تاکسونومی و بیوسیستماتیک

نشریه علمی پژوهشی دانشگاه اصفهان

سال ششم - شماره بیست و یکم - زمستان ۱۳۹۳

شاپای چاپی: ۲۰۰۸-۸۹۰۶ شاپای الکترونیک: ۲۳۲۲-۲۱۹۰

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علمی پژوهشی

سال ششم - شماره بیست و یکم - زمستان ۱۳۹۳

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تاکسونومی و سیستماتیک

علمی-پژوهشی

سال ششم - شماره بیست و یکم - زمستان ۱۳۹۳

نشـــریه تاکســـونومی و بیوسیســـتماتیک بـــر اســـاس ابلاغیـــه شـــماره **۳/۱۱/۹۵۵** مـــورخ **۱۳۸۸/۰۶/۳۱** کمیســـیون بررســـی نشـــریات علمـــی وزارت علـــوم تحقیقـــات و فنـــاوری، دارای درجـــه **علمـــی-پژوهشـــی** و شـــماره اســـتاندارد بـــین‌المللـــی (شـــاپا) **۸۹۰۶-۲۰۰۸** (نســـخه چـــاپی) و شـــماره اســـتاندارد بـــین‌المللـــی **۲۱۹۰-۲۳۲۲** (نســـخه الکترونیـــک) از ســـازمان اســـناد و کتابخانـــه ملـــی جمهوری اسلامی ایران می‌باشد.

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گوگل پژوهشگر	**http://scholar.google.com/citations?hl=en&user=dxidM-UAAAAJ**

چاپ و لیتوگرافی: انتشارات دانشگاه اصفهان

ناشر: دانشگاه اصفهان

انتشار: بهار ۱۳۹۴

تاکسونومی و بیوسیستماتیک
سال ششم - شماره بیست و یکم - زمستان ۱۳۹۳
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صاحب امتیاز: معاونت پژوهش و فناوری دانشگاه اصفهان
سردبیر: دکتر محمدرضا رحیمی‌نژاد رنجبر — استاد - دانشگاه اصفهان

ناشر: انتشارات دانشگاه اصفهان

نشانی: اصفهان- خیابان هزار جریب - دانشگاه اصفهان- ساختمان کتابخانه مرکزی- معاونت پژوهش و فناوری طبقه دوم- اداره چاپ، انتشارات و مجلات - کد پستی: ۸۱۷۴۶۷۳۴۴۱- دفتر نشریه تاکسونومی و بیوسیستماتیک
نشانی پست الکترونیک: TBJ@ui.ac.ir
پایگاه اختصاصی نشریه: http://uijs.ui.ac.ir/tbj
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هدف

دانشگاه اصفهان با هدف معرفی و نشر آخرین یافته‌های علمی پژوهشگران دانشگاه‌ها و مؤسسات علمی در زمینه تاکسونومی و بیوسیستماتیک با تأکید بر خزانهٔ وراثتی جانداران (یوکاریوت‌ها و پروکاریوت‌ها)، نشریه علمی-پژوهشی **تاکسونومی و بیوسیستماتیک** با عنوان انگلیسی **Taxonomy and Biosystematics** را به صورت فصلنامه منتشر می‌نماید.

محورهای موضوعی نشریه

نشریه تاکسونومی و بیوسیستماتیک، مقاله‌های اصیل پژوهشی در زمینه‌های: معرفی تاکسون‌های جدید، مرور نامگذاری تاکسون‌ها، طبقه‌بندی تاکسون‌ها، معرفی روش‌های نوین ایجاد و تحلیل داده‌ها، ژن اکولوژی، ژنتیک جمعیت‌ها و تنوع وراثتی، تنوع زیستی و فیلوژنی تاکسون‌ها را پس از داوری دقیق به صورت مقاله کامل (full paper) و مقاله کوتاه (short paper) به چاپ می‌رساند.

قوانین حق نشر

۱. مقالاتی که برای بررسی به این نشریه ارسال می‌گردد نباید قبلا در جایی به چاپ رسیده باشد و یا همزمان به مجلات دیگر ارائه شده باشد. همچنین، نبایستی نتایج آن در گردهمایی‌ها ارائه شده باشد.
۲. مسؤولیت صحت مطالب مقاله بر عهده نویسنده مسؤول مقاله است.
۳. تعداد و ترتیب اسامی نگارندگان بر اساس توافق بین آنها و نویسنده مسؤول مقاله صورت می‌گیرد.
۴. عدم رعایت شیوه نگارش مقاله باعث عدم پذیرش یا کندی مراحل پذیرش مقاله خواهد شد.
۵. نشریه در پذیرش، رد و اصلاح مقاله بر اساس قوانین مصوب این نشریه عمل می‌نماید.
۶. مقاله‌های دریافت شده توسط دبیران و داوران متخصص بررسی می‌شود و پس از پذیرش علمی توسط هیأت تحریریه با رعایت نوبت به چاپ می‌رسد.

تدوین مقاله در یک نگاه

متن مقاله بایستی به زبان فارسی باشد، رعایت قواعد دستور زبان فارسی و رسا بودن جملات مورد توجه است.

مقاله کامل (full paper) به ترتیب شامل: عنوان، نام نگارندگان، وابستگی سازمانی نگارندگان، چکیده فارسی، واژه‌های کلیدی، مقدمه، مواد و روش‌ها، نتایج، بحث، جمع‌بندی، سپاسگزاری، منابع، Title، Author(s) Name(s')، Author(s) Affiliation(s')، Abstract و Key words باشد و حداکثر در ۱۵ صفحه در فرمت نهایی نشریه تنظیم شود.

مقاله کوتاه (short paper) کاملا شبیه مقاله کامل است به طوری که دارای: عنوان، نام نگارندگان، وابستگی سازمانی نگارندگان، چکیده فارسی، واژه‌های کلیدی، مقدمه، مواد و روش‌ها، نتایج، بحث، جمع‌بندی، سپاسگزاری، منابع، Title، Author(s) Name(s')، Author(s) Affiliation(s')، Abstract و Key words است، با این تفاوت که بدون بخش‌بندی و حداکثر در ۴ صفحه تنظیم می‌شود.

تدوین مقاله با شرح جزییات (رعایت ترتیب در متن)

فایل مقاله با نرم‌افزار Microsoft Office Word در فرمت ذخیره 2003، در کاغذ A4، با حاشیه‌های ۳ سانتی‌متر از چهار سوی، فاصله خطوط ۱ (single) و به صورت یک ستونی تهیه شود.

عنوان: فارسی: 16 B Zar Bold، انگلیسی: 14 Times New Roman Bold

نام نگارندگان: فارسی: 11 B Zar، انگلیسی: 9 Times New Roman

درج شماره مربوط به وابستگی سازمانی هر نگارنده پس از نام نگارنده به صورت superscript

درج ستاره (*) برای نویسنده مسؤول (Corresponding Author)

وابستگی سازمانی نگارندگان: فارسی: 10 B Zar و انگلیسی: 8 Times New Roman

درج شماره مربوط به وابستگی سازمانی هر نگارنده پیش از نشانی به صورت superscript

نشانی پست الکترونیک نگارنده مسؤول: 10 Times New Roman

چکیده: فارسی: 11 B Zar، انگلیسی: 9 Times New Roman

حداقل ۱۰۰ و حداکثر ۲۵۰ واژه، از به کار بردن واژه‌های اختصاری اجتناب شود.

واژه‌های کلیدی: حداکثر ۷ واژه مرتبط و مرتب شده بر اساس حروف الفبا

متن مقاله: به ترتیب شامل: مقدمه، مواد و روش‌ها، نتایج، بحث، جمع‌بندی و سپاسگزاری است. فارسی: 13 B Zar، انگلیسی: 11 Times New Roman.

از درج پاورقی برای بیان توضیحات انگلیسی و فارسی و بالعکس خودداری شود و در صورت نیاز، در درون پرانتز و در متن مقاله آورده شود.

جدول‌ها

جدول‌ها به همراه توضیحات آنها در متن مقاله آورده شود.

شماره‌گذاری و توضیحات جدول‌ها به صورت بالانویس باشد. فارسی: 11 B Zar، انگلیسی: 9 Times New Roman.

فرمت جدول‌ها در بخش Text wrapping، به صورت None انتخاب شود.

جدول‌های طولانی به صورت جدولی یکپارچه طراحی شود.

شکل‌ها

شکل‌ها به همراه توضیحات آنها در متن مقاله آورده شود.

شماره‌گذاری و توضیحات شکل‌ها به صورت زیرنویس باشد. فارسی: 11 B Zar، انگلیسی: 9 Times New Roman.

فرمت شکل‌ها در بخش Layout، به صورت In line with text انتخاب شود.

شکل‌ها از حالت گروه‌بندی (group) خارج شود و به صورت یکپارچه باشد.

شکل‌های چند قسمتی فقط با حروف انگلیسی بزرگ (بالا، چپ) برچسب‌گذاری شوند.

شکل‌هایی که از جنس نمودار هستند به صورت دو بعدی، سیاه و سفید، بدون سایه، با بافت ساده و بدون خطوط افقی طراحی شوند.

شکل‌هایی که از جنس تصویر هستند به صورت دو بعدی، بدون سایه، با کیفیت بسیار بالا ارسال شوند.

زیرنویس شکل‌ها و بالانویس جدول‌ها به یکی از دو صورت زیر تنظیم شود:

مقادیر، میانگین ... تکرار ± SE (یا StD یا انحراف معیار یا خطای معیار) است. حروف یکسان بیانگر عدم اختلاف معنی‌دار با استفاده از آزمون (دانکن یا توکی یا ...) است.

مقادیر، میانگین ... تکرار ± SE (یا StD یا انحراف معیار یا خطای معیار) است. حروف یکسان بیانگر عدم اختلاف معنی‌دار در سطح P<XXX است.

منابع (بر اساس شیوه‌نامه انجمن روان‌شناسی آمریکا، APA)

منابع استفاده شده در سراسر مقاله فقط به زبان انگلیسی و سال میلادی باشد.

منابعی که در اصل فارسی زبان هستند، با درج عبارت (in Persian) در انتها مشخص شوند.

منابع استفاده شده در متن مقاله در چهار مورد با فهرست منابع کاملا منطبق باشد: استفاده شدن یا نشدن در متن یا انتها، داشتن املای صحیح و یکسان، داشتن یا نداشتن همکار، یکسان بودن سال.

استناد در متن (references in text): به صورت نام نویسنده یا نویسندگان (بدون نام کوچک) و سال انتشار نوشته شود.

ابتدای جمله

Ranjbar و Mahmoudian (۲۰۱۳) با مطالعه چهار گونه از جنس ...

Ashrafzadeh و همکاران (۲۰۱۰) با بررسی برخی ویژگی‌های بوم‌شناختی گونهٔ جربیل بلوچی ...

وسط جمله

مطالعه Mehdipour Moghaddam و همکاران (۲۰۱۲) برای شناسایی گونه‌هایی جدید از باکتری‌های ...

نتایج به دست آمده از بررسی‌های Kharazian (۲۰۱۰) بر روی طبقه‌بندی و ریخت‌شناسی ...

انتهای جمله (از قدیم به جدید)

(Ranjbar and Mahmoudian, 2013؛ Mehdipour Moghaddam *et al*., 2012؛ Kharazian, 2010؛ Ashrafzadeh *et al*., 2010)

عبارت *et al* بایستی به صورت مورب باشد (به دلیل لاتین بودن).

منابع (references in list)

منابع بر اساس حروف الفبا مرتب شود و به اندازه ۰/۵ سانتی‌متر به صورت Hanging تورفتگی داشته باشد. 11 Times New Roman

استناد به مقاله (paper)

به ترتیب شامل: عنوان نویسنده یا نویسندگان، سال، عنوان مقاله، عنوان نشریه، شماره مجلد، شماره صفحات است (به علامت‌های جدا کننده ویرایشی توجه شود).

پیش از عنوان آخرین نویسنده، واژه ربط and استفاده شود (استفاده از & مجاز نیست).

برای استناد به مقاله‌هایی که هنوز چاپ نشده‌اند به جای سال، از عبارت (in press) استفاده شود.

عنوان مقاله با حروف کوچک نوشته شود، به استثنای نخستین حرف از: نخستین واژه، اسامی خاص و اسامی علمی.

عنوان نشریه به صورت کامل (نه مخفف) نوشته شود.

حروف نخستین عنوان نشریه به صورت بزرگ (capital) نوشته شود.

Gholipour, A. and Sonboli, A. (2013) Rediscovery of *Acorus calamus* (Acoraceae) in Iran. Taxonomy and Biosystematics 5(15): 113-116 (in Persian).

Takano, A., Gisil, J. and Suleiman, M. (2013) Floral size variation causes differentiation of pollinators and genetic parameters in *Alpinia nieuwenhuizii*, a flexistylous ginger (Zingiberaceae). Plant Systematics and Evolution 299(5): 865-871.

استناد به کتاب (book)

با توجه به اینکه ترجمه اغلب کتاب‌ها بدون دریافت مجوز از نویسنده و ناشر اصلی انجام می‌شود، استفاده از آنها در مجامع بین‌المللی موجب بروز مشکلات عدیده‌ای می‌شود. لذا، استناد به ترجمه‌های فارسی مجاز نیست. در صورت نیاز، اصل کتاب تهیه، مطالعه و به آن ارجاع داده شود.

به ترتیب شامل: عنوان نویسنده یا نویسندگان، سال، عنوان کتاب، شماره ویرایش در صورت وجود، نام انتشارات، نام نخستین شهر محل انتشارات.

Ghahreman, A. and Attar, F. (1999) Biodiversity of plant species in Iran. Tehran University Press, Tehran (in Persian).

Stace, C. A. (1989) Plant taxonomy and biosystematics. Edward Arnold, London.

استناد به بخشی از کتاب (chapter in book) به طوری که هر بخش دارای نویسنده جداگانه باشد:

به ترتیب شامل: عنوان نویسنده یا نویسندگان بخش، سال، عنوان بخش، استفاده از واژه :In، عنوان اصلی کتاب، نام ویراستار اصلی، شماره ویرایش در صورت وجود، شماره صفحه آغاز و پایان بخش، نام انتشارات، نام نخستین شهر محل انتشارات.

توضیح: اگر ویراستار اصلی (chief editor) یک نفر باشد، از .Ed و اگر بیش از یک نفر باشد (chief editors) از .Eds استفاده می‌شود.

Morrison, L. A. (1993) *Triticum-Aegilops* systematics: taking an integrative approach. In: Biodiversity and wheat improvement (Ed. Damania, A. B.) 59-66. John Wiley & Sons, New York.

Sears, E. R. (1956) The systematic, cytology and genetics of wheat. In: Handbuch der Pflazenzuchtung. (Eds. Kapparet, H. and Rudorf, W.) 2: 164-187. Paul Parey, Berlin and Humburg.

استناد به کتاب چند جلدی دارای ویراستار اصلی با تاریخ نشر چند ساله

Rechinger, K. H. (Ed.) (1963-1998) Flora Iranica. vols. 1-176. Akademische Druck-U Verlagsanstalt, Graz.

Townsend, C. C. and Guest, E. (1966-1985) Flora of Iraq. vols. 1-9. Ministry of Agriculture and Agrarian Reform, Baghdad.

استناد به یک جلد از کتاب چند جلدی دارای ویراستار اصلی

Podlech, D., Zarre, Sh. and Maassoumi, A. A. (2001) Papilionaceae IV, Astragalus II. In: Flora Iranica (Ed. Rechinger, K. H.) vol. 175. Akademische Druck-U Verlagsanstalt, Graz.

استناد به پایان‌نامه کارشناسی ارشد و رساله دکتری

به ترتیب شامل: نام نویسنده، سال، عنوان پایان‌نامه، مقطع تحصیلی، نام دانشگاه، نام شهر، نام کشور.

Barzehkar, Gh. (1995) Flora and plant communities with their distribution according to ecological properties in Noor Forest Park. MSc thesis, Tarbiat Modares University, Tehran, Iran (in Persian).

Von Konrat, M. (2003) A biosystematic study of the liverwort genus *Frullania Raddi*: encompassing a worldwide monograph of subg. microfrullania (Schust.) Schust.; a revision of the New Zealand species and study of subsidiary species. PhD thesis, The University of Auckland, Auckland, New Zealand.

استناد به Patent

به ترتیب شامل: نام نویسنده، سال، عنوان، نام کشور و شماره patent.

Suzuki, T., Ohishi, N. and Yagi, K. (2000) Methods of obtaining a composition 9-cis β-Carotene in high purity. US Patent 6057484.

استناد به همایش (سمینار، سمپوزیوم، کنگره، میتینگ و ...)

به ترتیب شامل: نام نویسندگان، سال انتشار، عنوان مقاله، دوره و نام همایش، نام محل برگزاری، نام شهر، نام کشور.

Mason-Gamer, R. J. and Helfgott, D. M. (2002) Molecular phylogenetic investigation of allopolyploid *Elymus* in North America. 4th International Triticeae Symposium, Prague, Czech Republic.

استناد به مقاله کامل همایش (سمینار، سمپوزیوم، کنگره، میتینگ و ...): Proceedings

به ترتیب شامل: نام نویسندگان، سال انتشار، عنوان مقاله، دوره و نام همایش، نام محل برگزاری، نام شهر، نام کشور.

Mohsenzadeh, S. (1996) Study of nitrogen fertilizer time and amount on seed production and other characterizations of Sorgum. In: Proceeding of the 4th Iranian Congress of Agriculture and Plant Breeding, Isfahan, Iran (in Persian).

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فهرست

نشریه علمی - پژوهشی تاکسونومی و بیوسیستماتیک
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فهرست

ژنتیک جمعیت عروس‌ماهی اصفهانی (*Petroleuciscus esfahani*) (ماهیان استخوانی، کپورماهیان) در رودخانه زاینده‌رود، ایران

ندا شجاعی [۱]، سالار درافشان [۱]*، نوراله میرغفاری [۱] و مجید طالبی [۲]
[۱] گروه شیلات، دانشکده منابع طبیعی، دانشگاه صنعتی اصفهان، اصفهان، ۸۴۱۵۶-۸۳۱۱۱، ایران
[۲] گروه بیوتکنولوژی کشاورزی، دانشکده کشاورزی، دانشگاه صنعتی اصفهان، اصفهان، ایران

چکیده

جمعیت‌های عروس‌ماهی اصفهانی (*Petroleuciscus esfahani*) در رودخانه زاینده‌رود با استفاده از پنج جفت نشانگر مولکولی ریزماهواره بر روی ۱۲۰ نمونه بالغ صید شده از چهار ایستگاه: چشمه دیمه، خرسونک، چمگردان و پل صفائیه ارزیابی شد. تمامی جایگاه‌های بررسی شده چندشکل بودند. تعداد ۵۴ آلل برای پنج جایگاه تولید شد که در محدوده ۶ آلل در جایگاه CnaB-030 و ۱۷ آلل در جایگاه Ca3 قرار داشت. میانگین تعداد آلل مشاهده شده در جمعیت چشمه دیمه برابر با ۹/۶ عدد و در سایر جمعیت‌ها برابر با ۸/۶ عدد بود. میانگین هتروزیگوسیتی مشاهده شده برای ۵ جایگاه در گستره ۰/۹۲ تا ۱ قرار داشت که بیانگر سطح بالای تنوع ژنتیکی در هر جمعیت بود. در اغلب موارد، انحراف از تعادل هاردی-واینبرگ در آزمون جایگاه-جمعیت، عمدتا به علت افزایش هتروزیگوسیتی مشاهده شد. حداقل و حداکثر فاصله ژنتیکی به ترتیب بین جمعیت‌های چشمه دیمه-خرسونک و چشمه دیمه-چمگردان محاسبه شد. نتایج مقادیر اندک اما معنی‌دار F_{ST} را در مقایسه جفت جمعیت‌ها نشان داد. مطالعه حاضر، حضور چهار جمعیت متفاوت عروس‌ماهی اصفهانی را در رودخانه نشان داد که می‌تواند بیانگر اثر تکه‌تکه شدن زیستگاه بر جمعیت ژنتیکی عروس‌ماهی اصفهانی باشد.

واژه‌های کلیدی: ریزماهواره، عروس‌ماهی اصفهانی (*Petroleuciscus esfahani*)، جمعیت، رودخانه زاینده‌رود

* sdorafshan@cc.iut.ac.ir

مقایسه فیلوژنتیک ژنوم A در برخی از گونه‌های جنس *Triticum* با روش تحلیل کاریوتیپ

محمدحسین اهتمام *[۱]، محمدرضا رحیمی‌نژاد [۲]، حجت‌اله سعیدی [۲] و فاطمه ابراهیم [۱]
[۱] گروه زراعت و اصلاح نباتات، دانشکده کشاورزی، دانشگاه صنعتی اصفهان، اصفهان، ایران
[۲] گروه زیست‌شناسی، دانشکده علوم، دانشگاه اصفهان، اصفهان، ایران

چکیده

کاریوتیپ ۴۸ جمعیت از پنج گونه (*Triticum aestivum*، *T. durum*، *T. monococcum*، *T. turgidum* و *T. urartu*) و دو زیرگونه *T. boeoticum* subsp. *boeoticum* و *T. boeoticum* subsp. *thaodar* دارای ژنوم A از جنس *Triticim* تحلیل شد. کروموزوم‌ها با نرم‌افزار Micro Measure 3.3 اندازه‌گیری شد. ویژگی‌های کاریوتیپی شامل: تعداد کروموزوم، طول کلی کروموزوم، میانگین طول کروموزوم ها، درصد شکل کلی کاریوتیپ، اندیس سانترومری، طول بلندترین کروموزوم، طول کوتاه‌ترین کروموزوم، میانگین طول بازوهای بلند، میانگین طول بازوهای کوتاه، نسبت بازوها، اندیس عدم تقارن کاریوتیپی، درصد نسبت بین کوتاه‌ترین و بلندترین کروموزوم ها، تفاوت دامنه طول نسبی کروموزوم‌ها، اندیس تقارن درون کروموزومی، اندیس تقارن میان کروموزومی، طول نسبی کروموزوم‌ها و فرمول کاریوتیپی اندازه‌گیری و محاسبه گردید. تمام جمعیت‌ها در گروه A1 طبقه‌بندی Stebbines قرار گرفتند. تحلیل خوشه‌ای بر پایه ضرایب A1 و A2 سه گروه کاریوتیپی را در جمعیت‌های مطالعه شده ایجاد کرد: گروه نخست شامل *T. aestivum* با بیشترین عدم تقارن کاریوتیپی، گروه دوم شامل *T. boeoticum* subsp. *boeoticum*، *T. boeoticum* subsp. *thaodar* و *T. monococcum* و با کمترین عدم تقارن کاریوتیپی و گروه سوم شامل *T. durum*، *T. turgidum* و *T. urartu* و با جایگاهی مابین دو گروه قبل. گونه‌های دیپلوئید *T. monococcum* بر اساس ضرایب A1 و A2 قدیمی‌ترین و ابتدایی‌ترین کاریوتیپ را داشت. با توجه به نتایج به نظر می‌رسد *T. durum* ابتدایی‌تر از *T. turgidum* باشد و احتمالا *T. monococcum* می‌تواند به عنوان دهنده ژنوم A به *T. aestivum* و *T. durum* به حساب آید.

واژه‌های کلیدی: ژنوم A، تحلیل کاریوتیپ، فیلوژنی، *Triticum*

* hehtemam@cc.iut.ac.ir

انواع کرک دمبرگ جنس *Alchemilla* از تیره Rosaceae از ایران

مرضیه بیگم فقیر *، کبری چایچی خیرخواه و ربابه شاهی شاووان
گروه زیست‌شناسی، دانشکده علوم، دانشگاه گیلان، رشت، ایران

چکیده

انواع کُرک دمبرگ ۲۴ گونه از جنس *Alchemilla* با استفاده از میکروسکوپ الکترونی (SEM) و دیجیتالی مطالعه شد. تمام دمبرگ‌ها متعلق به برگ‌های کاملاً توسعه یافته قاعده‌ای هستند. دو نوع کُرک اصلی (استوانه‌ای و مسطح نواری) و هفت زیرگروه آنها شامل: کُرک‌های خوابیده/تقریباً خوابیده، تقریباً خوابیده/افراشته/پراکنده، افراشته/پراکنده انبوه، افقی انبوه، بدون کُرک، دارای کُرک افقی/افراشته/پراکنده/برگشته و کُرک برگشته شناسایی شد. نتایج این تحقیق نشان داد که صفات ریزریخت‌شناسی دمبرگ از نظر تاکسونومیکی دارای اهمیت است و می‌تواند برای رده‌بندی گونه‌های این جنس استفاده شود. یک کلید شناسایی برای تفکیک گونه‌ها نیز ارائه شده است.

