MgCl_2$, 200 µM of each dNTP, 1 U of *Taq* polymerase, primers 12.5 pmol and 50 ng of genomic DNA template. PCR amplification was carried out using a thermal cycler (Eppendorf, Germany), which was programmed for an initial step of 4 min at 94°C followed by 35 cycles of 1 min at 94°C, annealing temperature according to the primer (Table 2) for 45 sec and 1 min at 72°C was programmed for 10 min and the product was stored at 4°C until loading. SCAR-23 analysis was performed under the same conditions, but annealing temperatures varied from 43°C to 67°C depending on primers which are given in Table 2.

Electrophoresis was performed in 1.5% agarose gels. Band size was estimated by comparison to 100 bp ladder DNA standard (Fermantas). Female-diagnostic bands were extracted and purified from agarose gels using QIAquik Gel Extraction Kit (Qiagen) and quantified using the Saran wrap method[21].

Purified sex-linked fragment was cloned into the TA cloning vector pTZ57R/T, InsTAclone™ PCR cloning kit No. K1214 (Fermantas). Competent cells of *Escherichia coli* strain DH5α, prepared by calcium chloride method as described by Sambrook[22], were transformed with the recombinant vector and plated onto LB/ampicillin/IPTG/X-Gal plates. Positive colonies were determined by blue/white screening. Plasmids from randomly selected white colonies were extracted using the Plasmid DNA Isolation Kit (column-based, Cat. No. 1001, 1002) from Bioserve Pvt Ltd, Hyderabad, India. Vectors containing fragments of the correct size were sequenced using Eurofins Biotechnologies Pvt Ltd and in both reverse and forward directions with M-13 universal primers using ABI3700 DNA analyser (Applied Biosystems, USA). The sequence is thereafter used to design specific primer pairs of 15–30 bp which amplify single major bands of the size

Table 2. ISSR primers used for bulk segregant analysis

Primer name	Primer sequence	Size (bp)	Annealing temperature (°C)	Tm (°C)
UBC-813	CTCTCTCTCTCTCTCTT	17	51	45
UBC-817	CACACACACACACACAA	17	51	45
UBC-818	CACACACACACACACAG	17	55	47
UBC-820	GTGTGTGTGTGTGTGTC	17	55	47
UBC-821	GTGTGTGTGTGTGTGTT	17	51	45
UBC-822	TCTCTCTCTCTCTCTCA	17	51	45
UBC-827	ACACACACACACACACG	17	53	47
UBC-830	TGTGTGTGTGTGTGTGG	17	53	47
UBC-834	AGAGAGAGAGAGAGAGYT	18	53	46
UBC-836	AGAGAGAGAGAGAGAGYA	18	53	46
UBC-840	GAGAGAGAGAGAGAGAYT	18	53	48
UBC-841	GAGAGAGAGAGAGAGAYC	18	53	48
UBC-843	CTCTCTCTCTCTCTCTRA	18	53	46
UBC-844	CTCTCTCTCTCTCTCTRC	18	53	48
UBC-845	CTCTCTCTCTCTCTCTRG	18	53	48
UBC-850	GTGTGTGTGTGTGTGTYC	18	54	48
UBC-852	TCTCTCTCTCTCTCTCRA	18	53	46
UBC-853	TCTCTCTCTCTCTCTCRT	18	53	46
UBC-854	TCTCTCTCTCTCTCTCRG	18	53	48
UBC-855	ACACACACACACACYT	16	53	48
UBC-856	ACACACACACACACYA	16	54	46
UBC-858	ACACACACACACACYG	16	53	48
UBC-861	ACCACCACCACCACCACC	18	53	55
UBC-862	AGCAGCAGCAGAGCAGC	18	56.5	55
UBC-863	AGTAGTAGTAGTAGTAGT	18	51	41
UBC-867	GGCGGCGGCGGCGGCGGC	18	67	69
UBC-880	GGAGAGGAGAGGAGA	15	48	45
UBC-899	CATGGTGTTGGTCATTGTTCCA	22	62	53
ISSR-1	CTCTCTCTCTCTCTCTTG	18	46	48
ISSR-2	CTCTCTCTCTCTCTCTAC	18	46	48
ISSR-5	CACACACACACACGT	15	45	42
ISSR-7	CACACACACACACGG	15	50	45
ISSR-9	GTGTGTGTGTGTGG	14	42	40
ISSR-10	GAGAGAGAGAGACC	14	45	40
ISSR-15	GTGGTGGTGGC	11	46	38
ISSR-18	GATAGATAGATAGG	14	34	32
ISSR-23	GACAGACAGACACC	14	50	40
IT-1	GGTAACAAGGTTTCC	15	40	39
IT-2	AGTTTCTTCTCCTCC	15	40	39

Table 3. Putative sex-linked primers identified through BSA in betelvine

Primer name	Primer sequence (5′ to 3′)	Size (bp)	Sex linkage
ISSR-10	GAGAGAGAGAGACC	459	Male
ISSR-23	GACAGACAGACACC	636	Female
UBC-852	TCTCTCTCTCTCTCTCRA	1200	Male

similar to that of the cloned fragment. From the cloned sequence of ISSR-23, forward and reverse primers of SCAR-23 were designed manually.