واژه‌های کلیدی: *Alchemilla*، کُرک، دمبرگ، ریزریخت‌شناسی، ایران

* faghirmb@guilan.ac.ir

گزارش گونه *Potentilla ghazniensis* از تیره Rosaceae به عنوان گونه جدید از مرکز ایران

مرضیه بیگم فقیر [۱]*، علیرضا نقی‌نژاد [۲] و عبدالرحمان دهقانی [۲]
[۱] گروه زیست‌شناسی، دانشکده علوم، دانشگاه گیلان، رشت، ایران
[۲] گروه زیست‌شناسی، دانشکده علوم پایه، دانشگاه مازندران، بابلسر، ایران

چکیده

گونه *Potentilla ghazniensis* به عنوان گزارشی جدید از مرکز ایران معرفی می‌شود. این گونه با داشتن کرک‌های متنوع (بلند و کوتاه، افراشته/تقریبا گسترده) روی دمبرگ برگ‌های قاعده‌ای، خامه تقریبا انتهایی و در قاعده ضخیم و همچنین کلاله غیرگسترده قابل شناسایی است. *P. ghazniensis* دومین نماینده از بخش *Lipskyanae* در ایران است که از نظر دارا بودن خامه کوتاه‌تر از گونه وابسته خود *P. botschantzeviana* تفکیک می‌شود. تصاویر گیاه و ویژگی‌های ریخت‌شناسی آن ارایه شده است.

واژه‌های کلیدی: فلور ایران، گزارش جدید، بخش *Lipskyana*، *Potentilla*

* faghirmb@guilan.ac.ir

مطالعه فلوریستیک منطقه امن آق‌داغ در منطقه حفاظت شده مراکان: استان آذربایجان غربی، ایران

هانیه نفیسی و فرخ قهرمانی‌نژاد *
گروه زیست‌شناسی گیاهی، دانشکده علوم زیستی، دانشگاه خوارزمی، تهران، ایران

چکیده

منطقه امن آق‌داغ با ۵۱۸۴/۷ هکتار در استان آذربایجان غربی یکی از سه منطقه امن منطقه حفاظت شده مراکان است. بر اساس ضریب آمبرژه آب و هوای این منطقه نیمه‌خشک سرد و بر اساس ضریب دومارتون نیمه‌خشک است. طی سال‌های ۱۳۸۷ تا ۱۳۸۸ از این منطقه ۲۲۷ تاکسون (گونه، زیرگونه، واریته) متعلق به ۴۷ تیره و ۱۶۶ جنس شناسایی شد. از این بین، ۲۰۵ تاکسون دولپه و ۲۱ تاکسون تک‌لپه و یکی بازدانه هستند. تیره‌های Asteraceae (۳۰ گونه)، Lamiaceae (۲۶ گونه) و به دنبال آن تیره‌های Brassicaceae، Caryophylaceae و Fabaceae غنی‌ترین تیره‌ها هستند و متنوع‌ترین جنس‌ها *Astragalus* با ۹ گونه و *Gallium* با ۵ گونه هستند. این منطقه شامل ۲۱ تاکسون بومزاد ایران و ۶ تاکسون نادر و ۲ جنس مونوتیپیک است. همچنین، در پژوهش حاضر برای نخستین بار، ۱۱ تاکسون از شمال‌غرب ایران و ۲۹ تاکسون از آذربایجان غربی گزارش می‌شود. شکل زیستی اغلب گیاهان منطقه، تروفیت‌ها با ۳۶/۵۷ و همی‌کریپتوفیت‌ها با ۲۷/۷۵ درصد هستند. از نظر جغرافیای گیاهی، منطقه تحت تأثیر عناصر ایرانی-تورانی (۳۱/۵۳ درصد) و ایرانی-تورانی/ اروپا-سیبری (۲۷/۴۹ درصد) هستند. بالاترین کسر از گیاهان منطقه متعلق به عناصر دو، سه یا چند منطقه‌ای (۵۹/۰۱ درصد) هستند.

واژه‌های کلیدی: آذربایجان غربی، ارس، فلور، کورولوژی، ایران

* ghahremaninejad@khu.ac.ir

یادداشت‌هایی بر *Aegilops cylindrica* (Triticeae, Poaceae) در ایران

سید ذبیح‌اله حسینی * و محمدرضا رحیمی‌نژاد
گروه زیست‌شناسی، دانشکده علوم، دانشگاه اصفهان، اصفهان، ایران

چکیده

تحقیق حاضر مبتنی بر مطالعه تاکسونومی ۱۰۰ نمونه جمعیتی از گونه *Aegilops cylindrica* جمع‌آوری شده از سرتاسر کشور و همچنین مطالعه نمونه‌های تیپ و نمونه‌های مربوط موجود در هرباریوم‌های موزه تاریخ طبیعی وین و برلین است. نتایج این مطالعه نشان داد که صفات ریخت‌شناختی نظیر: طول سنبله، طول سیخک، تعداد سنبلک‌ها در هر سنبله، حضور/غیبت کُرک روی سنبلک‌ها و محور در بین نمونه‌های جمعیتی مطالعه شده تنوع بالایی دارد. بر اساس نتایج پژوهش حاضر، گونه *A. cylindrica* در ایران با سه واریته که همگی گزارش جدید برای ایران هستند، شناخته می‌شود.

واژه‌های کلیدی: *Aegilops cylindrica*، Triticeae، Poaceae، تاکسونومی، ایران

* zhossini@gmail.com

بررسی آناتومی، گرده‌شناسی و انواع کرک گونه *Phlomis olivieri* از تیره Lamiaceae

حسین یوسفی [۱]، عاطفه امیراحمدی [۲]، مرتضی عطری [۱] و رضا نادری [۲]*
[۱] گروه زیست‌شناسی، دانشکده علوم، دانشگاه بوعلی سینا، همدان، ایران
[۲] دانشکده زیست‌شناسی و پژوهشکده علوم زیستی، دانشگاه دامغان، دامغان، ایران

چکیده

در مطالعه حاضر، صفات آناتومی، گرده‌شناختی و انواع کرک گونه *Phlomis olivieri* به منظور شناخت آنها جهت به‌کارگیری در اهداف سیستماتیک بررسی شد. نتایج نشان داد که دستجات آوندی در ساقه در کنار هم قرار دارند. مزوفیل از یک لایه پارانشیم نرده‌ای و سه تا چهار لایه پارانشیم اسفنجی تشکیل شده است. دو دسته آوند همجوار در مرکز و دو دسته آوند فرعی کوچک در حاشیه دمبرگ و چهار دسته آوند کوچک در برگک وجود دارد. پنج نوع اصلی کرک (سپری، غده‌ای سرسان، ستاره‌ای، ساده تک سلولی و درختی) مشاهده شد. کرک‌های سرسان به سه گروه طبقه‌بندی شدند: نوع اول (پایه کوتاه)، نوع دوم (پایه متوسط یا سه سلولی) و نوع سوم (پایه بلند یا چهار سلولی). کرک‌های ستاره‌ای به دو گروه بدون پایک یا پایه کوتاه و پایه بلند طبقه‌بندی شدند. کرک‌های درختی نیز برای نخستین بار در این گونه گزارش می‌شود. دانه‌های گرده سه شیاری، نسبتا بزرگ، تخم‌مرغی و تزیینات سطح لایه خارجی دانه گرده مشبک و روزن‌دار هستند.

واژه‌های کلیدی: آناتومی، تیره نعناییان (Lamiaceae)، گرده‌شناسی، کرک، *Phlomis*، ایران

* rezanaderia@du.ac.ir

گزارش عدد کروموزومی از جنس *Astragalus* بخش *Onobrychoide* از تیره Fabaceae در ایران

مسعود رنجبر [۱]*، آناهیتا حدیدچی [۲] و حسین ریاحی [۲]
[۱] گروه زیست‌شناسی، دانشکده علوم، دانشگاه بوعلی سینا، همدان، ایران
[۲] گروه علوم گیاهی، دانشکده علوم زیستی، دانشگاه شهید بهشتی، تهران، ایران

چکیده

در مطالعه حاضر، گزارش عدد کروموزومی میتوزی برای ۱۰ جمعیت متعلق به شش گونه از جنس *Astragalus* بخش *Onobrychoidei* شامل: *A. aduncus*، *A. arguricus*، *A. cancellatus*، *A. lilacinus* و *A. vegetus* ارایه شده است. تمام گونه‌های مطالعه شده دیپلوئید هستند و عدد ۲*n*=۲*x*=۱۶ را نشان می‌دهند که با عدد کروموزومی پایه پیش‌بینی شده *x*=۸ مطابقت دارد. علاوه بر این، مطالعات میوز تعداد کروموزوم ۲*n*=۲*x*=۱۶ را برای *A. aduncus*21 و *A. brevidens* همچنین ۲*n*=۴*x*=۳۲ را برای *A. vegetus*99 نشان داد. اگرچه جفت‌شدگی بی‌والان‌ها و تفکیک کروموزوم‌ها در این گونه عادی است، لیکن برخی ناهنجاری‌ها در طی میوز در این گونه مشاهده می‌شود.

واژه‌های کلیدی: *Astragalus*، عدد کروموزومی، Fabaceae، رفتار میوز، میتوز، ایران

* ranjbar@basu.ac.ir

واریته‌ای جدید از گونه *Trigonella persica* از تیره Fabaceae از ایران

مسعود رنجبر *، زهرا حاج مرادی و رؤیا کرمیان
گروه زیست‌شناسی، دانشکده علوم، دانشگاه بوعلی سینا، همدان، ایران

چکیده

در مطالعه حاضر، *Trigonella persica* var. *gandomanica* به عنوان واریته‌ای جدید از جنوب ایران شرح داده شده است. این تاکسون متعلق به بخش *Bucerates* است که بزرگترین بخش از گونه‌های یک‌ساله از جنس *Trigonella* است. این تاکسون نخستین واریته معرفی شده برای گونه *T. persica* از ایران است. *T. persica* var. *persica* نزدیک‌ترین تاکسون به این واریته است. با این وجود، واریته جدید با داشتن میوه‌های بدون کرک در مقابل میوه‌های کرکی از *T. persica* var. *persica* متفاوت می‌شود.

واژه‌های کلیدی: واریته جدید، Fabaceae، Section *Bucerates*، *Trigonella persica*، ایران

* ranjbar@basu.ac.ir

بررسی مجدد ریخت‌شناسی و تشریحی *Psilotum nudum* در جنگل‌های پست خزری، شمال ایران

فاطمه بازدیدوحدتی [۱]، شهریار سعیدی مهرورز [۱]*، علیرضا نقی‌نژاد [۲] و ربابه شاهی شاوون [۱]

[۱] گروه زیست‌شناسی، دانشکده علوم، دانشگاه گیلان، رشت، ایران

[۲] گروه زیست‌شناسی، دانشکده علوم پایه، دانشگاه مازندران، بابلسر، ایران

چکیده

Psilotum nudum یک نهانزاد اپی‌فیت ابتدایی است که اخیرا از جنگل‌های هیرکانی گزارش شده است. در مطالعات پیشین این گیاه به عنوان گونه‌ای اپی‌فیت از زیستگاه‌های متعدد در ناحیه پست خزری گزارش شده بود. به علاوه، ویژگی‌های ریخت‌شناسی و تشریحی *P. nudum* در دو جمعیت از این گونه در بخش مرکزی ناحیه هیرکانی بازنگری شد. نتایج مطالعه حاضر گویای این است که هیچ اختلاف معنی‌داری در ویژگی‌های ریخت‌شناسی و تشریحی مشاهده نشده است. ساقه هوایی گیاه خطی، سبز با انشعابات دو شاخه‌ای است. برگ‌ها تقلیل یافته، ساده، فلس‌مانند بدون زبانک هستند. سینانژیوم از اتحاد سه اسپورانژیای کپسول‌مانند تشکیل یافته است. همچنین، نوع استوانه آوندی در رأس ساقه هوایی و ساقه هوایی اکتینواستل و در ریزوم هاپلواستل است. در بررسی حاضر، بستر رشد جدیدی از این گونه روی تخته سنگ، از جنگل عطاکوه در گیلان گزارش شده است.

واژه‌های کلیدی: تشریح، ریخت‌شناسی، تخته سنگ جنگلی، جنگل نیمه کوهستانی عطاکوه، *Psilotum nudum*، ایران

* saeidimz@guilan.ac.ir

روابط فنتیک *Lolium* s.l. از تیره Poaceae در ایران بر اساس پروفایل لکه‌های فلاونوئیدی و صفات ریخت‌شناسی کمی

سهیلا رئیسی چهرازی [۱]، حجت‌اله سعیدی [۱] و مجید شریفی تهرانی [۲]*
[۱] گروه زیست‌شناسی، دانشکده علوم، دانشگاه اصفهان، اصفهان، ایران
[۲] گروه زیست‌شناسی، دانشکده علوم، دانشگاه شهرکرد، شهرکرد، ایران

چکیده

روابط بین گونه‌های *Lolium* و *Festuca* از دیرباز موضوع قابل توجه در تاکسونومی زیرقبیله Loliineae بوده است. مطالعه حاضر به بررسی روابط فنتیک در *Lolium* s.l. (شامل زیرجنس *Schedonorus* از جنس *Festuca*) با استفاده از پروفایل لکه‌های فلاونوئیدی و صفات ریخت‌شناسی کمی می‌پردازد. اندازه‌گیری صفات ریخت‌شناسی کمی و دانسیتومتری لکه‌های فلاونوئیدی و رسم پلات پروفایل آنها با استفاده از تصاویر کالیبره شده دیجیتال و نرم‌افزار ImageJ و تحلیل‌های چند متغیره خوشه‌ای و رسته‌بندی با استفاده از نرم‌افزار NTSYS-pc صورت گرفتند. هر یک از گونه‌های مطالعه شده بر اساس پروفایل لکه‌های توصیف شده و مقادیر *Rf* و درصد هر یک از لکه‌ها در پروفایل مربوط گزارش شده‌اند. تنوع موجود در لکه‌های پروفایل گونه‌های *L. rigidum*، *L. perenne* و *F. pratensis* نشان داد که پروفایل لکه‌های فلاونوئیدی را می‌توان به عنوان صفات مفید برای مطالعه بیشتر تنوع درون سطح گونه نیز مطرح نمود. تحلیل خوشه‌ای صفات ریخت‌شناسی کمی، گونه‌ها را در گروه‌هایی مجزا تفکیک نمود و جمعیت اردبیل از گونه *L. persicum* را نیز از سایر جمعیت‌های این گونه تفکیک نمود. تفکیک جمعیت‌های *F. arundinacea* به دو گروه مجزا نیز نتیجه‌ای قابل توجه است که وجود دو شکل مجزا از این گونه در ایران را پیشنهاد می‌کند.

واژه‌های کلیدی: روابط فنتیک، فلاونوئید، ریخت‌شناسی کمی، *Festuca*، *Lolium*

* sharifi-m@sci.sku.ac.ir

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Bulinska-Radomska and Lester, 1988) may lead to uninterpretable results. Careful selection of morphological characters, along with adoption of proper methods for analysis, will have great impact on resulting phenograms. Measurements of morphological characters reported in Table 4 may be of interest for those researchers working on gene pools of these taxa using molecular markers or for plant breeders or physiologists who want to know how these taxa may vary in their different morphological characters. The grouping of Iranian *F. arundinacea* populations into two distinct subgroups was an interesting result in our study which supported for existence of two forms in mixed populations. The applicability of quantitative morphological data to reveal phenetic relationships in this taxonomically complex group, and the potential usage of flavonoids to further description of taxa with biochemical data, and to study the variation held in their populations, were reported in this study and are intended for a more extensive study in tribe Poeae.

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F. arundinacea was closer to *L. rigidum* than to other outbreeding *Lolium* spp.

Results obtained from analysis of quantitative morphological characters in our study were concordant to Aiken's results. *F. arundinacea* although divided into two separate groups, showed close relationships with *L. rigidum*. Analysis of quantitative morphological data provided more resolution in *F. arundinacea*, and the separation of *L. persicum* Acc.#799 (Ardabil, Khalkhal) from other populations in this study was also in concordance with the molecular analysis by Sharifi-Tehrani *et al.* (2008). The application of flavonoids spot profiles for classification of *Festuca* and *Lolium* species in this study was comparable to SDS-PAGE profiles of seed proteins Aiken *et al.* (1998). Flavonoids spot Profiles belonging to the members of *F.* subgen. *Shedonorus* were similar to profiles of their relatives in genus *Lolium*. Presence/absence of spot profiles were not used here as qualitative data to elucidate the relationships, or to classify taxa, as the homology of spots are to be certified. Observed variations in spot profiles of the studied species, specially, in *L. rigidum*, *L. perenne*, and *F. pratensis* claimed for their applicability for investigating the chemical variation between the populations within the species level. TLC chromatograms of flavonoid extracts in this study provided sharp-enough bands which could be scored and analyzed. Close relationships between *F. pratensis* and *L. multiflorum* which was demonstrated by Pasakinskiene *et al.* (1998) through analysis of GISH bands, was also confirmed by both morphological data (Figure 4C; PCO plot) and flavonoids spot profiles (Figures 2F, 2B and 2D).

Application of quantitative morphological characters for phenetic classification of *Lolium* spp. was reported in a recent work by Oshib-Nataj *et al.* (2011), where the resultant dendrogram clustered the 33 specimens into the 5 species. Relationships between some Iranian members of festucoids (including *Lolium*) using AFLPs (Majidi *et al.*, 2006; Majidi and Mirlohi, 2010) showed the close relationships between *F. arundinacea* and *F. pratensis* specimens, to which group, the specimens belonging to *L. perenne* and *L. rigidum* were connected. Results obtained from analysis of AFLPs were concordant with the previous foundings about relationships in this genus. The study also demonstrated the application of AFLPs for genetic relationships studies in cool season grasses. The phenetic analysis of Iranian species of *Lolium* based on 27 morphological characters, measured on 68 specimens (6 species) dispersed *L. perenne* and *L. multiflorum* (closely related taxa) among other *Lolium* species which claimed for the inapplicability of morphological characters for phenetic analysis of *Lolium*, i. e. (Mirjalili *et al.*, 2008). Relationships between *Lolium* species in the resultant clustering scheme could hardly be accepted (see also Mirjalili and Bennett, 2006) regarding the previous results from many other literatures (see Darbyshire, 1993 and refs. there in for review).

Results of the current study based on quantitative morphological data and flavonoids spot profiles, were in concordance with the previous foundings about species relationships in this group, and produced interpretable groupings in both cluster- and PCO analyses which also further confirmed our previous founding about *L. persicum* population Khalkhal (Ardabil province of Iran). Flavonoid spot profile of *F. gigantea* was different from those of *F. arundinaceae* and *Festuca pratensis*. These results did not support the results of seed protein electrophoresis analysis as they produced similar seed protein profiles (Bulinska-Radomska and Lester, 1985).

Conclusions

For complex plant groups (such as *Lolium* s.l.) misleading characters should be identified and avoided. Many non-reproductive characters and certain reproductive characters (see

of *F. arundinacea* specimens based on quantitative morphological data was interesting. Another interesting result was the misplacement of one specimen belonging to *L. persicum* (acc.#799, collected from Ardabil province, Khalkhal) which was grouped with *F. pratensis* specimens. Cophenetic analysis (Figure 4B) showed that there was moderate levels (r = 0.58) of correlation between resultant dendrogram and the underlying distance matrix. However, the results were strongly confirmed with resultant plot from PCO analysis (Figure 4C) which showed the separation of *L. persicum*, partitioning of *F. arundinacea* and misplacement of *L. persicum* acc.#799. Results of PCO analysis confirmed that the first three axes had collected and expressed 82 percent of the variation held in raw data matrix (Table 5).

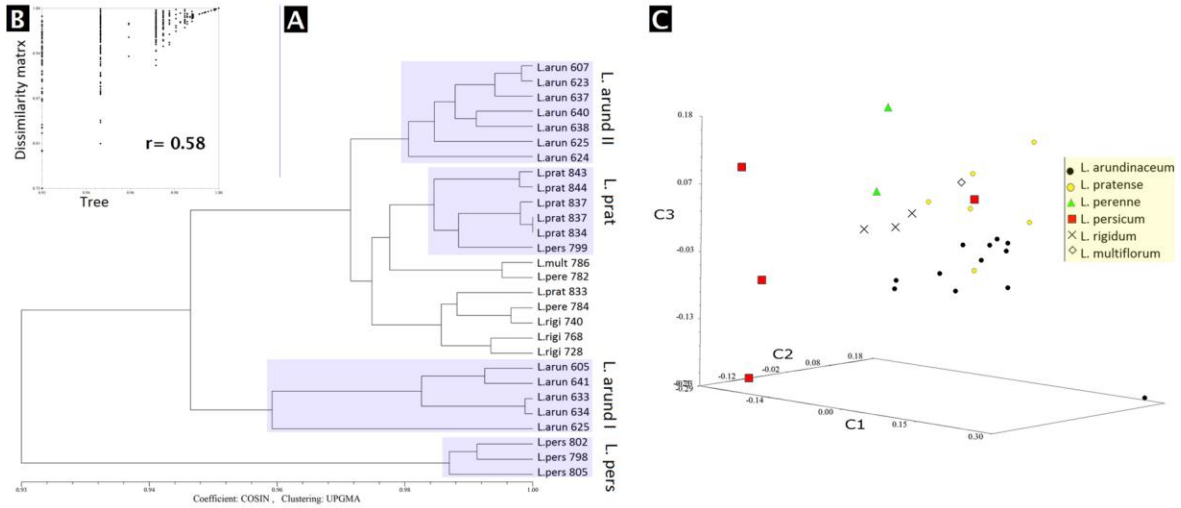

Figure 4. Multivariate analyses. A. Cluster analysis based on Cosine dissimilarity coefficient, and UPGMA as sorting method; B. Co-phenetic plot; C. Principal Coordinates analysis based on Cosine dissimilarity coefficient for quantitative data

Table 5. Axes loadings in PCO analysis. More than 82 percent of variation was expressed by three first axes

Axis	Eigen value	Percent	Cumulative%
1	1.70	33.70	33.70
2	1.41	27.89	61.59
3	1.05	20.78	82.37
4	0.77	15.19	97.56

Partitioning of *Lolium persicum* was reported in a previous work by Sharifi-Tehrani *et al.* (2008), so that the population Ardabil, Khalkhal (the population from which acc.#799 was collected), was distantly separated from other populations. Although population Khalkhal was clearly identified as *L. persicum* based on diagnostic characters and available keys, however, quantitative morphological studies and microsatellites provided evidence supporting for its separation from other *L. persicum* populations. To elucidate the taxonomic status of this population, more extensive studies are required.

SDS-PAGE analysis of seed protein profiles of taxa belonging to genera *Festuca* and *Lolium* proved to be useful for classification of festucoids (Aiken *et al*., 1998). Their method consisted scoring of each protein band identified based on R*f* values, and analyzing the qualitative data using Jaccard's coefficient. Their resultant dendrogram showed that members of *Festuca* subgen. *Schedonorus* (*F. arundinacea* and *F. pratensis*), had constructed a major group with *Lolium* spp., within which *F. pratensis* specimens were sub-grouped together, and

Results (Table 4) showed that quantitative morphological characters contained variations that could be used both for description of taxa and multivariate analysis to elucidate the phenetic relationships between them. Table 4 summarizes the data, as the first least- and most-variable characters for each taxon are reported, with annotations for their min, max, SD, and P-values (P is the standardized value, and is defined here as: range divided by the mean).