Molecular markers have become an indispensable tool and used extensively in genetic diversity and marker-assisted selection in plant breeding programmes. Molecular markers are also a powerful tool for early detection of sex in dioecious plants. Dioecy prevents intra-individual self-pollination and is one of the most extreme inbreeding avoidance mechanisms[23] and only around 6% of angiosperms are dioecious. Recently, they have been used to screen markers for ascertaining sex in papaya[24], *Humulus lupulus*[25] and *Cycas circinalis*[26]. ISSR markers have been reported to produce more complex marker patterns than the RAPD approach[27].

Thirty-five ISSR primers were used in BSA for identifying sex-linked markers and out of them, three putative sex-linked primers, viz. ISSR-10, UBC-852 and ISSR-23 showed sex-specific banding pattern (Table 3). The primer ISSR-10 (5′GAGAGAGAGAGACC3′) amplified

unique 459 bp fragment in the bulk and 10 individual male DNA, whereas similar fragment is absent in the bulk and 10 female individuals (Figure 1). The primer UBC-852 (5′TCTCTCTCTCTCTCTCRA3′) amplified unique 1200 bp fragment in the bulk and individual males of the bulk (Figure 2). Similar fragment is not observed in the bulk and individual females. The primer ISSR-23 (5′GACAGACAGACACC3′) amplified 636 bp fragment unique in the bulk and 10 individual females (Figure 3). The band is consistently absent in the bulk and 10 male individuals. These putative sex-linked markers are useful for determining sex in betelvine.

Figure 1. Male-specific DNA fragment produced by PCR with ISSR-10 in the male bulk and bulk individuals germplasm of betelvine.

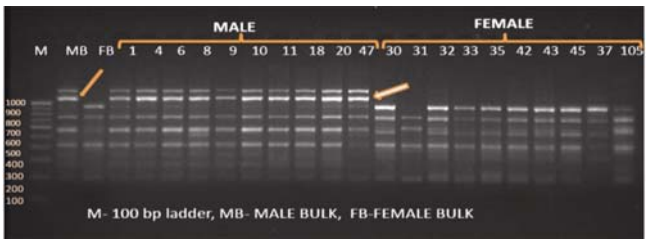

Figure 2. Male-specific DNA fragment produced by PCR with UBC-852 in the male bulk and bulk individuals germplasm of betelvine.

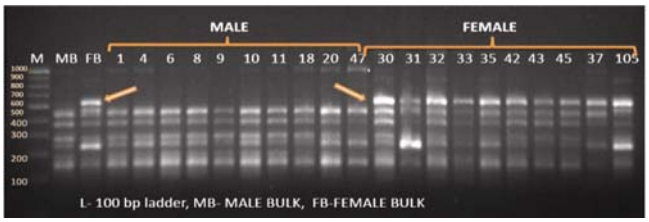

Figure 3. Female-specific DNA fragment produced by PCR with ISSR-23 in the female bulk and bulk individuals germplasm of betelvine.

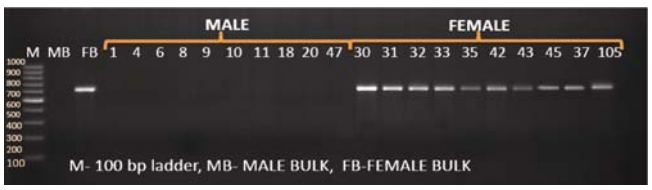

Figure 4. Female-specific DNA fragment produced by PCR with SCAR-ISSR-23 in the female bulk and bulk individuals betelvine germplasm.

In order to develop sex-linked SCAR markers in betelvine, putative markers identified by arbitrary marker analysis (ISSR, RAPD) were used. The arbitrary markers technique is sensitive to changes in the reaction conditions[28]. In order to bridge the gap between the ability to obtain linked markers to a gene of interest in a short-time, SCAR marker technique was developed and applied. SCARs are PCR-based markers that represent genomic DNA fragments at genetically defined loci that are identified by PCR amplification using sequence-specific oligonucleotide primers[24,29,30]. Three putative sex-linked markers are cloned and sequenced and the sequence is available on the National Centre for Biological Information (NCBI) public data with accession number ISSR-10 (BankIt1545465 Seq1JX186197), ISSR-23 (BankIt1545468 Seq JX186198) and UBC 852 (BankIt1547154 Seq1 JX220174).