Table 4. Measurement of morphological characters. The unit of all measures is mm

Less variable characters						**More variable characters**					
Character	**Min**	**Max**	**Mean**	**SD**	**P**	**Character**	**Min**	**Max**	**Mean**	**SD**	**P**
F. arundinacea											
Awn pubescent length	0.05	0.06	0.05	0.00	0.20	Rachilla internode length	2.75	8.15	5.33	1.88	1.01
Lemma thickness	0.08	0.09	0.09	0.01	0.11	Upper glume width in CS	1.37	5.31	2.00	1.19	1.97
Lemma width	1.23	1.55	1.40	0.11	0.23	Auricle length	0.60	2.84	1.79	0.88	1.25
Glume base cilia length	0.07	0.41	0.15	0.12	2.27	Palea length	5.03	6.54	5.83	0.76	0.26
Style width	0.17	0.74	0.35	0.17	1.63	Lemma width in CS	1.12	2.13	1.63	0.71	0.62
F. pratensis											
Base of glume width	0.50	0.53	0.52	0.02	0.06	Sheath width in CS	3.26	6.91	4.76	1.91	0.77
Ovary width	0.50	0.58	0.52	0.04	0.15	Lower glume number of veins	1	3	1.5	1	1.33
Upper glume thickness	0.09	0.20	0.13	0.05	0.85	Lower glume length	1.50	3.59	2.75	0.81	0.76
L. multiflorum											
Upper glume thickness	0.16	0.18	0.17	0.01	0.12	Upper glume length	6.14	9.00	7.57	2.02	0.38
Anther width	0.37	0.40	0.39	0.02	0.08	Leaf width	4.88	5.74	5.31	0.61	0.16
Style width	0.26	0.35	0.31	0.06	0.29	Anther length	2.70	3.54	3.12	0.59	0.27
L. perenne											
Upper glume width in CS	2.13	2.21	2.17		0.04	Leaf thickness	0.12	0.20	0.16		0.50
Stem width adjacent to node	1.55	1.63	1.59		0.05	Anther width	0.49	0.57	0.53		0.15
Stem node length	1.48	1.56	1.52		0.05						
L. persicum											
Lemma thickness	0.08	0.09	0.09	0.01	0.11	Upper glume length	3.56	13.50	9.52	5.26	1.04
Leaf thickness	0.16	0.34	0.23	0.08	0.78	Upper glume width	0.80	2.19	1.64	0.62	0.85
						Palea width in CS	1.14	2.09	1.53	0.50	0.62
L. rigidum											
Palea width	1.30	1.46	1.39	0.08	0.12	Auricle length	1.38	2.84	2.16	0.73	0.68
Stem node length	1.50	1.69	1.62	0.11	0.12	Upper glume length	9.00	10.00	9.50	0.71	0.11
Floret length	7.23	7.40	7.32	0.12	0.02	Ovary+stigma length	1.33	2.30	1.70	0.53	0.57

Multivariate analysis

Multivariate analysis (cluster and PCO analyses) of six *Lolium* s.l. based on quantitative morphological characters was performed to elucidate the phenetic relationships between them. Resultant dendrogram using Cosine coefficient and UPGMA method (Figure 4A) showed that *L. persicum* specimens were grouped together and distantly separated from the rest of specimens. Specimens belonging to *F. pratensis* were also grouped together (except for *F. pratensis* acc.# 833; a specimen collected from Mt. Dena in central Zagros region). Specimens belonging to *F. arundinacea* were divided into two well defined groups. Geographical location of populations from which specimens of *F. arundinacea* were collected, did not support the sub-grouping of these specimens. However, the partitioning

(profile F) for this species which R*fs* ranged from 0.12 to 0.78. The most prominent spot was R*f* = 0.6 which contained 33.2% of the total flavonoids in the corresponding extract. *Lolium perene* L. (syn. *L. marschallii* Steven): Up to 11 distinct spots were identified (profiles G, H) for this species which R*f*s ranged from 0.1 to 0.8. The most prominent spot was R*f* = 0.6 (profile G) which contained 38.4% of the total flavonoids in the corresponding extract. *Lolium rigidum* Gaudin: Up to 9 distinct spots were identified (profiles E, I, J) for this species which R*f*s ranged from 0.1 to 0.83. The most prominent spot was R*f* = 0.6 (profile I) which contained 59.6% of the total flavonoids in the corresponding extract. *Lolium persicum* Boiss. and Hohen. ex Boiss.: 4 distinct spots were identified (profile K) for this species which R*fs* ranged from 0.14 to 0.46. The most prominent spot was R*f* = 0.19 which contained 27.7% of the total flavonoids in the corresponding extract. *Vulpia myuros* (L.) C.C.Gmel.: 4 distinct spots were identified (profiles L, M) for this species which R*f*s ranged from 0.05 to 0.68. The most prominent spot was R*f* = 0.05 (profile M) which contained 27.8% of the total flavonoids in the corresponding extract.

Festuca gigantea (L.) Vill. (syn. *Lolium giganteum* (Linnaeus) Darb.): 4 distinct spots were identified (profile N) for this species which R*fs* ranged from 0.11 to 0.59. The most prominent spot was R*f* = 0.4 which contained 49.6% of the total flavonoids in the corresponding extract. *Festuca alaica* Drobow: 5 distinct spots were identified (profile O) for this species which R*fs* ranged from 0.06 to 0.61. The most prominent spot was R*f* = 0.45 which contained 44.6% of the total flavonoids in the corresponding extract.

Morphology

Phenetic relationships between species belonging to *Lolium* s. str. plus those *Festuca* spp. routinely hybridize them (i. e. *Lolium* s.l., excluding *F. gigantea*) were studied using quantitative morphological characters. Measurements were performed using digital images taken from different parts of specimens, while a millimeter paper was in background of each photo. After calibration of images in ImageJ software, measurements were performed and a scale bar (white on black background) was superimposed on each photo and backgrounds were replaced with black color. Fertile parts of florets in six *Lolium* s.l. species are shown in Figure 3.

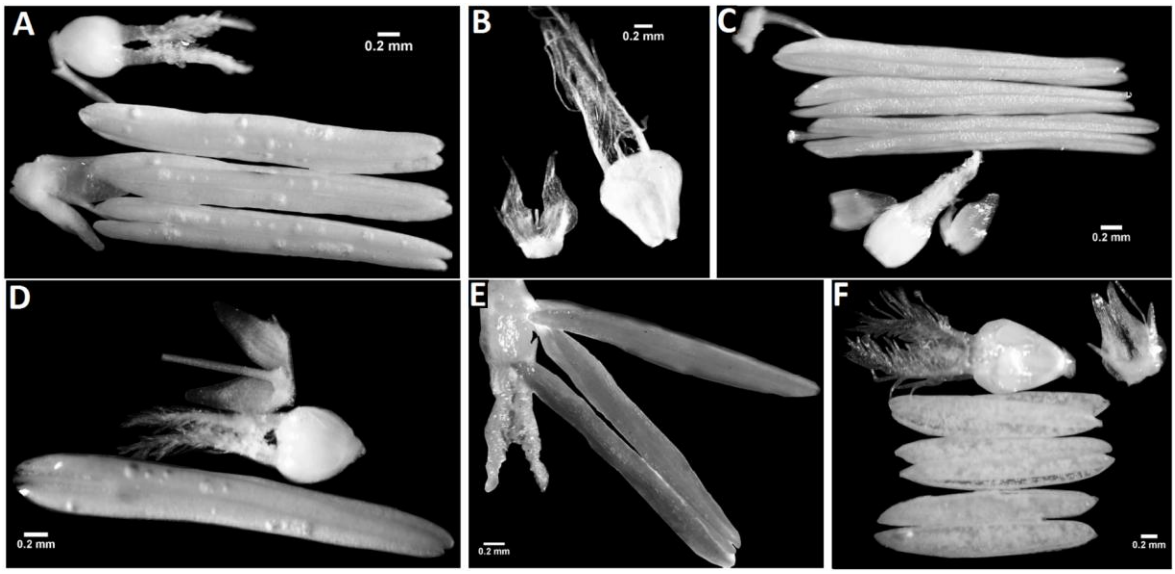

Figure 3. Fertile parts of florets in six *Lolium* s.l. species. Scale Bars length = 0.2 mm. A. *Festuca pratensis*; B. *L. multiflorum*; C. *L. rigidum*; D. *L. perenne*; E. *F. arundinacea*; F. *L. persicum*

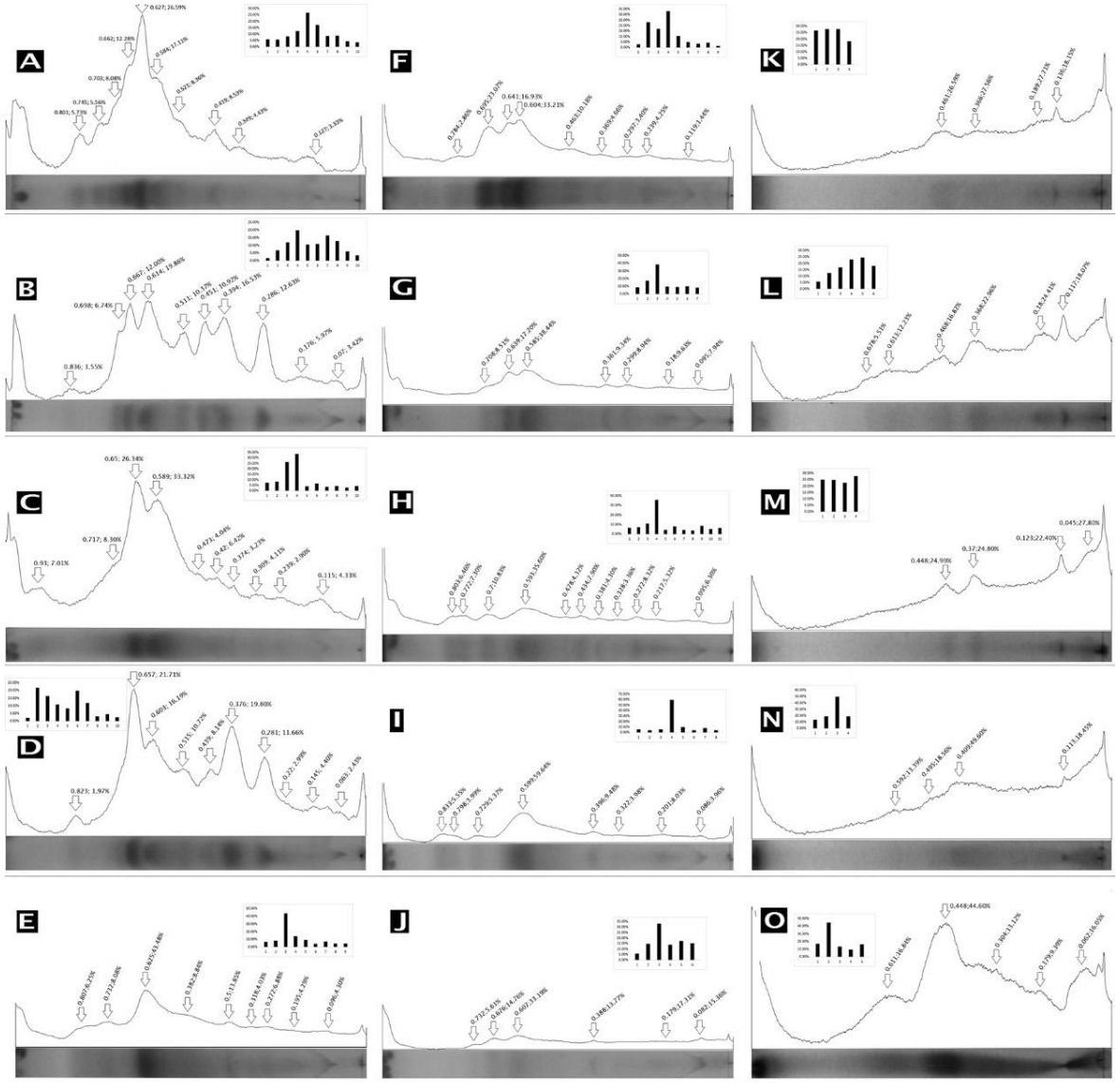

Figure 2. Plots showing location (R*f*), intensities and percentage of each flavonoid spot in selected specimens. Arrows point to pick of each spot, numbers above each arrow: first number corresponds to R*f* values, and second numbers are percentage of each spot in its profile. Bar charts are drawn according to percentage values. A. *Festuca arundinacea*; B. *F. pratensis*; C. *F. sclerophylla*; D. *F. pratensis*; E. *L. rigidum*; F. *L. multiflorum*; G. *L. perenne*; H. *L. perenne*; I. *L. rigidum*; J. *L. rigidum*; K. *L. persicum*; L. *Vulpia myuros*; M. *V. myuros*; N. *F. gigantea*; O. *F. alaica*.

Festuca arundinacea L. (syn.: *Lolium arundinaceum* (L.) Darb.): 10 distinct spots were identified (profile A) for this species which R*fs* ranged from 0.13 to 0.8. The most prominent spot had R*f* = 0.63 which contained 26.6% of the total flavonoids in the corresponding extract. *Festuca pratensis* Hudson (syn.: *Lolium pretense* (Hudson) Darb.): 10 distinct spots were identified (profiles B, D) for this species which R*f*s ranged from 0.06 to 0.84. The most prominent spot had R*f* = 0.66 (profile D) which contained 21.7% of the total flavonoids in the corresponding extract. *Festuca sclerophylla* Boiss. et Hohen. (syn. *Leucopoa sclerophylla* (Boiss. et Hohen.) Krecz. et Bobr.): 10 distinct spots were identified (profile C) for this species which R*fs* ranged from 0.11 to 0.93, and the most prominent spot had R*f* = 0.59 which contained 33.3% of the total flavonoids in the corresponding extract. *Lolium multiflorum* Lam. (syn. *L. italicum* Braun): 9 distinct spots were identified

Morphology

Sixty eight quantitative morphological characters from both vegetative (17) and reproductive (51) characters were used for morphological study (Table 2). Each character was measured 3 to 6 times (independent measures on same specimen) to calculate averages and standard deviations (Table 4). Measurements were performed on several digital images taken with 15 megapixels Cannon EOS 500D camera capable to connect to stereomicroscope, and using millimeter-papers to calibrate the images. Calibration of images was performed using ImageJ software, and measurements were transferred to Excel datasheets to calculate the basic statistics (min, max, average, and SD). Inapplicable characters such as 'upper glume length' for *Lolium* spp. specimens were considered as missing values. Data were converted to NTS format of NTSYS-pc software and analyzed using Simint, Njoin, SAHN, Eigen and Mod3D modules. Cosine distance (dissimilarity) coefficient ($C_{ij} = \sum_k (x_{ki} - x_{kj}) / \sum_k x_{ki}^2 \sum_k x_{kj}^2$) was used for calculating dissimilarity matrix, and UPGMA was used as the sorting method in cluster analysis. Same coefficient was used for PCO analysis.

Results and Discussion

Flavonoids spot profiles

Solvent system was optimized to achieve best separation of flavonoid spots in one dimensional TLCs (Figure 1). Two dimensional test TLCs moved spots on diameter of TLC and confirmed efficient separation of spots using one dimensional TLCs. To document the process of optimization of solvent system, a database system (unpublished) was designed to help storage and retrieval of our TLCs data containing components of each tested solvent, list of samples on each chromatogram, TLC images, and to making specialized reports.

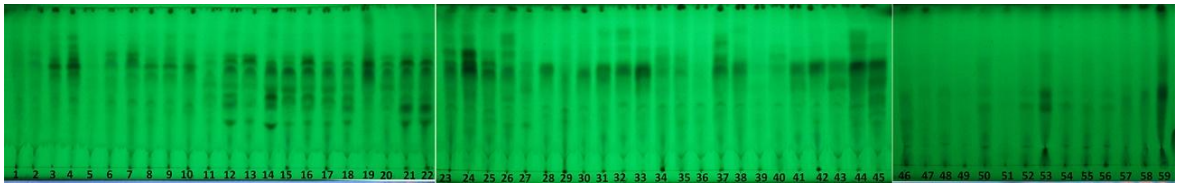

Figure 1. TLC chromatograms of selected specimens. Number beneath each lane refers to numbers in 'TLC lane' and corresponding specimen in Table 1

Flavonoids were separated in range R*f* = 0.045 to R*f* = 0.93. Number of identified flavonoid spots (separate R*f* values) in each extract ranged from 4 spots in *L. persicum* and *V. myuros* (K, M in Figure 2), to 11 spots in *L. perenne* (H in Figure 2). Percentage of spots in their corresponding extracts ranged from 1.44% (spot 1 in *L. multiflorum*; F) to 59.64% (spot 5 in *L. rigidum*; I).

Plots of *F. arundinacea*, *F. pratensis*, *F. sclerophylla* and *L. multiflorum* (A; B, D; C; F in Figure 2) were topologically similar, showing large spots in R*f* range 0.5-0.7. Plots of *L. perenne* samples (G, H in Figure 2) were close to this group, but with less strong spots (area under curve, or percentage of picks). Four plots belonging to *V. myuros*, *L. persicum* and *F. gigantea* were also similar, although the members of this group were distantly connected together. Plot of *F. alaica* was distinct, containing a large spot in R*f* 0.45. Flavonoid spot profile of *L. persicum* was most similar to that of *F. gigantea* (syn. *L. giganteum sensu* Darbyshire). Percentage of each spot and its corresponding R*f* value for all specimens are presented in Table 3.

water: 20, acetic acid: 20, iso-propanol: 10, butanol: 50. Separated flavonoid spots on TLCs were visualized under UV 254nm and 366nm and digitally photographed using a Canon EOS 500D digital camera. Skewness of images was corrected in Adobe Photoshop software ver. 13.0 CS6 x64 extended. Then, images were calibrated in ImageJ software ver. 1.47s (Rasband, 2008) and for each specimen, the flavonoid spots profile were plotted. Migration distance for each spot was measured in ImageJ software, and data transferred to Microsoft Excel 2013 to calculate the R*f* values (Table 3). The area under each flavonoid spot in each profile was measured in ImageJ software and data were transferred to Microsoft Excel 2013 to calculate the percentage of each spot in its corresponding profile, where the bar graphs for each profile were drawn.

Table 3. R*f* values and percentage of each identified flavonoid spot in its corresponding profile. Letters in first column are same as letters in Figure 2. Numbers in first row are numbers of spots on the chromatogram from small R*f*s to large R*f*s (right to left on chromatogram)

	Spots		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
A	*F. arundinacea*	R*f*	0.127	0.349	0.419	0.521	0.584	0.627	0.662	0.703	0.745	0.801	
		%	3.33%	4.43%	8.53%	8.36%	17.11%	26.59%	12.28%	8.08%	5.56%	5.73%	
B	*F. pratensis*	R*f*	0.07	0.176	0.286	0.394	0.451	0.511	0.614	0.667	0.698	0.836	
		%	3.42%	5.97%	12.63%	16.53%	10.92%	10.37%	19.86%	12.00%	6.74%	1.55%	
C	*F. sclerophylla*	R*f*	0.115	0.239	0.309	0.374	0.42	0.473	0.589	0.65	0.717	0.93	
		%	4.33%	2.90%	4.11%	3.23%	6.42%	4.04%	33.32%	26.34%	8.30%	7.01%	
D	*F. pratensis*	R*f*	0.063	0.145	0.22	0.281	0.376	0.439	0.515	0.603	0.657	0.823	
		%	2.43%	4.40%	2.99%	11.66%	19.80%	8.14%	10.72%	16.19%	21.71%	1.97%	
E	*L. rigidum*	R*f*	0.096	0.195	0.272	0.318	0.382	0.5	0.625	0.732	0.807		
		%	4.30%	4.29%	6.88%	4.03%	8.84%	13.85%	43.48%	8.08%	6.25%		
F	*L. multiflorum*	R*f*	0.119	0.239	0.297	0.369	0.463	0.604	0.641	0.695	0.784		
		%	1.44%	4.25%	3.40%	4.66%	10.18%	33.21%	16.93%	23.07%	2.86%		
G	*L. perenne*	R*f*	0.095	0.18	0.299	0.361	0.585	0.639	0.708				
		%	7.94%	9.63%	8.94%	9.34%	38.44%	17.20%	8.51%				
H	*L. perenne*	R*f*	0.095	0.217	0.272	0.328	0.381	0.434	0.478	0.593	0.7	0.772	0.803
		%	6.30%	5.32%	8.32%	3.36%	4.30%	7.90%	4.32%	35.60%	10.83%	7.30%	6.46%
I	*L. rigidum*	R*f*	0.086	0.201	0.322	0.396	0.599	0.729	0.798	0.833			
		%	3.96%	8.03%	3.98%	9.48%	59.64%	5.37%	3.99%	5.55%			
J	*L. rigidum*	R*f*	0.082	0.179	0.388	0.607	0.676	0.732					
		%	15.36%	17.31%	13.77%	33.19%	14.76%	5.61%					
K	*L. persicum*	R*f*	0.136	0.189	0.366	0.461							
		%	18.15%	27.71%	27.56%	26.59%							
L	*V. myuros*	R*f*	0.117	0.18	0.368	0.468	0.613	0.678					
		%	18.07%	24.41%	22.96%	16.82%	12.23%	5.51%					
M	*V. myuros*	R*f*	0.045	0.123	0.37	0.448							
		%	27.80%	22.40%	24.80%	24.99%							
N	*F. gigantea*	R*f*	0.113	0.409	0.495	0.592							
		%	18.45%	49.60%	18.56%	13.39%							
O	*F. alaica*	R*f*	0.062	0.179	0.304	0.448	0.611						
		%	16.05%	9.39%	13.12%	44.60%	16.84%						

Table 2. List of morphological characters

Organ	No.	Character name	Organ	No.	Character name
Stem	1	Stems; node length	Floret	35	Floret; width of callus
	2	Nodes; width		36	Floret; length
	3	Stem; width adjacent to node		37	Floret; width
Leaf	4	Leaf; length	Lemma	38	Lemma; length
	5	Leaf; width		39	Lemma; width
	6	Leaf; thickness		40	Lemma; length complete - in CS
	7	Leaf; number of leaf veins		41	Lemma; thickness
Flag leaf	8	Flag leaf; length		42	Lemma; number of veins
	9	Flag leaf; width	Palea	43	Palea; length
	10	Flag leaf; thickness		44	Palea; width
	11	Flag leaf; number of veins		45	Palea; length complete - in CS
Sheath	12	Sheath; width		46	Palea; thickness
	13	Sheath; width-complete		47	Palea; number of veins
	14	Sheath; thickness	Awn	48	Awn; length
	15	Ligule; length		49	Awn; lemma tip to awn base, distance
	16	Auricle; length		50	Awn; width
	17	Auricle; cilia length		51	Awn; length of pubescent
Rachilla	18	Rachilla; inter-node length	Stamen	52	Stamen; number
	19	Rachilla; inter-node width		53	Anther; length
Glume	20	Base of glume; width		54	Anther; width
	21	Base of glume; cilia length		55	Filament; length
	22	Lower glume; length	Gynoecium	56	Ovary+Stigma; length
	23	Lower glume; width		57	Ovary; width
	24	Lower glume; width-complete		58	Stigma; length
	25	Lower glume; thickness		59	Style; width
	26	Lower glume; number of veins		60	Lodicule; number
	27	Upper glume; length		61	Lodicule; length
	28	Upper glume; width		62	Lodicule; width
	29	Upper glume; width-complete		63	Caryopsis; length
	30	Lower glume; thickness		64	Caryopsis; width
	31	Lower glume; number of veins	Terminal spikelet	65	Terminal spikelet; lower glume length
Spikelet	32	Spikelet; length		66	Terminal spikelet; lower glume width
	33	Spikelet; number of florets		67	Terminal spikelet; upper glume length
	34	Spikelet; axis inter-node length		68	Terminal spikelet; upper glume length

Extraction of flavonoids and TLC

Plant materials (leaves) were ground to a fine powder using mortar and pestle. Extraction was performed using 80% MeOH for 36 h according to Markham (1982) with modifications. Solvent of the filtrate was evaporated using rotary evaporator in 50-60° C under relative vacuum. Dried extracts were dissolved in distilled water and filtered to discard fatty substances. Aqueous extract was dried again and dissolved in 5 ml MeOH. One dimensional thin layer chromatography was performed using 20 cm × 10 cm Aluminum sheets covered with silica gel 60F254 (Merck). Solvent system consisted of

Materials and Methods

Plant material

Plant materials were collected from wild populations throughout their distribution ranges in Iran (Table 1). Specimens were paper-dried, and determined using identification keys available in Flora Iranica and Flora of Turkey (Bor, 1970; Davis *et al.*, 1988). Seventy eight samples were chosen for flavonoid extractions or morphological studies (Tables 1, 2). Total flavonoids were extracted from 0.6 to 1.0 gram of dried leaves of 59 selected specimens belonging to ten species from three closely related genera.

Table 1. Plant material collected from wild populations in Iran. Acc: accession numbers of each specimen; TLC lane: corresponding flavonoid profile in Figure 1; S: corresponding plot in Figure 2

Alt. (m)	Loc	Acc	TLC Lane	S	Alt. (m)	Loc	Acc	TLC Lane	S
Festuca arundinacea					***Lolium rigidum***				
2095	Ardabil, Sabalan	643	1		2030	Sarab, Sabalan	724	28	
1880	Fars, Arzhan	633	2		2014	Ardabil, Sabalan	728	29	
2448	Hamadan, Tuyserkan	606	3		2014	Ardabil, Sabalan	730	30	
2448	Hamadan, Tuyserkan	607	4	A	1534	Urmia, Shahrchay	709	31	
2450	Kashan, Ghohroud	637	5		1534	Urmia, Shahrchay	727	32	I
2560	Kerman, Sardouyeh	624	6		1534	Urmia, Shahrchay	758	33	
2000	Yasouj, Sisakht	632	7		-	Fars, Sarvestan	747	34	
1790	Yasouj, Sisakht	669	8		-	Kerman, Estahban	752	35	
2150	Yasouj, Sisakht	671	9		-	Kerman, Estahban	759	36	
2450	Lurestan, Gahar	675	10		1790	Yasouj, Sisakht	712	37	
2898	Hamadan, Alvand	605	-		1326	Kud, Zaribar Lake	704	38	
2000	Yasouj, Sisakht	625	-		500	Ramsar	735	39	
2560	Kerman, Sardouyeh	623	-		40	Ramsar to Chaboksar	737	40	J
2095	Ardabil, Sabalan	641	-		2050	Semnan, Nekarman	740	41	
2104	Ardabil, Sabalan	640	-		2050	Semnan, Nekarman	767	42	
1700	Kashan, Ghamsar	638	-		1960	Tehran, Firouzkouh	718	43	
2450	Kashan, Ghohroud	637	-		1960	Tehran, Firouzkouh	738	44	E
1880	Fars, Arzhan	633	-		1960	Tehran, Firouzkouh	769	45	
1880	Fars, Arzhan	634	-		1960	Tehran, Firouzkouh	768	-	
festuca pratensis					***Lolium persicum***				
2000	Sarab, Sabalan	831	11		1618	Ardabil, Khalkhal	799	46	
2187	Chalaous, Moroud	841	12	B	35	Rasht, Parrehsar	821	47	
2196	Hamadan, Alvand	843	13		160	Tonekabon, Road 2000	804	48	K
2606	Isfahan, Semirom	835	14		500	Kheyroudkenar Jungle	819	49	
2000	Ramsar, Javaherdeh	826	15		900	Ramsar to Javaherdeh	802	-	
2000	Ramsar, Javaherdeh	834	16		1790	Yasouj, Sisakht	805	-	
2050	Semnan, Nekarman	824	17		1880	Fars, Arzhan	798	-	
1960	Tehran, Firouzkouh	830	18		***Vulpia myuros***				
2050	Semnan, Nekarman	878	20		1686	Asalem to Khalkhal	862	50	L
2050	Semnan, Nekarman	881	21	D	250	Asalem to Khalkhal	233	51	
2050	Semnan, Nekarman	885	22		460	Azadshahr	231	52	
Festuca sclerophylla					-	Herbarium Loan	1365	53	
2100	Karaj, Gachsar	852	19	C	460	Azadshahr	868	54	
Lolium perenne					1850	Yasouj, Sisakht	857	55	
1850	Chalous, Moroud	782	25	G	500	Kheyroudkenar Jungle	854	56	M
2050	Semnan, Nekarman	693	26	H	***Festuca gigantea***				
-	Herbarium loan	1369	27		-	Galougah to Timaj	1366	57	N
2847	Hamadan, Alvand	844	-		-	Asalem to Khalkhal	1367	58	
2187	Ardabil, Sabalan	837	-		***Festuca alaica****				
2000	Ramsar, Javaherdeh	834	-		-	Herbarium Loan	1368	59	O
2150	Yasouj. Mt. Dena	833	-		***Lolium multiflorum***				
2187	35 Km Chalous Road, Moroud village	782			-	Karaj	787	23	
1880	Fars, Arzhan	784	-		-	Karaj	786	-	
					1880	Fars, Arzhan	795	24	F

Darb., and *L. pratense* Darb. *Festuca* subgen. *Festuca* sect. *Ovinae* Fries (syn.: sect. *Festuca*), encompasses two controversial aggregates namely *F. ovina* L. and *F. rubra* L., according to the classification proposed by Hackel (1882), and with hundreds of species, subspecies, varieties, subvarieties, and formes, published so far. Stace *et al.* (1992) reduced all named morpho-anatomical variations into those two aggregates. He stated that only two characters (Sheaths of young tiller-leaves: fused/free, and tillers: extravaginal/ intravaginal) could definitely be used to distinguish between *Festuca ovina* and *F. rubra* aggregates; supporting the notion proposed by Hackel (1882) who divided the section into two main groups; *Intravaginales* and *Extravaginales vel Mixtae*. Phylogenetic studies based on cpDNA-RFLP (Darbyshire and Warwick, 1992) and ribosomal ITS (Charmet *et al.*, 1997; Gaut *et al.*, 2000; Torrecilla and Catalan, 2002) have demonstrated the paraphyly of *Festuca* and that, *Festuca* may include *Lolium* and *Vulpia* C.C.Gmel., therefore, choosing between the transfer of *F*. subgen. *Schedonorus* as a new genus (Soreng and Terrell, 1998), or realignment of it with genus *Lolium* (Darbyshire, 1993) remained to be studied. Torrecilla and Catalan (2002) worked on two main lineages in festucoids, namely "fine-leaved" and "broad-leaved fescues", and demonstrated that *Lolium* species were close relatives of broad-leaved fescues, and that the polyphyletic *Vulpia* was a close relative of fine-leaved fescues lineage. They confirmed that a monophyletic *Festuca* might encompass species from *Vulpia*, *Leucopoa* Griseb., *Schedonorus* and *Lolium*, while realignment of *Schedonorus* with *Lolium* would remain the rest of the clade as a large polyphyletic assemblage. Phylogeny of the festucoids based on nucleotide sequences of ITS and *trn*L-F regions (Catalan *et al.* 2004) showed that *Lolium*, *Mycropyropsis* and *Festuca* subgen. *Schedonorus* were close relatives, and they fell into a single monophyletic clade. Although several appreciated studies have been already performed in this complex group (Catalan *et al.*, 1997; Catalan, 2002; Torrecilla *et al.*, 2003; Catalan *et al.,* 2004; Torrecilla and Torrecilla *et al.*, 2004; Catalan, 2006; Muller and Catalan, 2006), the festucoids, *Festuca* s. str. and *Lolium* s.l. are still open and interesting subjects to be studied for more details.

Bor (1970) described six species of genus *Lolium* s. str. for Iran: *L. perenne* L., *L. multiflorum* Lam. (syn: *L. italicum* A.Br.), *L. rigidum* Gaud. (syn: *L. strictum* Presl.), *L. persicum* Boiss. and Hohen. ex Boiss., *L. temulentum* L. and *L. loliaceum* (Bory and Chaud.) Hand.-Mazz which was later considered as a synonym for *L. rigidum* subsp. *lepturoides* Sennen and Mauricio (legitimate name). They coincided with members of *Festuca* subgen. *Shedonorus* in high mountain elevations and mesic habitats along Alborz and Zagros chains. Flavonoids have long been proved as important characters in plant systematics and biosystematics researches, and they were still continue to take part specially in biosystematics researches (Sharifi-Tehrani and Ghassemi Dehkordi, 2011; Ghassemi Dehkordi *et al.*, 2012; Sharifi-Tehrani *et al.*, 2012).

This study was aimed to evaluate the relationships in *Lolium sensu* Darbyshire in Iran, using flavonoids spot profiles and quantitative morphological characters. *Lolium* specimens were studied here along with specimens from *Festuca* subgen. *Schedonorus* and from more distantly sister group, *Festuca* subgen. *Festuca*. This was the first report on the numerical analysis of quantitative morphological characters of *Lolium* s.l. in Iran. This study was also the first one to report application of digitally measured flavonoids spot profiles in the chemotaxonomy of the group in Iran. Relevance of this study was due to the importance of members of *Lolium* s.l. as economic hay and forage plants in Iran and the neighboring countries in the west of Mediterranean region, and also the relative absence of *F. sclerophylla* Boiss. ex Bisch. and *L. persicum* in previous studies.

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Phenetic relationships among *Lolium* s.l. (Poaceae) in Iran based on flavonoids spot profiles and quantitative morphology

Soheila Raeisi Chehrazi [1], Hojatollah Saeidi [1] and Majid Sharifi-Tehrani [2]*

[1] Department of Biology, Faculty of Sciences, University of Isfahan, Isfahan, Iran
[2] Department of Biology, Faculty of Sciences, University of Shahrekord, Shahrekord, Iran

Abstract
Relationships between species of *Lolium* and *Festuca* have long been an interesting subject in taxonomy of the subtribe Loliineae. This study was concerned with the phenetic relationships of *Lolium* s.l. (including *Festuca* subgen. *Schedonorus*) using flavonoids spot profiles and quantitative morphological characters. Measurement of morphological characters and densitometry of flavonoids spots and their profile plots were performed by using calibrated digital images and ImageJ software package. Multivariate analyses (clustering and ordination) performed by using NTSYS-pc software package. Each species was described based on its flavonoid spot profile, and R*f* values and percentage of each spot in the corresponding profile were reported. Variation in flavonoid spot profiles of *Lolium rigidum*, *L. perenne* and *Festuca pratensis* revealed that flavonoids spot profiles revealed that they may be useful characters for further studying the variations within the species level. Cluster analysis of quantitative morphological characters separated the species in well defined groups and further separated *L. persicum* population Ardabil from other *L. persicum* populations. Separation of *F. arundinacea* populations into two distinct groups was also interesting which suggested that the existence of two forms of this species in Iran is probable.
Key words: *Festuca*, Flavonoids, *Lolium*, Morphology, Phenetics, Quantitative

Introduction

Relationships between species of *Lolium* L. and *Festuca* L. have long been remained as a controversy in taxonomy of the subtribe Loliineae. *Lolium* which was first classified in the tribe Triticeae Dumortier based on the morphology of inflorescence, was later transferred to the tribe Poeae (= Festuceae) by Nevski (1934). Many non-morphological evidences from different sources of data supported this transfer (see Darbyshire, 1993). Inter-generic hybridization between *Festuca* subgen. *Schedonorus* (P. Beauvois) Petermann and outbreed species of *Lolium* (specially, *L. perenne* L.) along with other evidences from cytology, anatomy, molecular markers and genomic and plastid DNA, supported the union of *F.* subgen. *Schedonorus* and *Lolium*. Darbyshire (1993) suggested the realignment of *F.* subgen. *Schedonorus* with the genus *Lolium*, and introduced new combinations: *Lolium* subgen. *Schedonorus* Darb., *L. arundinaceum* Darb., *L. giganteum* Darb., *L. mazzettianum*

* Corresponding Author: sharifi-m@sci.sku.ac.ir

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stele was a protostele (haplostele), which supported our observations in this research.

The size and shape of the stomata are taxonomically important characters (Tahir and Rajput, 2009). In our study, stomata type was anomocytic; this is in agreement with observations reported by Roux (2003) and Mickle (2012).

Conclusion

In the current investigation, the morphological and anatomical studies on *P. nudum* indicate similarity between the samples collected from the two habitats, and a comparison of our results with other researcher's studies on *Psilotum* species indicates apparent differences with the *P. flaccidum* species and confirms the existence of *P. nudum* in forest ecosystem of Guilan.

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Discussion

Habitat preferences

Psilotum nudum was discovered in a lowland Hyrcanian forest of Ramsar in 2003 (Rezaei, 2003). It was mainly growing on the trunks slots and roughness of *Parrotia persica* trees in a very shady habitat. During last ten years after the first discovery of the species, this species was also reported elsewhere as epiphyte on some other species e.g. *Alnus glutinosa* (L.) Gaertn. in Chalus valley (Nazarian *et al.*, 2010), Nowshahr (Dr. Habib Zare, personal communication) and in Ramak valley in Ramsar (Naqinezhad and Zarrei, in preparation). In all mentioned habitats, *P. nudum* was found as epiphyte on lowland trees. Moreover, Khullar (2008) stated that it grew on the ground or in rock crevices, and Morgan (1962) reported that *P. triquetrum* had been found in rock fissure through which water is flowing. In the current study, a new different habitat, rocks, was found for this species in Ata-Kuh forest and also, as an epiphyte on *Parrotia persica*, in the Ramsar forest, confirming previous findings.

These habitats are under the threat of damage and destruction by road building and human activites. Therefore, they should be regarded as threatened species according to IUCN categories and criteria.

Anatomical and morphological reexamination

We decided to investigate *Psilotum nudum* morphologically and anatomically in detail. Our morphological findings of *P. nudum* were consistent with the morphological description of the taxon, of Ford (1904), Stiles (1910), Sporne (1962), Khoshravesh *et al*. (2009), Nazarian *et al*. (2010), and Singh *et al*. (2010).

According to Singh *et al*. (2010) *P. flaccidum* was 90 cm long and the leaves were in opposite position on the edges of plane branches, while *P. nudum* was 25-35 cm long and the leaves were placed in alternate position. Another comparable feature with respect to *P. nudum* and *P. flaccidum* in stem morphology was in the form of the aerial stem. In *P. flaccidum* the lower part was triangular and the upper part flattened in one plane, while in *P. triquetrum* this is multiangular in the lower parts and triangular in the ultimate branches (Stiles, 1910). Our results showed that the cross section of *P. nudum* stem supports this idea. Moreover, in another report by Sporne (1962), *P. nudum* had upright habit and *P. flaccidum* had pendulous habit.

Our results, like those of Ford (1904), Sporne (1962), Khullar (2008), Khoshravesh *et al*. (2009), Nazarian *et al*. (2010), and Singh *et al*. (2010), showed that the cortex in the stem was differentiated into three zones (outer photosynthetic, middle sclerenchymatous and inner parenchymatous) and the sclerenchymatous layers were responsible for mechanical protection of the plant. The result of this study showed that stele in the aerial stem was protostele (actinostele). The stele has been interpreted as a protostele (actinostele) by Schulte *et al*. (1987), Khoshravesh *et al*. (2009), and Singh *et al*. (2010) and as a siphonostele by Nazarian *et al*. (2010) and Gifford and Foster (1988). Khullar (2008) also mentioned that if the pith was considered as composed of sclerenchymatous cells, then the stele was a siphonostele, but if these sclerenchymatous cells belonged to the xylem, then it was a protostele. Also, our results showed that the cortex in the rhizome was differentiated into three zones (outer, middle and inner); this finding was in agreement with results obtained by Sporne (1962) and Ford (1904). Also, we found that stele in the rhizome was a protostele (haplostele), since non-differentiated tracheids to protoxylem and metaxylem in the pith are surrounded by phloem. Nazarian *et al*. (2010) have reported the stele is protostel (actinostele) in the rhizome. However, Singh *et al*. (2010) have reported that the

Table 1. Anatomical characters of *Psilotum nudum* in Ata-Kuh and Ramsar forests

	Characters	*P. nudum* (Ata-Kuh)	*P. nudum* (Ramsar)
Aerial stem	Cross section shape	Starlike	Pentagonal
	Cuticle diameter (µm)	12.5	11
	Epidermal cell shape	Oblong	Oblong
	Epidermis diameter (µm)	23	24
	Stomata type	Anomocytic	Anomocytic
	Outer parenchyma layer number	2-4	1-3
	Outer parenchyma diameter (µm)	32	31.5
	Sclerenchyma layer number	4-6	3-6
	Sclerenchyma diameter (µm)	100	98
	Inner parenchyma layer number	8-12	10-12
	Inner parenchyma diameter (µm)	131	132
	Endodermis cells	+	+
	Stele type	Protostele (Actinostele)	Protostele (Actinostele)
Apex aerial stem	Cross section shape	Triangular	Triangular
	Cuticle diameter (µm)	7.5	7.5
	Epidermal cell shape	Quadrangular	Quadrangular
	Epidermis diameter (µm)	25	23.5
	Stomata type	Anomocytic	Anomocytic
	Cortex diameter (µm)	179	181
	Endodermis cells	+	+
	Stele type	Protostele (Actinostele)	Protostele (Actinostele)
Rhizome	Cross section shape	Elliptic-Circle	Elliptic-Circle
	Cuticle diameter (µm)	7.5	7.5
	Epidermal cell shape	Quadrangular	Quadrangular
	Epidermis diameter (µm)	40	37
	Cortex diameter (µm)	475	468
	Phlopaphn	+	+
	Endodermis cells	+	+
	Pericycle	+	+
	Stele type	Haplostele	Haplostele

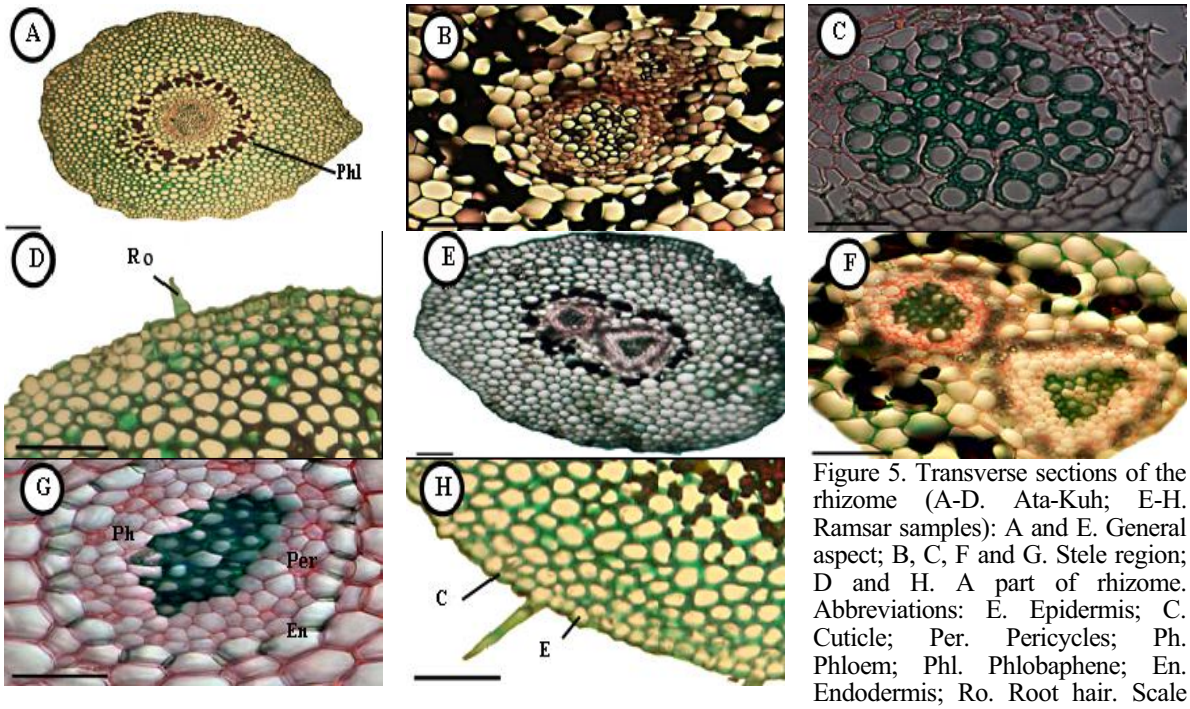

Figure 5. Transverse sections of the rhizome (A-D. Ata-Kuh; E-H. Ramsar samples): A and E. General aspect; B, C, F and G. Stele region; D and H. A part of rhizome. Abbreviations: E. Epidermis; C. Cuticle; Per. Pericycles; Ph. Phloem; Phl. Phlobaphene; En. Endodermis; Ro. Root hair. Scale Bar = 0.1 mm

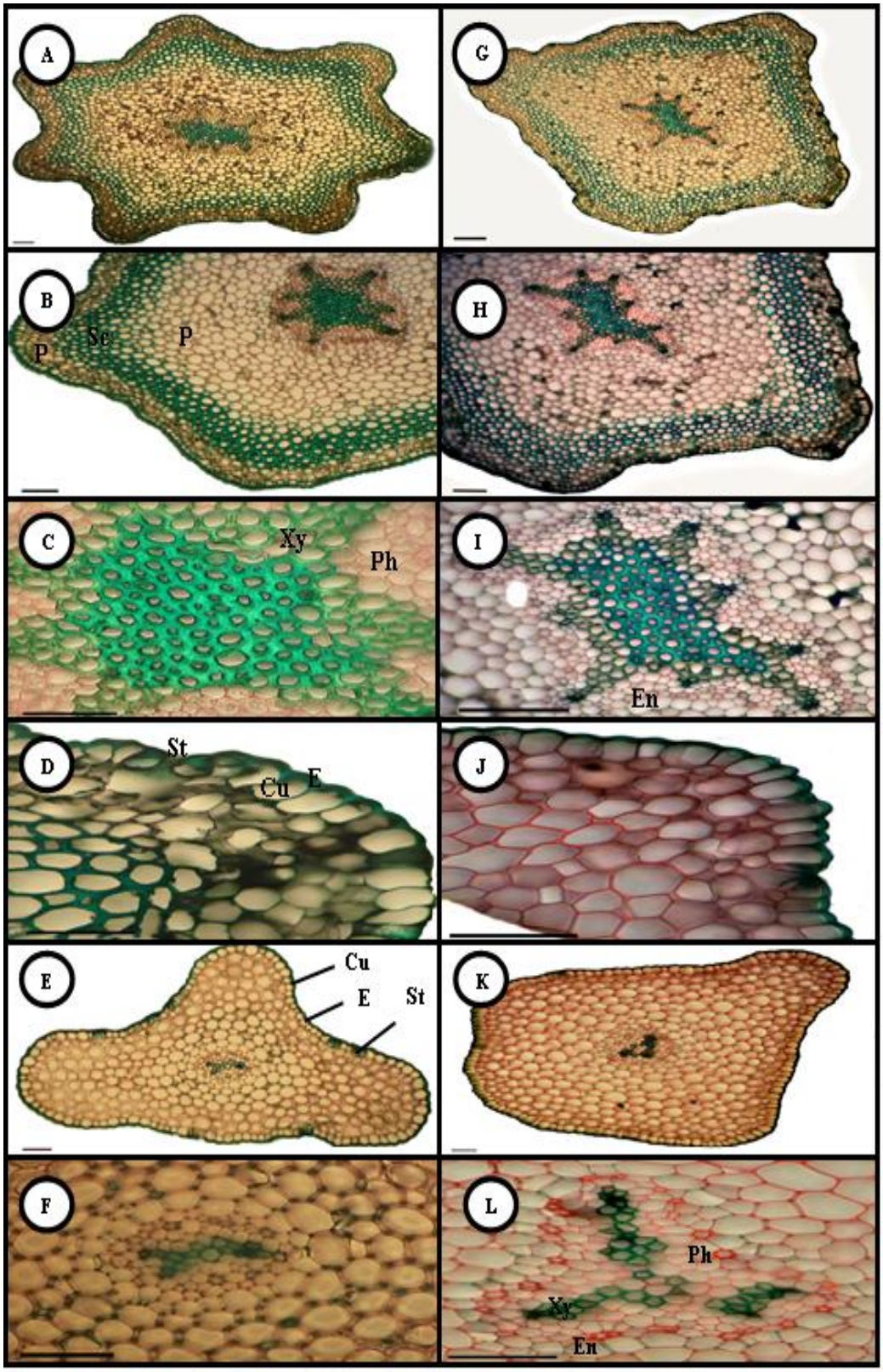

Figure 4. Transverse sections of the aerial stem (A-D. Ata-Kuh; G-J. Ramsar samples): A and D. General aspect; B and H. A part of aerial stem; C and I. Stele region. D and J. Stoma cell with cutinized walls. Transverse sections of the apex aerial stem (E-F. Ata-Kuh; K-I. Ramsar samples): E and K. General aspect; F and I. Stele region. Abbreviations: E. Epidermis; C. Cuticle; St. Stomata; Ph. Phloem; Xy. Xylem; P. Parenchyma; Sc. Sclerenchyma; En. Endodermis. Scale bar = 0.1 mm

Anatomical studies

Aerial stem

Cross sections of the aerial stem were irregular in outline (pentagonal in Ramsar samples and star-like in Ata-Kuh samples) due to the presence of grooves and ridges. Epidermis was composed of oblong cells that were heavily cutinized and interrupted by large numbers of stomata. Stomata were of the anomocytic type (Figure 3). Each stoma had two dumbbell shaped guard cells that are somewhat sunk below the level of the other epidermal cells and had heavily cutinized walls like those of epidermal cells (Figure 4D, J). The cortex was differentiated into three zones (I) outer cortex consisting of 2-4 layers of chlorophyllous cells (since the leaves did not perform their photosynthetic function, the green stem capture this activity), (II) middle cortex comprising 3-6 layers of sclerenchymatous cells, and (III) inner cortex, including thin-walled parenchymatous cells (Figure 4B, H). The stele was a protostele (actinostele) with xylem rays (star-like and pentarch to octarch) and surrounded by distinct layers of endodermis with characteristic casparian bands on the radial walls of its cells. The center of the stele was occupied by sclerenchymatous pith (Figure 4C, I). The characteristics of the aerial stem, apex aerial stem and rhizome anatomy of *Psilotum* species of 2 localities were given in Table 1.

Apex aerial stem

In cross section, apex aerial stem was triangular. Epidermal cells are quadrangular. Cuticle was present on the epidermis. Stomata were of the anomocytic type. The cortex was undifferentiated and the stele was a protostele (actinostele) that was surrounded by a clear layer of endodermis. In the apical part there were only 2-3 radiating rays (diarch or triarch) (Figure 4E, F, K and L).