Out of the three primers, ISSR-23 which has shown very clear and consistent co-segregation with female sex in the bulk and in the individuals of the bulk was selected for converting it to SCAR marker. ISSR 23 primer linked to female sex of plant was developed into SCAR based on isolated, cloned and sequenced female-specific fragments. Amplified female-specific fragment product of ISSR-23 is cloned and sequenced. From the sequence, female-specific SCAR primers (SCAR-23-F5′ GACAGACAGACACCAAGTTCAAGC3′ and SCAR-23-R 5′ATATATTTAGTGGTGTCTGTCTGTCA3′) are designed and these primers are efficient in identification of female plants. These specific primers were able to amplify a single unique 636 base-pair fragment which is present in all female individuals and absent in male individuals (Figure 4). SCAR from ISSR-23 (IS-23 F/R) amplifies a discriminating size (636 bp) band in bulk and individual female betelvine plants, but not in male bulk and individual male plant DNA. Development of sex-specific SCAR markers was also reported in dioecious crops such as papaya[31], *Rumex nivalis*[32].

In the present study, ISSR markers proved to be a powerful method to detect sex-specific fragments in dioecious betelvine. Sex was correctly predicted with this marker in each of the 20 betelvine accessions tested. The sex-linked SCAR marker is being validated further in all the available germplasm and segregating populations. Molecular markers linked with sex in betelvine help in identification of the genders in early growth stage of the plants (germplasm and hybrids), which facilitates classifying the germplasm and its utilization in crop improvement programmes. Moreover, sexual dimorphism is reported to be linked to many economically important traits such as disease resistance, leaf quality, etc. Exploring the relationship between sex-linked markers and desirable traits (especially to disease resistance, as betelvine crop is affected by many pathogens) in future will assist targeted genetic improvement.