Rhizome

Cross sections of the rhizome are elliptic-circle in outline. Rhizome epidermis is composed of quadrangular cells that are cutinized. Beneath this layer is a cortex that is differentiated into three zones. The outer and middle cortex includes parenchymatous cells. Inner cortex is dark brown in colour because of the presence of phlobaphene. Endodermis is distinguishable. It is followed by single layered pericycles which encircle the stele; the stellar type is a protostele (haplostele) (Figure 5).

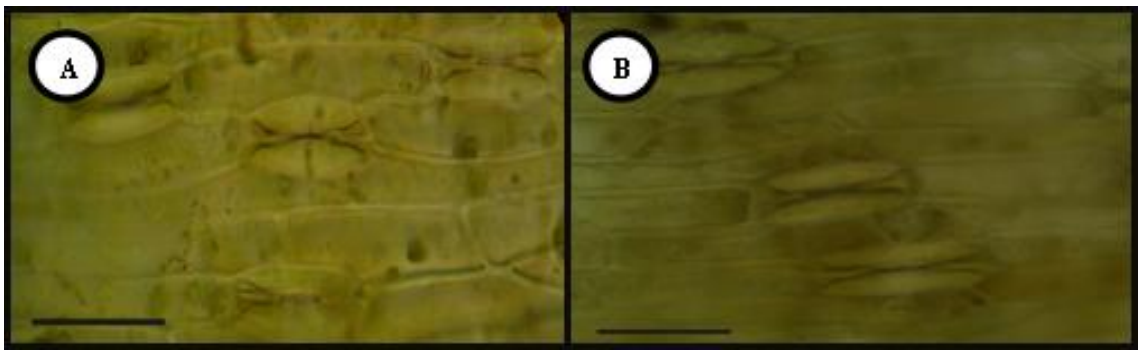

Figure 3. Stomatal type in the aerial stem in two studied localities (A. Ata-Kuh and B. Ramsar samples). Scale Bar = 0.1 mm.

cm (Ramsar) long, to 1.5 mm in diameter, cylindrical, lack chlorophyll (non-photosynthetic), subterranean, rootless, with short and long rhizoids and mycorrhizal. This is the first report of the species in the Hyrcanian forests of Guilan (Figure 2).

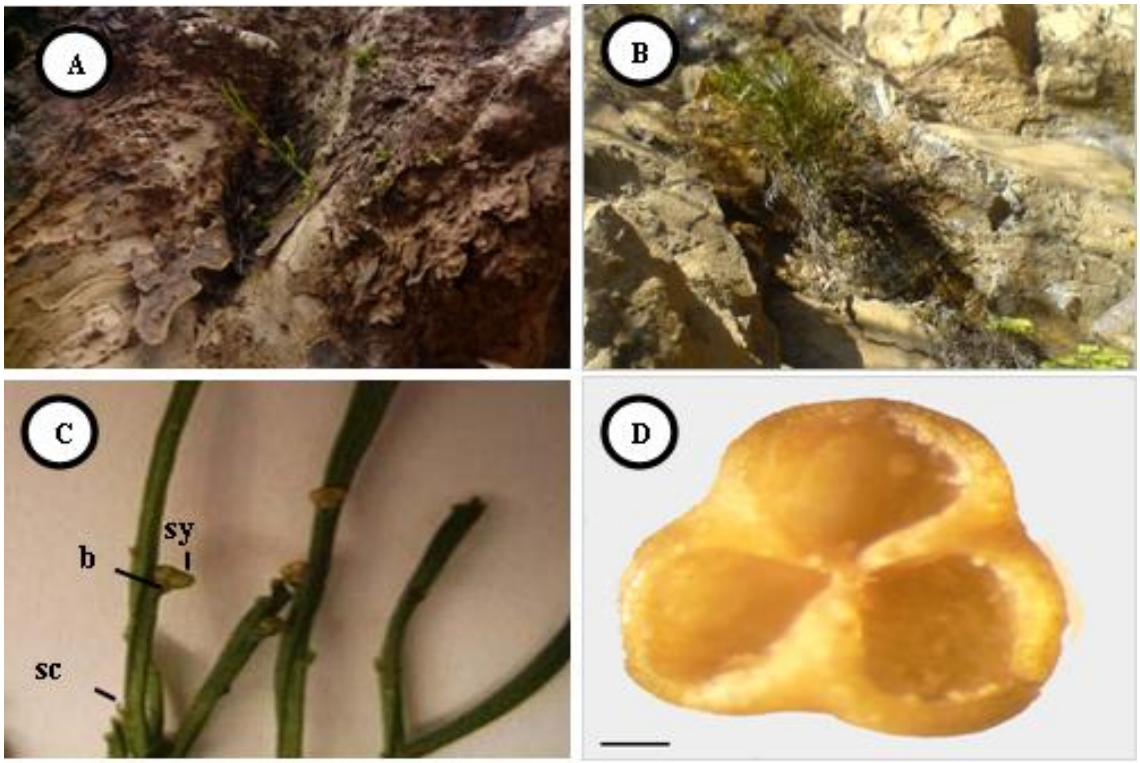

Figure 1. *Psilotum nudum* (L.) P. Beauv. A and B. Habit and Habitat (Ramsar on *Parrotia persica* C.A.Mey.); C. Synangia (b. Bracts; Sc. Scaly leaves; Sy. Synangium); D. Close up of synangium. Scale Bar D = 0.1 mm

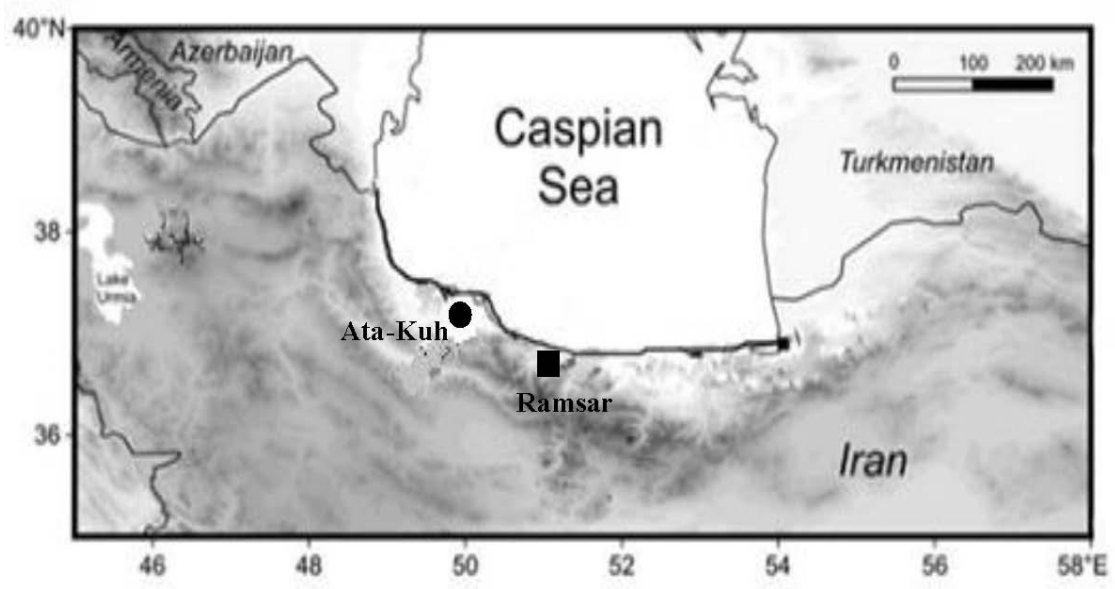

Figure 2. Map showing the distribution of the sampled population

commonly known as the Whisk Fern; this name comes from the likeness of the branched stems to whisks and alludes to its use in the past as a small broom.

Recently, study of its morphology and anatomy suggests that the ancestral features present in living Psilophytes represent a reduction from a more typical modern fern plant. Likewise, molecular evidence strongly confirms that *Psilotum* is a fern (Smith *et al*., 2006).

The genus *Psilotum* is characterized by dichotomously branched stems, the absence of roots, a relatively simple vascular structure, small reduced leaves and thick-walled, homosporous synangia (Roux, 2003). These small plants consist of a brown underground rhizome which is likewise much branched. *Psilotum* gametophytes apparently have a lipid coating (Bierhorst, 1971), and they are also subterranean, mycorrhizal, and not ephemeral. Likewise, it has two cytological races, a diploid with a chromosome number $2n = 52$; and a tetraploid with $4n = 104$ (Khullar, 2008).

So far, only a few anatomical and morphological studies have been published on the genus *Psilotum* (Ford, 1904; Stiles, 1910; Sporne, 1962; Schulte *et al*., 1987; Khoshravesh *et al*., 2009; Nazarian *et al*., 2010; Singh *et al*., 2010). However, the current study can relate these data to other available morphological and anatomical data of this genus and will certainly enrich the botanical information on this species.

In the present study, firstly we report a new locality with a new habitat in the Hyrcanian area. Then, we systematically examined the morphology and anatomy of aerial stem and rhizome in *Psilotum* species growing in two lowland forests (Ata-Kuh and Ramsar), in order to determine the presence of *Psilotum nudum* in forest ecosystem of Guilan.

Materials and Methods

Plant samples were collected from natural habitats and the voucher specimens were deposited in the Herbarium of Guilan University. For each sample, replicates were collected from two populations. Morphological data evaluated based on 10-15 plant specimens. Anatomical studies carried out on the samples conserved in a solution of alcohol-water-glycerine. The cross sections were stained with methylene blue and congo red and mounted with glycerine jelly to make permanent slides (Vardar, 1987). Well-stained sections of the aerial stem, apex aerial stem and rhizome were studied under the light microscope (using ocular and stage micrometer) and photographed by camera. The aerial stem was placed in a tube filled with 70 % lactic acid for 4-5 days. The sections were then prepared by a sharp scalpel or by hand.

Results

Habitat preferences and morphological evidences

Psilotum nudum is a perennial plant which grows as an epiphyte on the trunk of *Parrotia persica* C.A.May. in the Ramsar riparian forest or river bank (586 m a.s.l.) and as a lithophyte in crevices among rocks near streams in the Ata-Kuh forest (207 m a.s.l.) (Figure 1A, B). Aerial stems are 26 cm (Ramsar population) to 31 cm (Ata-Kuh population) long, and 2 mm in diameter, repeatedly dichotomously branched above with 20-36 branches, often pentagonal towards the first dichotomy and in the most distal portions triangular, erect, glabrous and chlorophyllous, with longitudinal parallel lines and dichotomous bract. Sporangia are 2.5 mm in diameter, large, with walls two cells thick, two or three sporangia fused to form a synangium, and orange-brown when mature. Leaves are 1.5-2 mm long, with spiral phyllotaxy on stem, lanceolate to ovate in outline, scale-like, pointed, with no stomata, lacking a midrib and without ligules (Figure 1C, D). Rhizomes are 3-4 cm (Ata-Kuh) and 2-4

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The morphological and anatomical reinvestigation of the *Psilotum nudum*, in Hyrcanian forests, N Iran

Fatemeh Bazdid Vahdati [1], Shahryar Saeidi Mehrvarz [1]*, Alireza Naqinezhad [2] and Robabeh Shahi Shavvon [1]

[1] Department of Biology, Faculty of Sciences, University of Guilan, Rasht, Iran
[2] Department of Biology, Faculty of Basic Sciences, University of Mazandaran, Babolsar, Iran

Abstract
Psilotum nudum is an ancestral epiphytic of the monilophytes which has been recently reported from Hyrcanian forests. It was already introduced as an epiphytic species from some patchy habitats in the lowland parts of Hyrcanian forests. Moreover, *Psilotum nudum* was reviewed using morphological and anatomical characteristics in two different populations in central parts of Hyrcanian zone. The results showed that there were no significant differences between morphological and anatomical characteristics. The aerial stems of the plant were linear, chlorophyllous, and dichotomously branched. Leaves were much reduced, simple, scale-like, and without ligules. Synangium was composed of 3-locular capsule-like sporangia. Also, stele type in apex aerial stem and aerial stem was actinostele; and it was haplostele type in rhizome. In this paper, a new locality of this species growing on the rocky sites was reported in Ata-kuh of Guilan.
Key words: Anatomy, Morphology, Forest rock, Submountain, Hyrcanian forest, *Psilotum nudum*, Iran

Introduction

Psilotaceae, as a family of Psilotales, comprises two genera (*Psilotum* Sw., *Tmesipteris* Bernh.) and about 15 species of fern-like plants (Kenrick, 2000). The genus *Psilotum* consists of two species, *P. nudum* (L.) P. Beauv. (= *P. triquetrum* Sw) and *P. flaccidum* Hook. & Grev. (= *P. complanatum* Sw.). Most commonly, it grows erectly on the ground or in crevices among rocks, but it may also grow as an epiphyte on tree-ferns or among other epiphytes on tree branches (Sporne, 1962). It is sometimes found in botanical gardens and in greenhouses (Nazarian *et al*., 2010). *Psilotum nudum* is fairly common in tropical and subtropical parts of both hemispheres (Singh *et al*., 2010).

This genus is often grouped with the extinct Psilophytes, the Rhyniales and Zosterophyllales dating from the Devonian some 400 million years ago (Roux, 2003). Among the vascular plants, they considered as one of the oldest and the simplest. The word *Psilotum* is derived from the Greek word Psilos which means naked or bare which is in regard to its lack of true leaves on the stem (Amanda, 2010). *Psilotum nudum* is more

* Corresponding Author: saeidimz@guilan.ac.ir

2247 m a.s.l., 27 April 2011, *Ranjbar and Hajmoradi 23878* (BASU!). Fars province: Sepidan to Nor Abad, 5 km after first neck of Nor Abad, 20 km before Dowlat Abad Hasani emergency, 1882 m a.s.l., 7 April 2010, *Ranjbar and Hajmoradi 23530* (BASU!). Markazi province: Tafresh, 1963 m a.s.l., 22 April 2010, *Ranjbar and Hajmoradi 23697* (BASU!).

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tip; claw ca. 4 mm long, keel ca. 8 × ca. 2 mm; stamen ca. 8 mm long. Legume linear, 20-40 × 1 1.5 mm, rarely compressed, semicircular-incurved, transversely reticulate, beak very short.

Etymology

The specific epithet refers to Gandoman city, where the new variety is found

Taxonomic and distributional remarks

Trigonella persica var. *gandomanica* is a narrowly endemic variety known only from the stony-steppe zone of the Kallar mountain, south Gandoman in Chaharmahal va Bakhtiari province (Figure 2). New variety shows remarkable similarities to *T. persica* var. *persica* in the shape and size of petals and pods and also in the sculpturing of pods. Both varieties are distributed in southwest Iran. However, *T. persica* var. *gandomanica* can be recognized well by its glabrous pods (Figure 1 H-K).

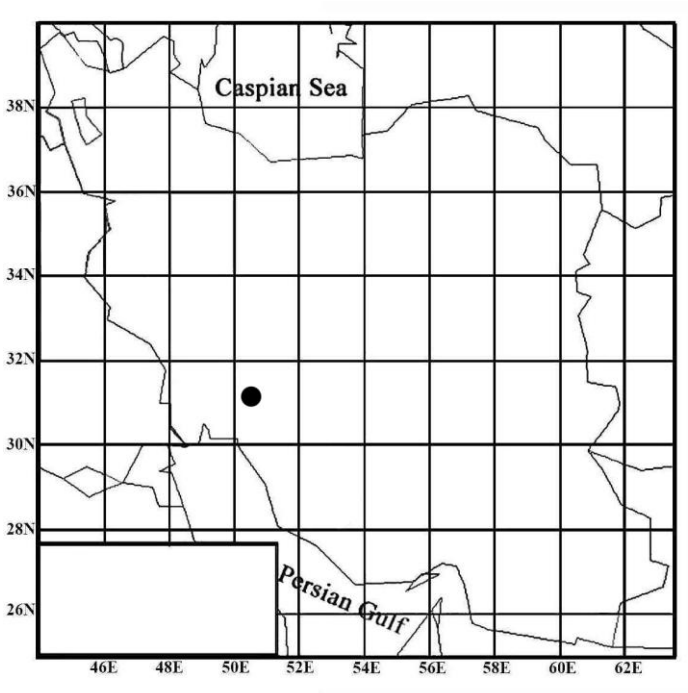

Figure 2. Distribution map of *Trigonella persica* Boiss. var. *gandomanica* Ranjbar and Hajmoradi

Specimens examined

Trigonella persica var. *persica*

Iran. Lorestan province: Dow Rud toward lake, 1450 m a.s.l., 22 April 2009, *Ranjbar and Hajmoradi 23664* (BASU!); Lorestan university, 1250 m a.s.l., 22 April 2009, *Ranjbar and Hajmoradi 23661* (BASU!). Kordestan province: 5 km after Sanandaj toward Divandarreh, 1500 m a.s.l., 16 May 2011, *Ranjbar and Hajmoradi 24923* (BASU!); Sanandaj toward Divandarreh, after Khamush Abad village, 1560 m a.s.l., 16 May 2011, *Ranjbar and Hajmoradi 26493* (BASU!). Hamedan province: Hamedan toward Qorveh, before Veynesar village, 1830 m a.s.l., 16 May 2011, *Ranjbar and Hajmoradi 24770* (BASU!); 30 km before Qorveh, 1800 m a.s.l., 21 May 2005, *Ranjbar 7081* (BASU!); Hamedan toward Malayer, after Ekbatan dam, before Ghahavand bifurcate, 1950 m a.s.l., 7 Jan 2004, *Ranjbar 5889* (BASU!). Kohgiluyeh va Boyer Ahmad province: Dena mountain,

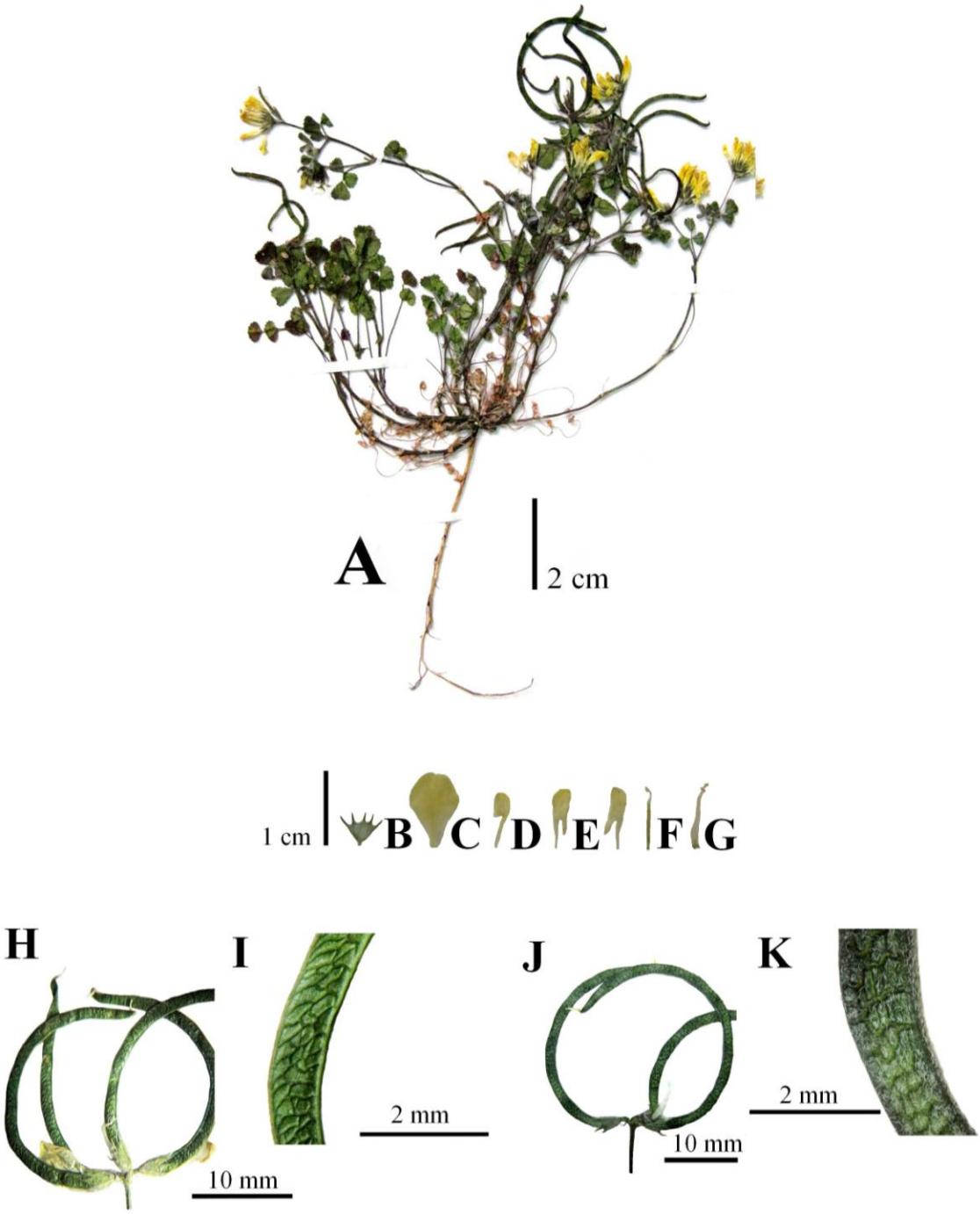

Figure 1. *Trigonella persica* Boiss. var. *gandomanica* Ranjbar and Hajmoradi (*Ranjbar and Hajmoradi 23536*, BASU). A. Habit (with flowers and pods); B. Calyx; C. Standard; D. Keel; E. Wings; F. Ovary; G. Androecium; H and I. Glabrous pods. J and K. Pubescent pods in *T. persica* var. *persica*

Annual herbs, stem usaully branched from base, often ascending, 8-16 cm tall, sparingly pilose. Stipules semisagittate, 2-4 × 1-1.3 mm. Leaves 3-26 mm long, sparsely to loosely covered with hairs, 0.3-0.5 mm long, petiole 2-23 mm long. Leaflets obdeltoid, sharp dentate, 2-6 ×2 -5 mm, sparsely covered with appressed white hairs on both sides, 0.3-0.5 mm long. Peduncle slender, 12-26 mm long. Flowers 2-6, umbellate, subsessile. Calyx shorter than corolla, 3.5-4 mm long, sparsely or loosely hairy; teeth lanceolate, ca. 1 mm long. Corolla yellow, standard 9-10 × ca. 5 mm, wings ca. 9 × ca. 2 mm, oblong, round at

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A new variety of *Trigonella persica* (Fabaceae) from Iran

Massoud Ranjbar *, Zahra Hajmoradi and Roya Karamian

Department of Biology, Faculty of Sciences, Bu-Ali Sina University, Hamedan, Iran

Abstract
Trigonella persica var. *gandomanica* is described as a new variety from southern part of Iran. This taxon belongs to section *Bucerates*, which is the largest section of annual *Trigonella* species. This taxon is the first variety of *T. persica* introduced from Iran. *T. persica* var. *persica* is closely related taxon to the variety. However, the new variety differs from *T. persica* var. *persica* by its glabrous pods (vs. hairy pods).
Key words: New variety, Fabaceae, Section *Bucerates*, *Trigonella persica*, Iran

Introduction

Trigonella L. (Fabaceae) includes about 135 species in worldwide, and the most of the species are distributed in the dry regions around Mediterranean, West Asia, Europe, North and South Africa, North America, and with only two species being present in South Australia (Mabberly, 1997). The members of *Trigonella* are hairless annual or perennial herbs or sometimes covered with simple hairs originate in the Mediterranean and Irano-Turanian regions (Yilmaz *et al*., 2009; Ranjbar *et al*., 2010, 2011). After revising the genus for the flora of Iran by Rechinger (1984), six new species and one new variety have been described from the country (Ranjbar *et al*., 2011, 2012) and seven taxa were added as new records (Hamzeh'ee, 2000; Janighorban, 2004; Badrzadeh *et al*., 2009; Ranjbar and Hajmoradi 2012).

Trigonella sect. *Bucerates* (Boiss.) Sirj. is represented with one endemic species and four subspecies in Iran (Rechinger, 1984). *Trigonella persica* Boiss. is only endemic species of the section in Iran. In this paper, *T. persica* var. *gandomanica* Ranjbar and Hajmoradi is described as a new variety from Iran. It was collected in a locality along with *T. persica* var. *persica*. Now with this variety, the total number of *Trigonella* taxa occurring in Iran increased to 47.

Taxonomy

Trigonella persica Boiss. var. ***gandomanica*** Ranjbar and Hajmoradi, **var. nov.** (Figure 1)

Trigonellae persicae var. *persicae affinis, a qua leguminis glabrescentibus (nec pilis appresso-albis) differt.*
Holotype: Iran. Gandoman to Yasuj, Gereh-Bass bridge, 31°75´N, 51°25´E, 1734 m a.s.l., 5 April 2010, *Ranjbar and Hajmoradi 23536* (BASU!; isotypes W, TARI).

* Corresponding Author: ranjbar@basu.ac.ir

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meiosis. However, some abnormalities which were observed in the taxa are discussed. 3905 cells were examined in this study. Although chromosomes of the investigated species showed regular behavior during meiosis, some abnormalities were observed as laggard chromosomes in diakinesis/methaphase I; anaphase I/telophase I; B chromosomes in diakinesis/methaphase I; anaphase I/telophase I; fragmented chromosomes in diakinesis/methaphase I; unequal distribution chromosome in telophase I; multipolar cells in telophase II; channels in the cytoplasmic anaphase I/telophase I; cytomixis phenomenon in prophase I and anaphase I/telophase I.

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polypolar in anaphase II/telophase II; asynchronous nucleus intelophase I; some abnormalities in meiotic behavior of the taxa are described (Table 2, Figure 4: M-X). The phenomenon of cytomixis consists of the migration of chromosome between meiocytes through cytoplasmic channel. Since cytomixis creates variation in the chromosome number of the gametes, it could be considered as a mechanism of evolutionary significance (Ghaffari, 2006). Cytomixis is not considered to be of great evolutionary importance, but it may lead to production of aneuploid plants, or results in the production of unreduced gametes, as reported in several grass species (Falistocco *et al.*, 1995). Unreduced gamete formation is of evolutionary importance as it can lead to the production of plants with higher ploidy levels. It was found in 2.38% P in *A. brevidence* of Ghochan, 0.03% P in *A. aduncus*21, 0.05% AI/TI in *A. vegetus*99 of Toyserkan (Table 2, Figure 4). Migration of chromatin material among the adjacent meiocytes occurs through cytoplasmic channels originated from the pre-existing system of plasmodesmata formed within the tissues of the anther. It was found in 0.13% AI/TI in in *A. vegetus*99 of Toyserkan.

According to Nicklas and Ward (1994), non-oriented bivalents may be related to impaired attachment of kinetochores to the spindle fibers. Pagliarini (1990) reported that laggards may result from late chiasma terminalization. Ascending chromosomes are the result of precocious migration and, according to Utsunomiya *et al.* (2002), generally consist of univalent chromosomes formed during late prophase stages by precocious chiasma terminalization in early metaphase I or may even result from low chiasma frequency or from the presence of asynaptic or desynaptic genes (Pagliarini, 2000). Laggards and non-oriented chromosomes may produce micronuclei, if they fail to reach the poles in time to be included in the main telophase nucleus (Koduru and Rao, 1981; Utsunomiya *et al.*, 2002), leading to the formation of micro-pollen and probably to gametes with an unbalanced chromosome numbers (Mansuelli *et al.*, 1995), such as aneuploids (Defani-Scoarize *et al.*, 1995). The highest score for laggard chromosomes expressed in 0.5% D/MI in *A. brevidence* of Ghochan, 0.06% D/MI, 1.01% AI/TI in *A. aduncus*21 and 0.41% D/MI, 0.02% AI/TI in *A. vegetus*99 of Toyserkan.

B chromosomes or accessory chromosomes that occur in addition to the standard or A chromosomes in some of the plants are smaller than other chromosomes and do not form any association with them. B chromosomes, when present in high numbers affect negatively the growth and vigor of the plants, while in low numbers may benefit the plant possessing them (Jones and Houben, 2003). The highest percent of B chromosomes were observed in 1.71% D/MI and 0.03% AI/TI, in *A. brevidence* of Ghochan, 1.23 % D/MI in *A. aduncus*21 of Abgarm, 1.06% D/MI in *A. vegetus* 99 of Toyserkan.

Fragmented chromosomes, because of being unable to orient at the metaphase plate were observed during diakinesis and metaphase I and II. The highest percent of fragmented chromosomes were observed in 0.03% D/MI in *A. aduncus*21 of Abgarm.

A considerable number of cells showed the unequal distribution of chromosomes that might be attributed to abnormalities in spindle formation causing unequal distribution of chromosomes. The highest percent of unequal distribution chromosome 1.2% AI/TI in *A. aduncus*21 of Abgarm.

In conclusion, original mitotic chromosome counts were presented for 10 populations belonging to 6 species of *Astragalus* sect. *Onobrychoidei*: *A. cancellatus*, *A. aduncus*, *A. arguricus*, *A. vegetus* and *A. lilacinus*. All taxa were diploid and possessed $2n = 2x = 16$ chromosome number, consistent with the proposed base number of $x = 8$. In addition, meiotic chromosome number of $2n = 2x = 16$ for *A. aduncus*21 and *A. brevidens* $2n = 4x = 32$ for *A. vegetus*99. All studied taxa displayed regular bivalent pairing and chromosome segregation at

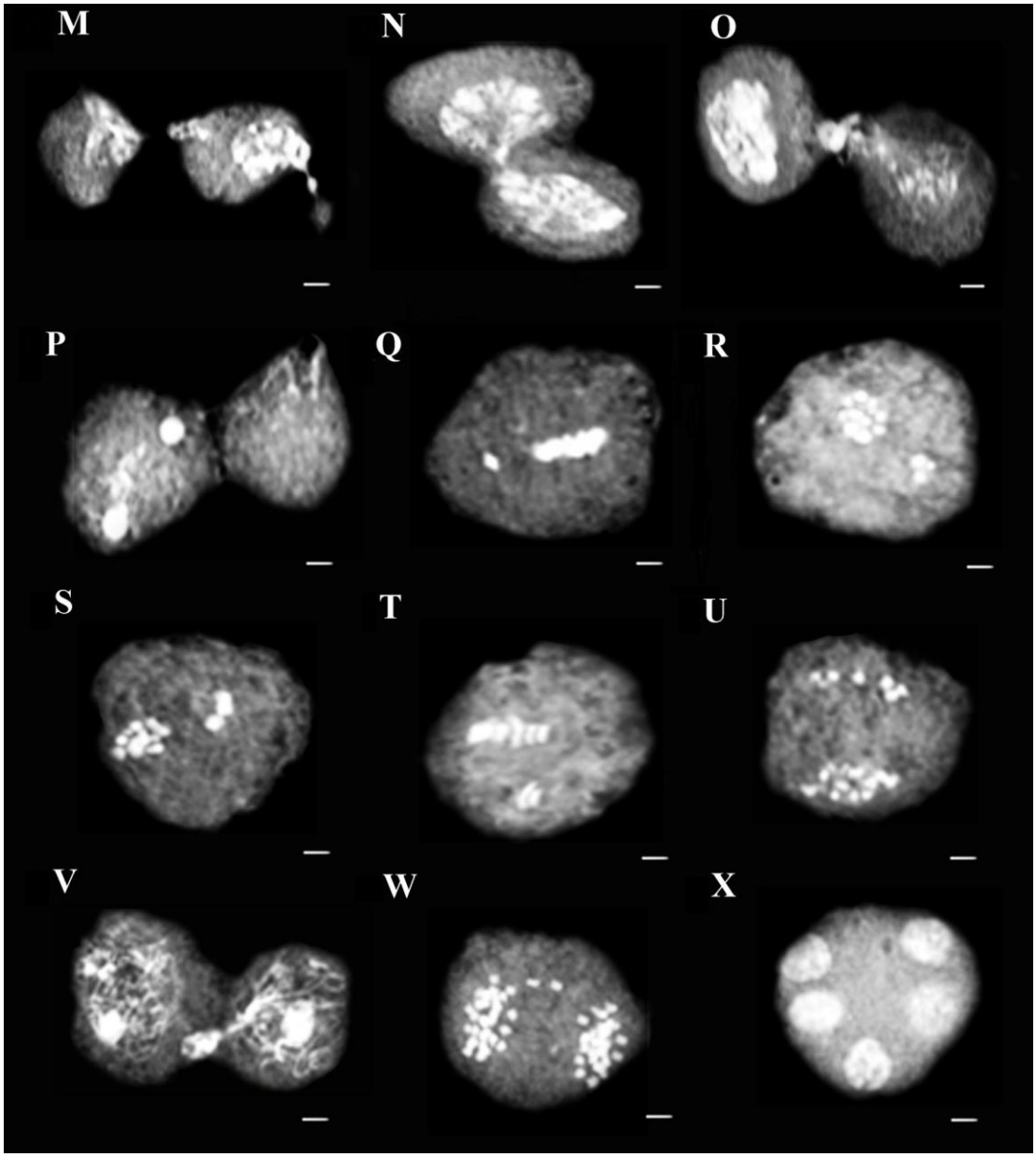

Figure 4. Meiotic behavior in *Astragalus brevidence* with $2n = 2x$=16: M, N and O. Cytomixis in prophase I. Meiotic behavior in *A. aduncus*21 with $2n = 2x = 16$: P. Cytomixis in prophase I; Q. B chromosome in methaphase I; R and S. Unequal distribution chromosome in telophase I; T. Fragmented chromosome in methaphase I; U. Unequal distribution chromosome in telophase I. Meiotic behavior in *A. vegetus*99 with $2n = 4x = 32$: V. Cytomixis in prophase I; W. laggard chromosome in anaphase I; X. Pentapolar.

The meiotic irregularities included the occurrence of various degree of binuclear in prophase; anucleus in prophase; desynapsis in diakinesis/metaphase I; fragmented chromosomes in metaphase I; sticky chromosome in anaphase I; cytomixis indiakinesis/metaphase I, anaphase I/telophase I, anaphase II/telophase II; cytoplasmic channel in prophase, diakinesis/metaphase I, anaphase I/telophase I, metaphase II, anaphase II/telophase II; B Chromosome in diakinesis/metaphase I; micronucleus in anaphase I/telophase I, anaphase II/telophase II; laggard chromosome in anaphase I/telophase I, anaphase II/telophase II; forword chromosome in anaphase I/telophase I, anaphase II/telophase II; bridge in anaphase I/telophase I, anaphase II/telophase II; tripolar and

Meiotic characters	***A. brevidence***	***A. aduncus*21**	***A. vegetus*99**
%B chromosome	1.71	1.23	1.06
%Fragmented chromosome	0	0.03	0
AI/TI	199	425	208
%AI/TI	14.57	29.6	18.7
%Cytomixis	0	0	0.05
%B chromosome	0.03	0	0
%Cytoplasmic channel	0	0	0.13
%Laggard chromosome	0	1.01	0.02
%Unequal distribution chromosome	0	1.2	0
MII	45	22	98
%MII	3.29	1.53	8.85
AII/TII	232	104	296
%AII/TII	16.1	7.25	26.73
%Tripolar	0	0	0.02
%Pentapolar	0	0	0.04
N	8	8	32

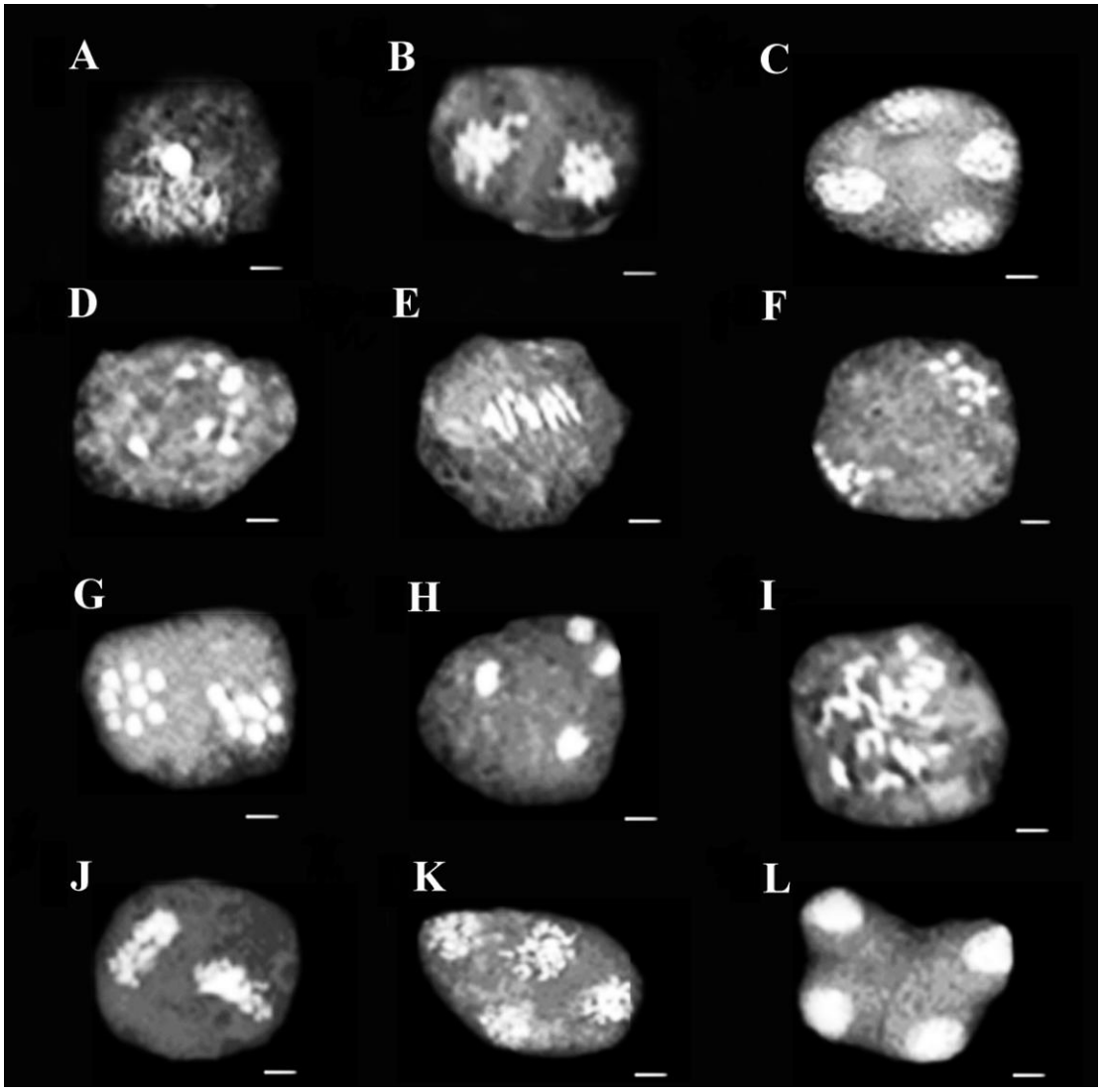

Figure 3. Different stages of meiosis in *Astragalus brevidense* with 2*n* = 2*x* = 16: A. Porophase; B. Telophase; I and C. Telophase II. Different stages of meiosis in *A. aduncus*21 with 2n = 2x = 16: D. Diakinesis; E. Metaphase; I and F. Anaphase; I and G. Telophase; I and H. Anaphase II. Different stages of meiosis in *A. vegetus*99 with 2*n* = 4*x* = 32: I. Diakinesis; J. Metaphase II; K. Telophase II; L. Tetrad in telophase II

Meiotic

The cytological data for the examined taxa is summarized in Table 2 and Figures 3-4. Chromosome numbers based on the basic number of $x = 8$ are found in the majority of the studied taxa, a diploid number of $2n = 2x = 16$ is recorded in the majority taxa, whereas a tetraploid number ($2n = 4x = 32$) is recorded in only one sample of *A. vegetus*99. Ledingham (1960) found that the *Astragalus* species from the old world have a basic chromosome number of $x = 8$, while those from the new world have $x = 11$, 12 and 13. This report was substantiated by Ledingham and Rever (1963). The chromosome number of *Astragalus*, based on $x = 8$ have been reported in the vast majority of the old world in *Astragalus* genus while counts based on base numbers $x = 7$ or $x = 6$ have been encountered in only a few species (Maassoumi, 1987; Badr *et al.*, 1996; Malallah *et al.*, 2001; Badr and Sharawy, 2007). The preponderance of *Astragalus* species with a basic number of $x = 8$ led Badr *et al.* (1996) to conclude that it is the primary basic number of *Astragalus* species. They claimed that the $x = 7$ and $x = 6$ numbers have been derived from $x = 8$ by aneuploid loss of chromosomes. Polyploidy in this species have been reported by Badr and Sharawy (2007). They determined the chromosome number in a karyological study on 24 species of Egyptian *Astragalus* and found different ploidy levels such as diploid ($2n = 16$, 14 and 12), triploid ($2n = 24$), tetraploid ($2n = 32$, 30 and 28), pentaploid ($2n = 30$), hexaploid ($2n = 48$) and octaploid ($2n = 64$) and Sheidai *et al.* (2009) have reported $2n = 16$, 32 and 48, in some species of Iranian *Astragalus*, indicating the role of polyploidy in the evolution of this genus. Only *Astragalus* exhibits aneuploidy, and it does so consistently only in the New World (though sporadically elsewhere). Some 95% of all Eurasian *Astragalus* species have euploid numbers based on $n = 8$. Recent molecular systematic studies (Liston, 1992a, 1992b; Sanderson and Doyle, 1993; Wojciechowski *et al.*, 1993; Wojciechowski *et al.*, 1999), indicating the use of cytological data in studying the phylogenetic relationship of *Astragalus* species (Hu *et al.*, 2008). Chromosome migration may also occur through cell wall dissolution among the neighboring meiocytes and forming syncyte (Falistocco *et al.*, 1995). The distribution of several meiotic abnormalities observed include a various degree of fragmented chromosomes; cytomixis; asynchronous nucleus; laggard chromosome; B chromosome and unequal distribution chromosome, which is likely to result in many structural changes and rearrangements at meiosis, and which could then lead to speciation. In this study, a total of 754 prophase (55.2%), 135 diakinensis/metaphases I (D/MI) (9.89%), 199 anaphase I/telophase I (AI/TI) (14.57%), 45 metaphase II (MII) (3.29%), 232 anaphase II/telophase II (AII/TII) (16.1%) cells in *A. brevidence* of Ghochan (Figures 3: A-C). A total of 697 prophase (48.63%), 185 diakinensis/metaphases I (D/MI) (12.9%), 425 anaphase I/telophase I (AI/TI) (29.6%), 22 metaphase II (MII) (1.53%), 104 telophase II (TII) (7.25%) cells in *A. aduncus*21 of Abgarm of Qazvin (Figures 3: D-H). A total of 372 prophase (33.60%), 133 diakinensis/metaphases I (D/MI) (12.01%), 208 anaphase I/telophase I (AI/TI) (18.7%), 98 metaphase II (MII) (8.85%), 296 anaphase II/telophase II (AII/MII) (26.73%) cells in *A. vegetus*99 of Toyserkan of Hamedan (Figure 3: I-L).

Table 2. Number of pollen mother cells (PMCs) analyzed and percentage of PMCs meiotic behavior

Meiotic characters	***A. brevidence***	***A. aduncus*21**	***A. vegetus*99**
Total cell number	1365	1433	1107
P	754	697	372
%P	55.2	48.63	33.60
%Cytomixis	2.38	0.03	0
D/MI	135	185	133
%D/MI	9.89	12.9	12.01
%Laggard chromosome	0.05	0.06	0.41

4. ***Astragalus vegetus*** Bunge, Mém. Acad. Imp. Sci. Saint Pétersbourg 11(16): 102 in clave et l.c. 15(1): 181. 1869].
Iran. East Azerbaijan: Varzaqan, 1415 m, *Ranjbar 15798* (BASU).
For this species, chromosome count of $2n = 2x = 16$ (Figures 1-2) is the first report.

5. ***Astragalus lilacinus*** Boiss., Diagn. Pl. Orient., ser. 1, 9: 42. 1849.
Iran. East Azerbaijan: Bostan Abad to Sarab, 1660 m, *Ranjbar 15451* (BASU).
For this species, chromosome count of $2n = 2x=16$ is the first report (Figures 1-2).

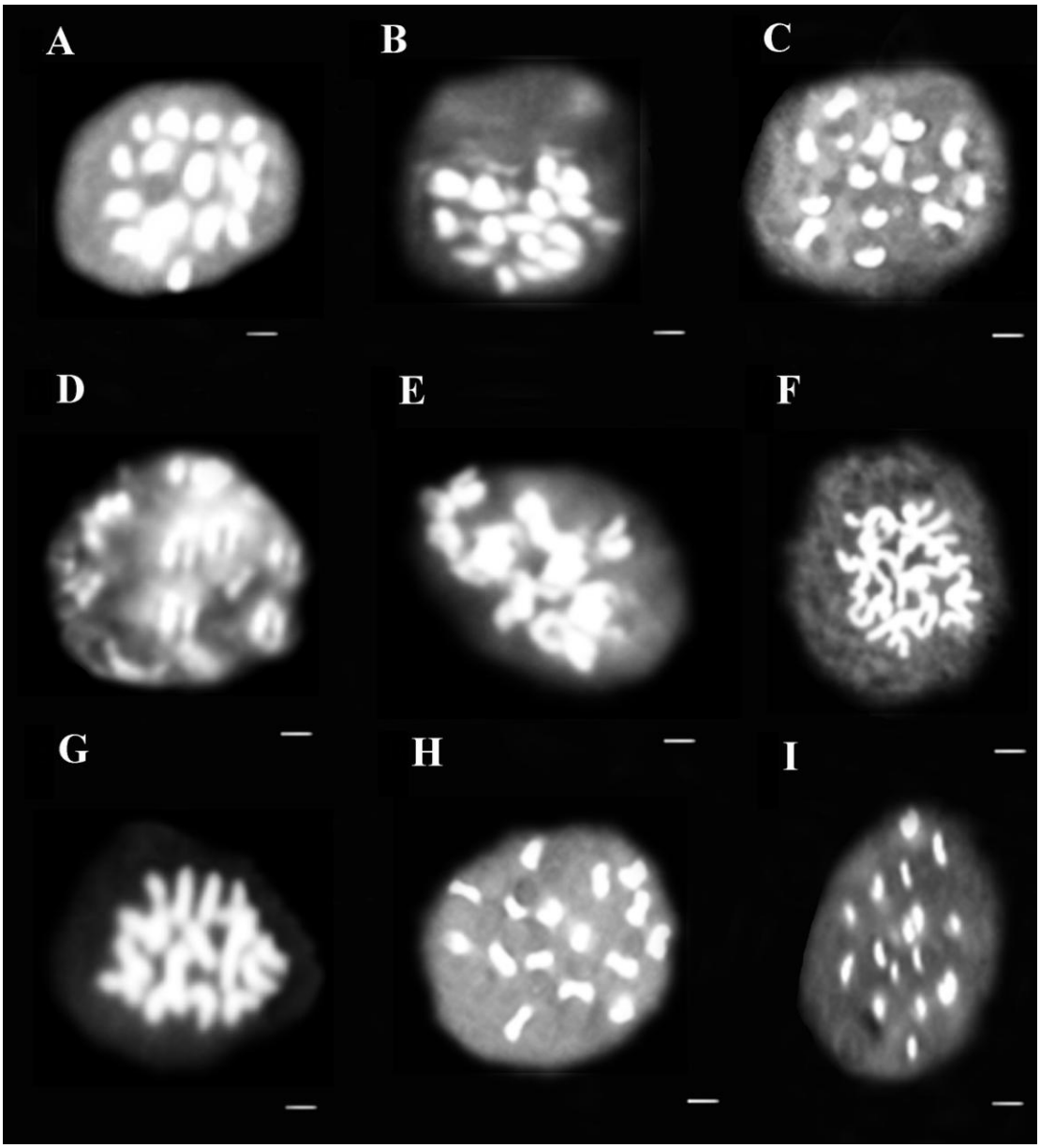

Figure 2. Mitosis in *Astragalus cancellatus* (15751) species studied: A. Prophase. Mitosis in *A. cancellatus* (4933) species studied: B. Prophase. Mitosis in *A. aduncus* (5498) species studied: C. Prophase. Mitosis in *A. aduncus* (15822) species studied: D and E. Prophase. Mitosis in *A. aduncus* (4953) species studied: F. Prophase. Mitosis in *A. aduncus* (7821) species studied: G. Prophase. Mitosis in *A. arguricus* (4947) species studied: H. Prophase. Mitosis in *A. vegetus* (15798) species studied: I. Prophase

Table 1. Taxa studied and acronyms

Taxa	Locality	Altitude (m)	Voucher specimen	Coordinate
A. cancellatus	East Azerbaijan: Misho Dagh	1830	4933	45°47'5.54"E 38°19'9.27"N
A. cancellatus	East Azerbaijan: Kaleybar	1215	15751	47°2'14.70"E 38°52'4.13"N
A. aduncus	West Azerbaijan: 25 km to Urmieh	1300	4953	45°7'53.48"E 37°34'51.35"N
A. aduncus	East Azerbaijan: Kandavan	2010	15822	47°54'48.37"E 38°7'27.16"N
A. aduncus	West Azerbaijan: Oshnaviyeh	2150	4949	45°5'54.00"E 37°2'23.00"N
A. aduncus	East Azerbaijan: 25 km to Marand	1300	5498	45°50'36.53"E 38°20'36.58"N
A. aduncus	Hamedan: Tuyserkan	2000-2200	7821	48°26'49.00"E 34°32'53.00"N
A. vegetus	East Azerbaijan: Varzaqan	1415	15798	46°39'16.00"E 38°30'35.00"N
A. arguricus	East Azerbaijan: Misho Dagh	1830	4947	45°47'5.54"E 38°19'9.27"N
A. lilacinus	East Azerbaijan: Bostan Abad to Sarab	1660	15451	46°49'59.99"E 37°51'0.01"N
A. brevidence	Khorasan: Ghochan	1412	18424	58°30'42.94"E 37°6'22.90"N
A. vegetus	Hamedan: Toyserkan	1294-1370	18799	8°26'49.00"E 34°32'53.00"N
A. aduncus	Qazvin: Abgarm	1540	18321	49°17'13.00"E 35°45'26.00"N

Results and discussion

Mitosis

1. *Astragalus cancellatus* Bunge, Mém. Acad. Imp. Sci. Saint Pétersbourg 11(16): 102 in clave [et l.c. 15(1): 178. 1869].