1. Kumar, N., Misra, P., Dube, A., Bhattacharya, S., Dikshit, M. and Ranade, S., *Piper betle* L., A maligned pan-Asiatic plant with an array of pharmacological activities and prospects for drug discovery. *Curr. Sci*., 2010, **99**, 922–932.
2. Parthasarthy, V. A., Chattopadhyay, P. K. and Bhose, T. K., *Plantation Crops (2)*, Naya Udyog, 2006.
3. Chattopadhyay, S. B. and Maity, S., *Diseases of Betelvine and Spices*, ICAR, New Delhi, 1967.
4. Rawat, A. K. S., Tripthi, R. D., Khan, A. J. and Balasubrahmanyam, V. R., Essential oil components as markers for identification of *Piper betle* L. cultivars. *J. Biochem. Syst. Ecol.*, 1989, **17**, 38–55.
5. Bhide, S. V., Zariwala, M. B. A., Amonlar, A. J. and Azuine, M. A., Chemo-preventive efficacy of betle leaf extract against benzo(a)pyrene induced fore-stomach tumors in mice. *J. Ethnopharmacol.*, 1991, **34**, 207–213.
6. Chopra, R. N., Nayar, S. L. and Chopra, I. C., *Glossary of India Medicinal Plants*, Council of Scientific and Industrial Research, New Delhi, 1956, 3rd reprint, 1992.
7. Sandhya, P. M., Patel, K., Saraswathi, G. and Srinivasana, K., Effect of orally administered betel leaf (*Piper betle* L.) on digestive enzymes of pancreas and intestinal mucosa and on bile production in rats. *Indian J. Exp. Biol*., 1995, **13**, 752–756.
8. Khozirah, S., Ling, S. K. and Nik, M., Second International Symposium on Chromatography of Natural Products, Herba-polonica, 2000.
9. Raghavendra, R. and Maiti, S., Occurrence of flowering in betel vine (*Pipe betle* L.). *Indian J. Agric. Sci*., 1989, **59**, 665–666.
10. Mulcahy, D. L., Weeden, N. F. and Kesseli, R. V., DNA probes for the Y chromosome of *Silene latifolia*, a dioecious angiosperm. *Sex Plant Reprod*., 1992, **5**, 86–88.
11. Jiang, C. S. K., RAPD and SCAR markers linked to the sex expression locus M in asparagus. *Euphytica*, 1997, **94**, 329–333.
12. Gill, G., Harvey, C., Gardner, R. and Fraser, L., Development of sex-linked PCR markers for gender identification in Actinidia. *Theor. Appl. Genet*., 1998, **97**, 439–445.
13. Hormaza, J., Dollo, L. and Polito, V., Identification of a RAPD marker linked to sex determination in *Pistacia vera* using bulk segregant analysis. *Theor. Appl. Genet*., 1994, **89**, 9–13.
14. Mandolino, G., Carboni, A., Forapani, S., Faeti, V. and Ranalli, P., Identification of DNA markers linked to the male sex in dioecious hemp (*Cannabis sativa* L.). *Theor. Appl. Genet*., 1999, **98**, 86–92.
15. Polley, A., Seigner, E. and Ganal, M., Identification of sex in hop (*Humulus lupulus*) using molecular markers. *Genome*, 1997, **40**, 357–361.
16. Dalbó, M. A., Ye, G. N. N. F., Weeden, H., Steinkellner, C. S. and Reisch, B. I., Gene controlling grape flower sex placed on a molecular marker-based genetic map. *Genome*, 2000, **43**, 333–340.
17. Alstrom-Rapaport, C., Lascoux, M., Wang, Y. C., Roberts, G. and Tuskan, G., Identification of RAPD marker linked to sex determination in the basket willow (*Salix viminalis* L.). *J. Hered*., 1998, **89**, 44–49.
18. Doyle, J. J. and Doyle, J. L., Isolation of plant DNA from fresh tissue. *Focus*, 1990, **12**, 13–15.
19. Saji, K. V., Taxonomic and genetic characterization of black pepper and related species, Ph D thesis, University of Calicut, Kerala, India, 2006.
20. Himabindu, K., Reetha, B., Suryanarayana, M. A., Sukanya, D. H., Vasantha Kumar, T., Sex linked ISSR markers in dioecious betelvine (*Piper betle* L.). In National Seminar on Contemporary Approaches on Crop Improvement, ISGPB, Bangalore, 2011.
21. Sambrook, J., Fritstch, E. F. and Maniatis, T., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, 1989, 2nd edn.
22. Sambrook, J. and Russel, D., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, 2001, 3rd edn.
23. Ainsworth, C., Boys and girls come out to play: the molecular biology of dioecious plants. *Ann. Bot*., 2000, **86**, 211–221.
24. Parasnis, A. S., Ramakrishna, W., Chowdari, K. V., Gupta, V. S. and Ranjekar, P. K., Microsatellite (GATA)n reveals sex specific differences in Papaya. *Theor. Appl. Genet*., 1999, **99**, 1047–1052.
25. Danilova, T. V. and Karlov, G. I., Application of inter simple sequence repeat (ISSR) polymorphism for detection of sex-specific molecular markers in hop (*Humulus lupulus* L.). *Euphytica*, 2006, **151**, 15–21.
26. Gangopadhyay, G., Roy, S. K., Ghose, K., Poddar, R., Bandyopadhyay, T., Basu, D. and Mukherjee, K. K., Sex detection of *Carica papaya* and *Cycas circinalis* in pre-flowering stage by ISSR and RAPD. *Curr. Sci*., 2007, **92**, 524–526.
27. Chowdhury, M. A., Vandenberg, B. and Warkentin, T., Cultivar identification and genetic relationship among selected breeding lines and cultivars in chickpea (*Cicer arietinum* L.). *Euphytica*, 2002, **127**, 317–325.
28. Agarwal, M., Shrivastava, N. and Harish, P., Advances in molecular marker techniques and their applications in plant sciences. *Plant Cell. Rep*., 2008, **27**, 617–631.
29. Mcdermott, J. M., Brandle, U., Dutly, F., Haemmerli, U. A., Keller, S., Muller, K. E. and Wolf, M. S., Genetic variation in powdery mildew of barley: development of RAPD, SCAR and VNTR markers. *Phytopathology*, 1994, **84**, 1316–1321.
30. Paran, I. and Michelmore, R. W., Development of reliable PCR-based markers linked to downy mildew resistance genes in lettuce. *Theor. Appl. Genet*., 1993, **85**, 985–999.
31. Urasaki, N. *et al*., A male and hermaphrodite specific RAPD marker for papaya (*Carica papaya* L.). *Theor. Appl. Genet*., 2002, **104**, 281–285.
32. Stehlik, I. and Blattner, F. R., Sex-specific SCAR markers in the dioecious plant *Rumex nivalis* (Polygonaceae) and implications for the evolution of sex chromosomes. *Theor. Appl. Genet*., 2004, **108**, 238–242.

ACKNOWLEDGEMENTS. G.N.K. acknowledges the JRF fellowship received from ICAR. He thanks IIHR for providing research facilities.

Received 3 February 2012; revised accepted 16 July 2012

Fossil batoid and teleost fish remains from Bhuban Formation (Lower to Middle Miocene), Surma Group, Aizawl, Mizoram

R. P. Tiwari* and Victor Zochhuana Ralte

Department of Geology, Mizoram University, Aizawl 796 004, India

Intraformational conglomeratic horizon within the Upper Bhuban unit of Bhuban Formation, Surma Group (Lower to Middle Miocene) exposed at two localities in the vicinity of Aizawl, Mizoram has yielded fish remains mostly in the form of isolated teeth, dental plates and spines. Majority of these belong to diverse selachians and have already been described. We describe here dental plates and a cau-

*For correspondence. (e-mail: rptmzu@rediffmail.com)