Iran. West Azerbaijan: Misho Dagh, 1830 m, *Ranjbar 4933* (BASU). East Azerbaijan: Kaleybar, 1215 m, *Ranjbar 15751* (BASU).

The basic chromosome number of $2n = 2x = 16$ is reported for *A. cancellatus*. According to our literature review, this is the first chromosome count for this taxon (Figures 1-2).

2. *Astragalus aduncus* Willd. 1802, Sp. Pl. 3: 1269.

Iran. West Azerbaijan: Urmieh, 1300 m, *Ranjbar 4953* (BASU); Oshnaviyeh, 2150 m, *Ranjbar 4949* (BASU). East Azerbaijan: Kandavan, 2010 m, *Ranjbar 15822* (BASU); 25 km to Marand, 1300 m, *Ranjbar 5498* (BASU). Hamedan: Tuyserkan, 2000-2200 m, *Ranjbar 7821* (BASU).

In this study, chromosome count for this taxon reported with $2n = 2x = 16$ (Figures 1-2).

3. *Astragalus arguricus* Bunge, Mém. Acad. Imp. Sci. Saint Pétersbourg 11(16): 103 in clave [et l.c. 15(1): 181. 1869] .

Iran. West Azerbaijan: Misho Dagh, 1830 m, *Ranjbar 4947* (BASU).

Morphologically, it is a variable species, normally found in open fields and along roadsides, and flourishes in spring. Chromosome count of $2n = 2x = 16$ (Figures 1-2).

A. sect. *Onobrychoidei*. It also shows the existence of polyploidy in *A. vegetus*99.

Materials and Methods

For mitosis, materials of 10 populations belonging to 5 species of *Astragalus* sect. *Onobrychoidei* were collected from different localities in Iran (Figure 1), in 2000 through 2008 and pods were separated from healthy plants. Voucher specimens were deposited at the Herbarium of the Bu-Ali Sina University (BASU), Hamedan, Iran (Table 1). Then, pods were left to dry at room temperature, and seeds obtained from dry pods and kept at 4 ℃ until used. Young root tips were obtained from seeds germinated in Petri dishes pretreated with 0.05% colchicines for 3 h and fixed in 3 : 1 ethanol: glacial acetic acid for 24 h. Root tips were hydrolyzed for 6 minuts in 1 M HCl at 60 ℃ , washed briefly in dd H_2O and stained in Feulgen's solution for 1 -2 h. All permanent slides were made using Venetian turpentine (Wilson, 1945). The slides were examined under an Olympus BX-41 photomicroscope.

Also chromosome number and meiotic behavior were analyzed in three species of *A. vegetus* and *A. brevidens* and *A. aduncus*. 15 flower buds from at least 5 plants at an appropriate stage of development were fixed in Piennr's fluid containing ethanol (96%) , chloroform and propionic acid, 6 : 3: 2 (v/v/v), for 24 h at room temperature and then stored in 70% alcohol at 4 ℃ until used. Anthers were squashed and stained with 2% acetocarmine. All permanent slides were made using Venetian turpentine (Wilson, 1945).

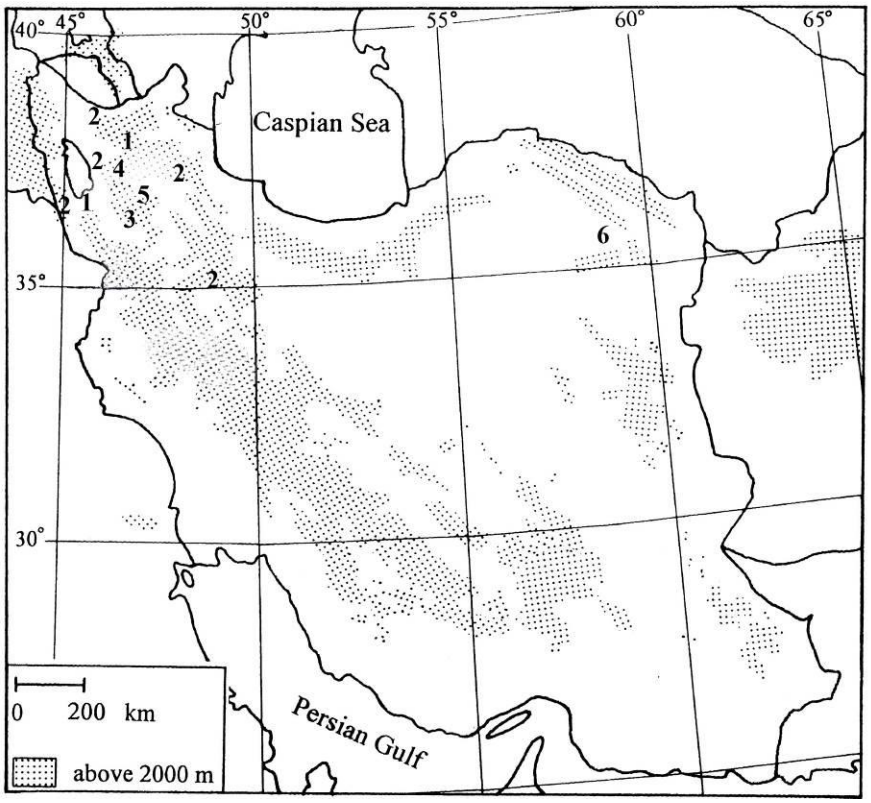

Figure 1. Distribution map of *Astragalus cancellatus* (1), *A. aduncus* (2), *A. arguricus* (3), *A. vegetus* (4), *A. lilacinus* (5), and *A. brevidence* (6)

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Chromosome number reports in *Astragalus* sect. *Onobrychoidei* (Fabaceae) from Iran

Massoud Ranjbar [1]*, Anahita Hadidchi [2] and Hossein Riahi [2]

[1] Department of Biology, Faculty of Sciences, Bu-Ali Sina University, Hamedan, Iran
[2] Department of Botany, Faculty of Biosciences, Shahid Beheshti University, Tehran, Iran

Abstract
In this study, original mitotic chromosome counts have been presented for 10 populations belonging to 6 species of *Astragalus* sect. *Onobrychoidei*: *A. aduncus*, *A. arguricus*, *A. cancellatus*, *A. lilacinus* and *A. vegetus*. All taxa were diploid and possessed $2n = 2x = 16$ chromosome number, consistent with the proposed base number of $x = 8$. In addition, meiotic studies revealed chromosome number of $2n = 2x = 16$ for *A. aduncus*21 and *A. brevidens* and also $2n = 4x = 32$ for *A. vegetus*99. Although this taxon displayed regular bivalent pairing and chromosome segregation at meiosis, some abnormalities were observed.
Key words: *Astragalus*, Chromosome number, Fabaceae, Meiotic behavior, Mitosis, Iran

Introduction

Astragalus L. (Gavan in Persian) is probably the largest genus of flowering plants, containing up to 3000 species (Polhill, 1981; Lock and Simpson, 1991; Yakovlev *et al*., 1996; Ranjbar and Karamian, 2002, 2003; Bagheri *et al*., 2011). Iran is one of the largest centers of diversity for this genus, with approximately 700 species and an endemic rate of 57% (Ghahreman *et al.*, 2002; Podlech, 2001). *Astragalus* sect. *Onobrychoidei* DC. with more than 80 species is a rather large section within genus. The section was revised for the flora of the former Soviet Union (Gontscharov, 1946) and for certain areas, e.g. the flora of Iran (Rechinger *et al.*, 1958), the flora of Turkey (Chamberlain and Matthews, 1970), and the flora of Iraq (Townsend and Guest, 1974). In the course of the work on *A.* sect. *Onobrychoidei* in Iran have been investigated (Rechinger *et al*., 1958; Ranjbar and Maassoumi, 1998; Podlech and Sytin, 2002).

Most of the cytological studies in the genus have concentrated on the chromosome count (Aryavand, 1983; Maassoumi, 1987, 1989; Sheidai *et al.*, 1996, 2000; Ghaffari, 2006; Akhavan and Saeidi, 2010; Faramarzi and Saeidi, 2011; Jalilian and Rahiminejad, 2011; Ranjbar and Mahmoudian, 2013). The basic chromosome number ($x = 8$) and four ploidy levels ($2n = 2x = 16$, $2n = 4x = 32$, $2n = 6x = 48$, $2n = 8x = 64$ and $2n = 12x = 96$) are present in the genus. The present study reports that there are chromosome abnormalities in

* Corresponding Author: ranjbar@basu.ac.ir

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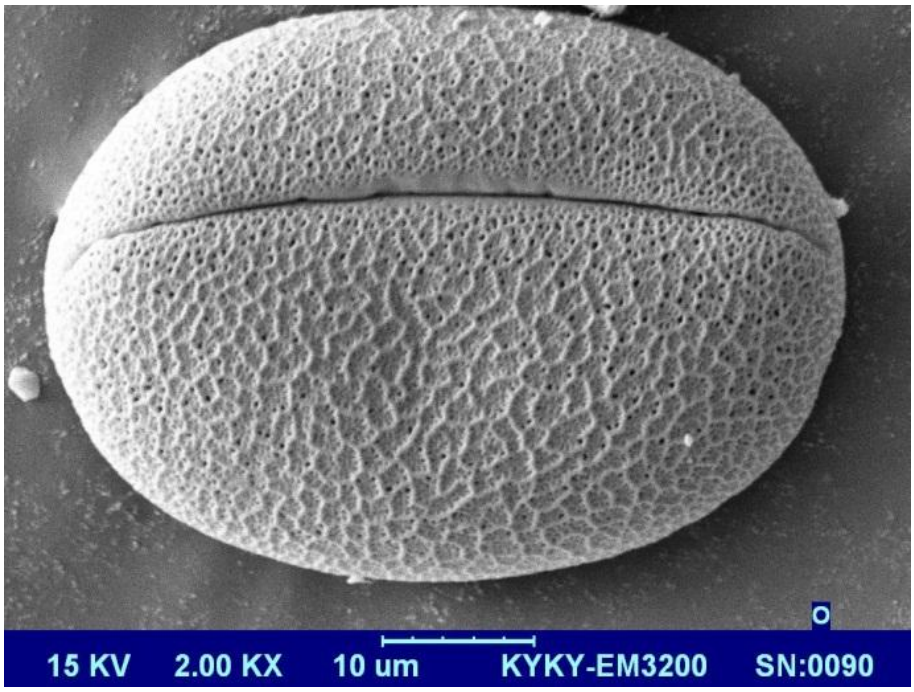

Figure 6. General appearances of the pollen grains in *Phlomis olivieri*

Table 4. Pollen morphological data of *Phlomis olivieri* (μm) showing minimum-maximum ranges and mean value ± standard deviation. All measurements are in μm except for ratio of polar axis/equatorial axis

Characters	**Minimum-maximum values**	**Mean ± Standard deviation values**
Polar axis	38.15-41.26	39.72 ± 1.13
Equatorial axis	26.05-29.45	27.88 ± 1.26
Ratio of polar axis/equatorial axis	1.39-1.48	1.43 ± 0.03
Exine thickness	2.95-3.15	3.05 ± 0.08

Discussion

The *Phomis olivieri* is an important rangeland plant in Iran also the anatomical characters, palynology and trichome types of this species were exactly examined. This is the first report from the anatomical characters of calyx and bracteole in this species. Metcalfe and Chalk (1972) pointed out that the structure of the vascular bundles in the petiole of the species of Lamiaceae could be used as a diagnostic character. In the petiole of this species, there is two-lobed vascular bundle in the centre and 1 small subsidiary bundle in petiolar wings. Therefore, the anatomical properties of the petiole may be useful characters for distinguishing and separation of this species from other species in the genus. Four vascular bundles were observed in bracteole. The number of vascular bundles in bracteole could be considered as a remarkable character. Five main types of trichomes were observed. Our findings about trichomes types are not consistent with Azizian and Cutler (1982). Our study about trichomes showed the presence of dendroid trichomes on abaxial surface of calyx. The dendroid trichomes are reported for the first time in *Ph. olivieri* from Iran whereas its importance remains unsound.

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along the biserrate long stalk cells. Type 5, this type was composed of unicellular simple trichomes that were observed nearly in all of parts. The sessile or short stalk and multiseriate stalked stellate trichomes were on the adaxial surface of leaf but uni- and biseriate stellate trichomes mostly were observed on the abaxial leaf surface. The density of the stellate (especially, sessile and multiseriate) trichomes were in abundance on the abaxial surface of calyx. The glandular trichomes were present in abundance on the abaxial surface of leaf. The most density of glandular trichomes was observed on the adaxial surface of petiole and the most density of stellate trichomes was observed on the adaxial surface of bracteole (Table 3). The dendroid trichomes were only observed on abaxial surface of calyx. The dendritic trichomes were reported for the first time in this species.

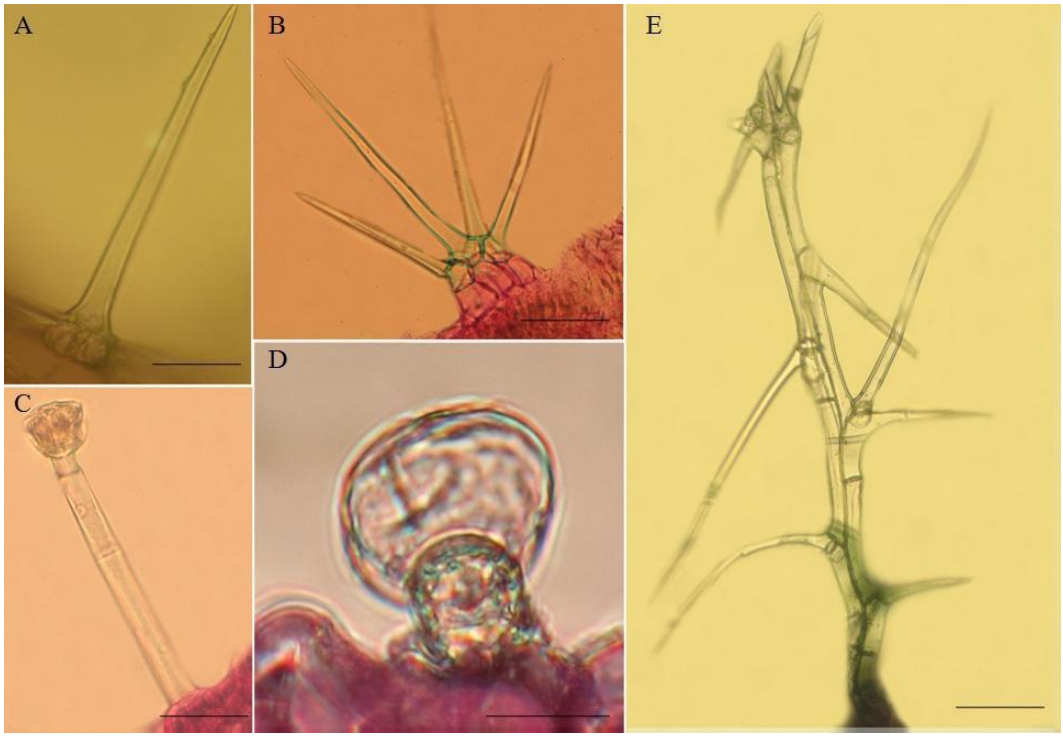

Figure 5. Types of trichome in *Phlomis olivieri*. A. Unicellular simple; B. Stellate; C. Capitate glandular; D. Peltate glandular; E. Dendroid. Scale Bar = 10 µm

Table 3. Distribution and density of trichome types in *Phlomis olivieri*. - absent, ± scarce, + present, ++ abundant

Material	Peltate	Glandular type 1 capitate	Glandular type 2 capitate	Glandular type 3 capitate	Type 1 stellate	Type 2 stellate	Dendroid	Unicellular
Stem	±/+	+	+	-	-	+	-	+
Adaxial leaf side	++	++	±	±	±	++	-	+
Abaxial leaf side	++	++	±	++	++	±	-	+
Petiole	++	+	±	±	±	+	-	+
Abaxial calyx side	+	±	++	±	++	+	++	+
Adaxial bracteole side	+	±	-	-	++	++	-	+

Pollen characteristics

The pollen grains were tricolpate. Their shape was protate-spheroidal. The dimensions of polar axis and equatorial axis were 38.15-41.26 and 26.05-29.45 µm, respectively. The ratio P/E was 1.39-1.48. The exine thickness was 2.95-3.15µm. Exine ornamentation was reticulate and perforate (Figure 6, Table 4).

of 4-5-layer cells. A single layer of elongated continuous fiber cells was observed under parenchyma cells. A one small parenchyma bundle was visible in middle of fiber cells. The expanded parenchyma cells had located under fiber cells (see Table 2).

Bracteole

The upper and lower epidermises were one layer and covered with a thick cuticle layer. 3-4 parenchyma layers had placed after vascular bundles. The fiber cells were observed among of xylem cells (see Table 2).

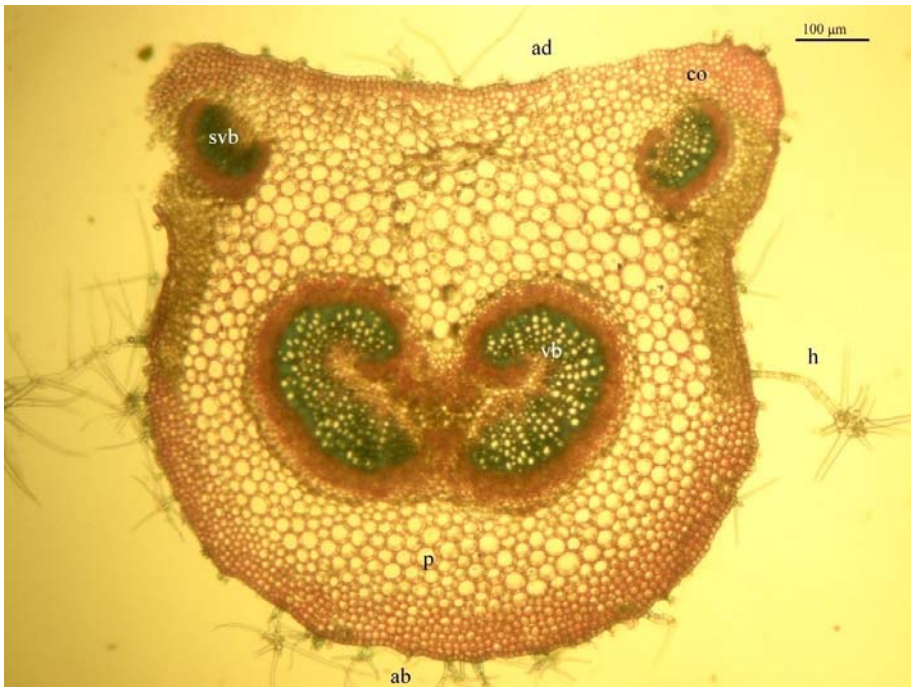

Figure 4. Cross-section of the petiole of *Phlomis olivieri*. ab. Abaxial epidermis; ad. Adaxial epidermis; co. Collenchyma; h. Hair; p. Parenchyma; svb. Subsidiary vascular bundles; vb. Vascular bundles. Scale Bar: 100 µm

Trichome morphology

Five different trichome types on the stems, leaf blades, petioles, calyces and bracteoles of *Phlomis olivieri* were observed: peltate, capitate glandular, stellate, unicellular simple and dendroid trichomes (Figure 5). Capitate glandular and peltate trichomes could be distinguished by head size and stalk length (Ascensão and Pais, 1998). In the capitate trichome, the length of the stalk should be more than half the height of the head, whereas peltate trichomes were short with a uni- or bicellular stalk and a large secretory head with 4 to 18 cells arranged in one or two concentric circles (Werker *et al*., 1985). Type 1 is the typical peltate glandular trichome and consisted of a basal epidermal cell, a very short unicellular stalk and a round multicellular secretory head consisting of four or eight cells. Type 2 was a capitate glandular trichome composed of a basal epidermal cell, unicellular to bicellular stalk of variable length and a large, unicellular or bicellular secretory head. Type 3 was a non-glandular (stellate) trichomes consisted of several basal epidermal cells, and branched at the tip of the stalk cell to form star-shaped that divided to two groups: group 1 (sessile or short stalked stellate trichomes) and group 2 (long stalked trichomes). The First group trichomes did not have stalk or have short stalk but the second group trichomes had long stalk to subdivided into three subgroups: stalks are uni-, bi- and multiseriate. Type 4 was dendroid trichomes that this kind of trichomes was branched along the stalk cell and the branching was

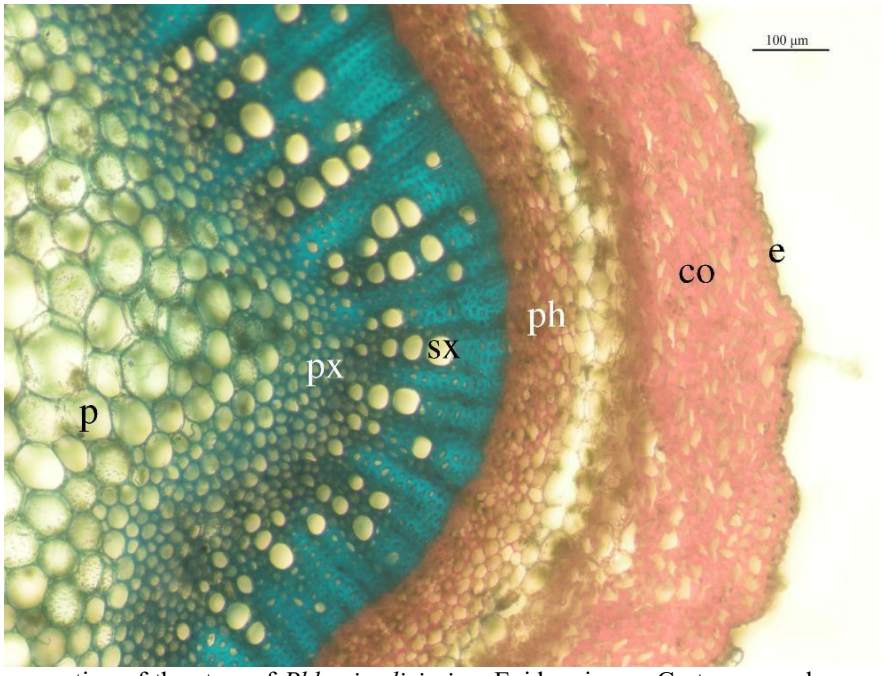

Figure 2. Cross-section of the stem of *Phlomis olivieri*. e. Epidermis; cp. Cortex parenchyma; ph. Phloem; sx. Secondary xylem; px. Primary xylem; p. Pith. Scale Bar= 100 µm

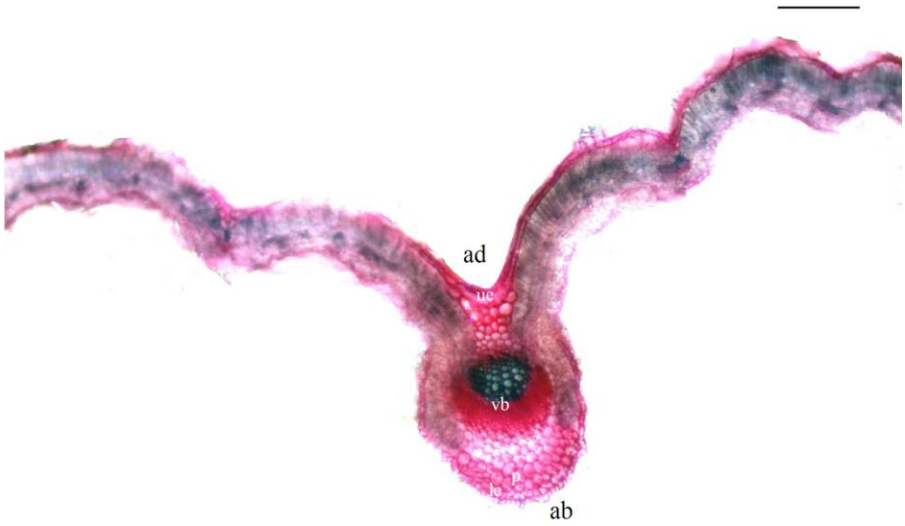

Figure 3. Cross-sections of the leaf blades of *Phomis olivieri*. ab. Abaxial epidermis of midrip; ad. Adaxial epidermis of midrip; le. Lower epidermis; ue. Upper epidermis; vb. Vascular bundle. Scale Bar = 200 µm

Petiole

The adaxial surface was concave and the abaxial surface was convex. Both epidermises were one layer and covered with a thick cuticle layer. The epidermal cells of both surfaces were more or less rectangular to oval. Under epidermis, 2-7 collenchyma layers had placed, as well as collenchyma density at the corner was more than other parts. Parenchyma cells were hexagonal or orbicular. There were two collateral vascular bundles in the centre and two small subsidary bundles in the corners of petiole (Table 2, Figure 4).

Calyx

The both epidermises were one layer and covered with a thick cuticle layer. The upper epidermal cells were nearly longer than the lower epidermal cells. Parenchyma cells consisted

Anatomical characteristics

Stem

The stem was clearly quadrangular. The epidermis was covered by a thin cuticle. The epidermis consisted of a single layer of oval, squarish and rectangular cells. Underneath the epidermis, multi-layered collenchyma cells were located at the corners of the stem. The cortex was composed of 2-5 layers of irregular oval and rectangular parenchymatic cells with intercellular spaces. Vascular bundles were next to each other. Those located at the corners were slightly bigger in size than the others. Cambium was not visible. Primary and secondary xylem could be differentiated. Tracheae in the secondary xylem were denser and larger than in the primary xylem. The pith was large and comprised of hexagonal or orbicular parenchymatic cells with intercelular spaces in the centre of stem (Table 2, Figure 2).

Leaf

Transverse section of the lamina indicated that the upper and the lower epidermis were covered with a nearly thick cuticle layer. The thickness of both epidermises cuticles was nearly equal. Both epidermises consisted of 1 layered oval and rectangular cells. Upper epidermal cells were larger than lower epidermal cells or nearly equal to them. The mesophyll was composed of elongated rectangular palisade parenchyma and spongy parenchyma cells. The palisade parenchyma was 1 layered. The spongy parenchyma cells were 3-4 layers. The spongy parenchyma cells had intercellular spaces. Transverse section of the midrip showed that the adaxial surface was concave and the abaxial surface was convex. The epiderm was 1 layer. There was one large vascular bundle in the center that was surrounded by parenchymatic cells (Table 2, Figure 3). Stomata are diacytic.

Table 2. Anatomical measurement of various tissues of *Phlomis olivieri*

Material	Length Min-Max	Mean ± S.D.	Width (µm) Min-Max	Mean ± S.D.
Stem				
Epidermis cell	0.5-1.1	0.78 ± 0.25	0.4-0.7	0.54 ± 0.11
Collenchyma cell	0.5-3	1.54 ± 1.03	0.4-1.6	0.88 ± 0.50
Parenchyma cell	1-2	1.42 ± 0.37	0.5-1.1	0.8 ± 0.25
Pith cell	1-3.5	2.48 ± 0.97	1-3.3	2.16 ± 0.90
Leaf				
Upper epidermis cell	0.7-1.6	1.26 ± 0.35	0.3-1	0.68 ± 0.25
Lower epidermis cell	0.4-1	0.72 ± 0.21	0.4-0.7	0.52 ± 0.13
Palisade parenchyma	2.5-2.8	2.70 ± 0.12	0.4-0.5	0.44 ± 0.05
Spongy parenchyma	-	-	0.5-1.2	0.88 ± 0.25
Pith cell	1.1-2.1	1.64 ± 0.37	0.8-2	1.52 ± 0.44
Petiole				
Adaxial epidermis cell	0.6-1.4	0.86 ± 0.31	0.4-0.7	0.54 ± 0.11
Abaxial epidermis cell	0.4-1.4	0.84 ± 0.37	0.5-0.9	0.66 ± 0.15
Fiber cell	0.4-0.8	0.54 ± 0.16	0.2-0.8	0.48 ± 0.23
Parenchyma cell	1.5-3	2.14 ± 0.65	1.3-2.1	1.72 ± 0.37
Calyx				
Upper epidermis cell	0.2-0.6	0.48 ± 0.16	0.2-0.5	0.34 ± 0.11
Lower epidermis cell	0.7-1.5	1.26 ± 0.39	0.6-0.7	0.66 ± 0.05
Parenchyma upper fiber cell	0.5-0.8	0.64 ± 0.13	0.3-0.7	0.53 ± 0.17
Parenchyma lower fiber cell	2.1-3.9	3.12 ± 0.79	1.5-1.9	1.64 ± 0.19
Fiber cell	0.6-0.8	0.72 ± 0.08	0.4-0.5	0.44 ± 0.05
Bracteole				
Adaxial epidermis cell	0.7-2.5	1.26 ± 0.72	0.3-0.8	0.64 ± 0.20
Abaxial	0.5-0.8	0.60 ± 0.12	0.4-0.5	0.44 ± 0.05
Parenchyma cell	0.3-1.1	0.58 ± 0.33	0.2-0.7	0.42 ± 0.19

Table 1. Voucher specimens of *Phlomis olivieri* used in this study

No.	Collection site	Altitude (m)	Collector	Date
1	Hamedan: Asada Abad mountains	2180	Yousefi 19013 (HUI)	4 June 2011
2	Hamedan: Malayer, Lashkardar	2118	Yousefi 19011 (HUI)	7 June 2011
3	Hamedan: Nahavand, Giyan	1737	Yousefi 19005 (HUI)	5 June 2011
4	Hamedan: Razan, Boghaty Mountains	2148	Yousefi 19012 (HUI)	12 June 2011
5	Hamedan: Heidare village	2114	Yousefi *s.n.* (HUI)	4 June 2011
6	Hamedan: road of Touyserkan to Malayer	1891	Yousefi 19010 (HUI)	7 June 2011
7	Kermanshah: 95 km from Kermanshah to Kerned Gharb	1425	Yousefi *s.n.* (HUI)	1 June 2011
8	Kermanshah: road of Biseton to Sonqor, Karkasar, Mooineh village, Dalakhani mountains	1800	Yousefi *s.n.* (HUI)	1 June 2011
9	Kurdestan: road of Hamedan to Sanandaj	1848	Yousefi *s.n.* (HUI)	2 June 2011
10	Kurdestan: Salavat Abad mountains	2016	Yousefi *s.n.* (HUI)	2 June 2011

Results

Phenology and habitat

Phlomis olivieri is a perennial herb species distributed in Iran and Iraq, and flowering in June to August. This plant grows on mountainous regions, adjacent to rocky slopes, steppe vegetation and the overgrazed rangeland soils of Irano-Turanian region and Hyrcanian district of Iran (Jamzad, 2012) and could be as one of important destroyed rangeland indicator together with *Stachys inflata* Benth. (Mozaffarian, 2005) (Figure 1).

Figure 1. General appearance of *Phlomis olivieri* (yellow flower) together with *Stachys inflata*

phytogenetics are well known in Lamiaceae and in the related families Acanthaceae, Bignonicaceae, Scrophulariaceae and Verbenaceae (Abu-Asab and Cantino, 1987; Ahmad, 1974, 1978; Cantino, 1990; El-Gazzar and Watson, 1968, 1970; Elias and Newcombe, 1979; Gairola *et al*., 2009; Mathew and Shan, 1983; Metcalfe and Chalk, 1972; Rahn, 1992). Trichome micromorphology has been suggested to be useful in the phylogeny reconstruction (Abu-Asab and Cantino, 1987) and has been widely used in the systematics of Lamiaceae (Cantino, 1990; Kaya *et al*., 2000; Erken, 2005; Satil *et al*., 2007; Dinç and Özturk, 2008; Güvenç and Duman, 2010; Celep *et al*., 2011) as well as specific and subspecific levels (Bruni *et al*., 1987; Giuliani *et al*., 2008; Bini Maleci *et al*., 1992; Sebebe and Harley, 1992; Servettaz *et al*., 1992).

The importance of pollen morphology within the Lamiaceae has been proven as supplementary data to classification (Abu-Asab and Cantino, 1992, 1993, 1994; Dinç *et al*., 2009). A comprehensive overview of the pollen survey within subfamily Lamioideae sensu Erdtman (1945) has been published by Abu-Asab and Cantino (1994). Moreover, some further examinations of pollen have been carried out, e.g. by Sebebe and Harley (1992: for *Stachys* L.), Dönmez *et al*. (1999: for *Teucrium* L.), Satil *et al*. (2005: for *Thymus* L.), Dinç and Özturk (2008: for *Stachys* sect. *Ambleia* Benth.), Dinç *et al*. (2009: for *Lallemantia* L.) and Asadollahi *et al.* (2014: for *Salvia* L.). The pollen morphology generally supported the segregation of some genera of Lamiaceae such as *Marrubium* L., *Phlomis* and *Stachys*. However, its taxonomic value for infrageneric classification varies in the family Lamiaceae.

Up to now, some studies on *Phlomis* have been conducted (Bech, 1963; Azizian and Cutler, 1982; Azizian and Moore, 1982). But neither of them exactly investigated the anatomy, palynology and trichome tpyes of *Ph. olivieri*. Therefore, the present study aims to investigate the trichome types, palynology and anatomy of *Ph. olivieri* as an important rangeland plant of Iran.

Materials and Methods

Specimens of *Phlomis olivieri* were prepared from fresh material collected from various wild populations from west of Iran and herbarium specimens. Voucher specimens (Table 1) were deposited in the herbarium of the University of Isfahan. Anatomical studies were performed using an average of 40 fresh specimens kept in 70% ethanol. All sections were made from the stems, leaves, petioles, calyces and bracteoles using commercial razor blades. Sections were stained with Methyl blue and carmine and mounted on the slides using Canada balsam. Samples were studied using an Olympus microscope model BX-41 light microscope with 40X to 400X magnifications. For identification of trichome types, trichomes were obtained from surface of stems, leaves, petioles, calyces and bracteoles were studied using an Olympus microscope model BX-41 light microscope with 40X to 400X magnifications. For palynological study, pollen grains were obtained from herbarium samples. The pollen slides were prepared according to Wodehouse (1935) technique. For LM investigation, measurements and observations were made using the Olympus microscope model BX-41 LM. For the scanning electron microscopy (SEM), the pollen grains were observed and photographed with a KYKY-3200 SEM to determine their exine ornamentation. Also pollen terminology has been used based on Punt *et al*., 2007.

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An investigation of the anatomy, palynology and trichome types of *Phlomis olivieri* (Lamiaceae)

Hossein Yousefi [1], Atefe Amirahmadi [2], Morteza Atri [1] and Reza Naderi [2]*

[1] Department of Biology, Faculty of Sciences, Bu-Ali Sina University, Hamedan, Iran
[2] School of Biology and Institute of Biological Sciences, Damghan University, Damghan, Iran

Abstract
The anatomy, palynology and trichome types of *Phlomis olivieri* were studied in order to understand the usefulness of these characteristics for systematic purposes. Results showed that vascular bundles were next to each other in the stem, the mesophyll was composed of 1-layered palisade and 3-4 layered spongy parenchyma. There were two collateral vascular bundles in the centre and two small subsidary bundles in the corners of petiole and there were 4 small vascular bundles in the bracteole. Five main types of trichomes (peltate, capitate glandular, stellate, unicellular simple and dendroid trichomes) were observed. The capitate trichomes were subdivided into three groups: type 1 (short stalk), type 2 (medium or tri-cellular stalk) and type 3 (long or four-cellular stalk). Stellate trichomes were subdivided into two groups: group 1, sessile or short stalked trichomes and group 2, long stalked trichomes. The dendritic trichomes also were reported for the first time in this species. The pollen grains were tricolpate, relatively large, ovate and the exine ornamentation was reticulate and perforate.
Key words: Anatomy, Lamiaceae, Palynology, Trichome, *Phlomis*, Iran

Introduction

The genus *Phlomis* L. is one of the largest genera of subfamily Lamioideae (Lamiaceae) with more than 100 recognized species distributed in Asia, south Europe and north Africa that have been divided into two main sections: *Phlomis* and *Phlomoides* (Moench) Briq. (Rechinger, 1982). Section *Phlomis* was subdivided by Bentham (1834) into three subsections: *Dendrophlomis* Benth., *Gymnophlomis* Benth. and *Oxyphlomis* Benth. The diagnostic character for separating sections is corolla shape. Corolla in sect. *Phlomis* have a curved upper lip and a trifid lower lip with large median and smaller lateral lobes as opposed to the presence of a straight upper lip and a trifid lower lip with subequal lobes in sect. *Phlomoides* (Azizian and Moore, 1982). The genus represented by nearly 19 species in Iran including *Ph. olivieri* Benth. which grows wildly in the north, northwest, west and centre of Iran (Rechinger, 1982; Jamzad, 2012).

The taxonomic value of the indumentum as well as its implication in systematics and

* Corresponding Author: rezanaderia@du.ac.ir

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localities; e.g., var. *prokhanovii* and var. *cylindrica* grow side by side but still distinct in Yasouj to Babameydan site.

Based on the results of this study we concluded that *A. cylindrica* grows in Iran with three varieties: *cylindrica, prokhanovii* and *rumelica*. A taxonomic key to these varieties was constructed and presented as below:

Key to the varieties of *A. cylindrica* in Iran

1- Awns of the uppermost spikelet longer than the total length of spike; spike usually with 3-5 spikelets ……..... var. *rumelica*
- Awns of the uppermost spikelet shorter than the total length of spike; spike with more spikelets 2
2- Spikelets and rachis densely and brownish pilose ..... var. *prokhanovii*
- Spikelets and rachis scabrid, spikes green …..... var. *cylindrica*

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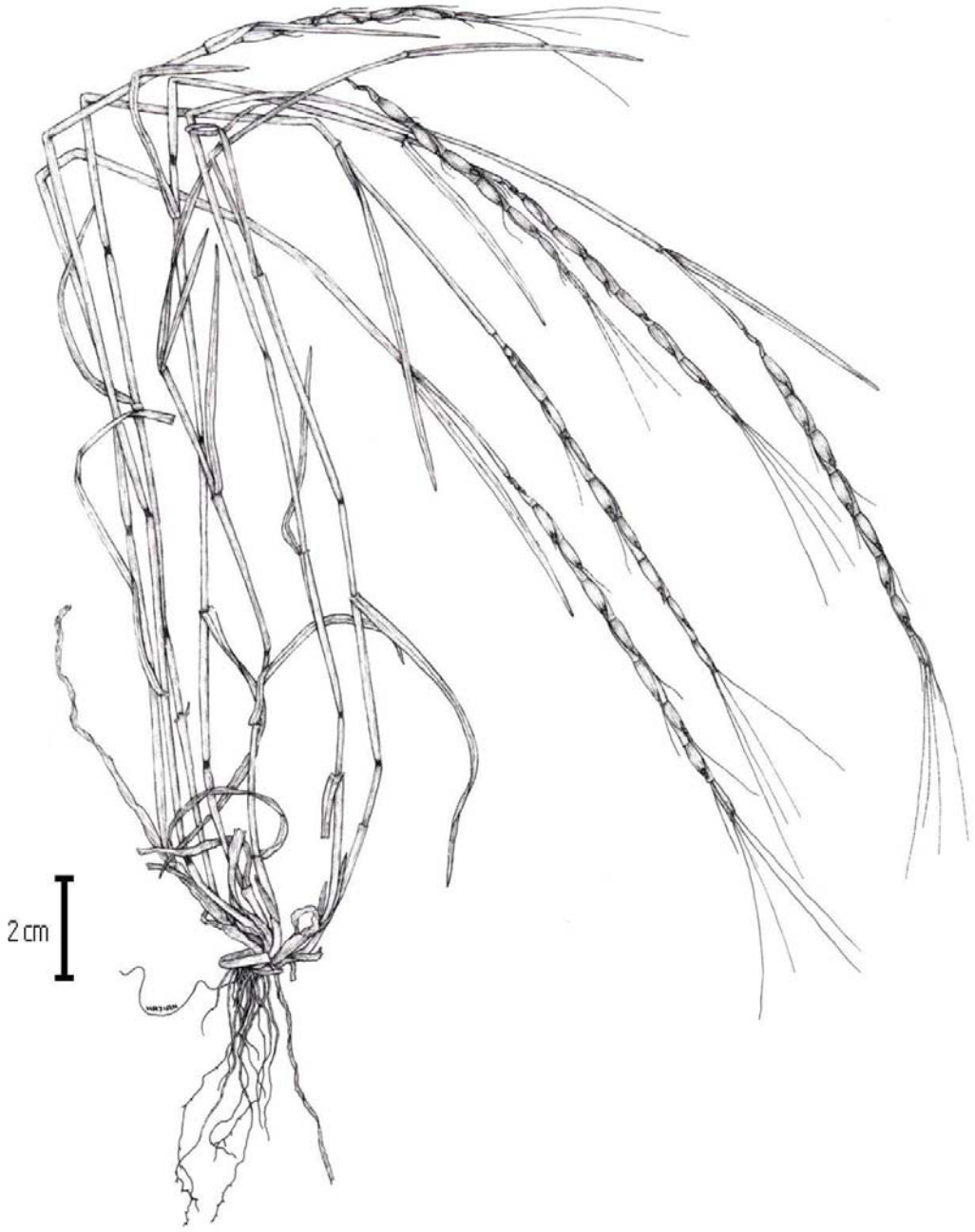

Figure 3. A general habit of *Aegilops cylindrica* Host (var. *cylindrica*)

The presence of scabrid glumes and rachis was the normal traits in *A. cylindrica*; neverthless a collected accession from Yasouj to Babameydan (HUI 17985) showed hairy glumes and rachis which is in accordance with Tzvelev (1976) who took the densely short pilose spikelets and rachis variant to describe his new variety (var. *prokhanovii*) (see Figures 2B, 2D, 2E). Plants of this accession were characterized by brownish spikes and rachis (Table 2).

According to the results of this study, despite the prevalence of hybridization in Poaceae (even inter-generic); from which natural and artificial hybrids between *A. cylindrica* and *T. aestivum* are well known and documented (Morrison *et al*., 2002 and our unpublished data); taxonomic identity of infra-specific taxa of *A. cylindrica* could be conserved in the sympatric

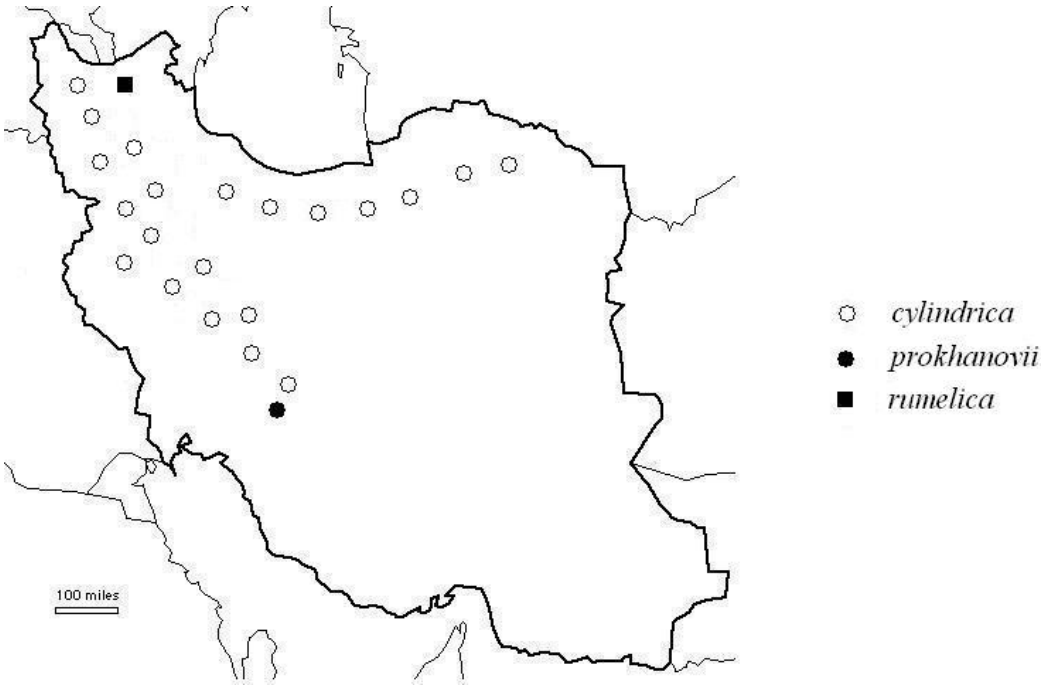

Figure1. A distribution map of *Aegilops cylindrica* varieties in Iran

Also, this study showed that spike shape among the studied materials vary from oblong to cylindrical. All plants having the longest awn and the least spikelets number per spike showed oblong spike shape compared with those having cylindrical spike. These characters were diagnostic features for var. *rumelica* which we collected from Azarbaijan (Ahar to Kalibar, 35 km to Kalibar, 1716 m) (see Figures 2C, 3, Tables 1, 2).

Figure 2. *Aegilops cylindrica* varieties. A. *cylindrica* (HUI 17989); B. *prokhanovii* (HUI 17985); C. *rumelica* (HUI 17986); D. Scabrid surface of glume in var. *cylindrica* and var. *rumelica*. E: Densly pilose in var. *prokhanovii*.

Table 2. Quantitative and qualitative characters considered in study of *Aegilops cylindrica* varieties in Iran (in quantitative characters means have calculated)

No.	Character	***A. cylindrica* var. *cylindrica***	***A. cylindrica* var. *prokhanovii***	***A. cylindrica* var. *rumelica***
1	Length of spike (cm)	9.36	8.1	5.22
2	Length of spikelet (mm)	10.66	9	10
3	Length of lower most glume (mm)	9	9.1	8.5
4	Width of lower most glume (mm)	3.66	3.4	3.1
5	Length of upper most spikelet awn (cm)	5	4.8	6.63
6	Length of lower most spikelet awn (mm)	2	2.7	3
7	Length of second spikelet awn (mm)	5.33	3	10.7
8	Length of second spikelet glum (mm)	10.33	7	7.2
9	Length of caryopsis (mm)	8.33	6	5.5
10	Width of caryopsis (mm)	2.16	2	2
11	Number of florets in spikelet	3.66	3	3
12	Number of seeds in spikelet	1.66	2	2
13	Number of spikelets in each spike	8	8	5.1
14	Length of second spikelet awn/ Length of second spikelet glum	0.51	0.42	1.48
15	Length of uppermost spikelet awn/ Length of spike	0.53	0.59	1.27
16	Length of lower most spikelet awn/ Length of lower most glume	0.22	0.29	0.35
17	Compact hair on rachis (absent: - / present: +)	-	+	-
18	Hair on glume (absent: - / present: +)	-	+	-
19	Spike shape (oblong: - / cylindric: +)	+	+	-
20	Spike colour (green: -/ brown: +)	-	+	-

Results and discussion

The results of this study showed that the geographical distribution of *Aegilops cylindrica* in Iran was restricted to an area from northwest (Azarbaijan province) eastwardly along Elburz mountains toward northeast (Khorasan province) and southwardly along Zagros mountains toward southwest of Iran with an elevation ranging from 500-2300 m (Figure 1). In comparison with Bor (1970) our observations expanded the distributional range of this species in Iran. Jaask (1978) considered *A. cylindrica* as a Mediterranean element, where the two putatively diploid progenitor i. e. *A. caudata* and *A. tauschii* underwent the hybridization and allopolyploidy processes to create *A. cylindrica* in Turky; which then emigrated to Europe and Iran.

In *A. cylindrica* usually the length of awns gradually increased from lowermost spikelet towards the uppermost one in each spike, so that the uppermost spikelet showed the longest awn. One of the studied accessions collected from NW of Iran showed interesting state in which not only was the awn of the uppermost spikelet the longest but also was longer than the total spike's length. On the other hand, in this specimen all the spikelets in the middle part of the spike showed awns longer than the accessions collected from the other areas. The majority of accessions studied possessed spikes with an average of eight spikelets per spike, however, the least number of spikelets per spike (five spikelets) was observed in above (Table 2).

Materials and Methods

A selection of 25 representetives (Table 1) belonging to *Aegilops cylindrica* from 100 accessions collected during the years 1996-2000 all around Iran were studied. The selected accessions included all distribution areas and main phenotypic variations. In order to provide enough plant materials for the morphological and taxonomic studies, the accessions were grown in the research field of the University of Isfahan. Voucher specimens are deposited in the herbarium of the University of Isfahan (HUI). In addition, all the related materials including the Iranian specimens collected for this study, type specimens of *A. cylindrica* var. *cylindrica* (W 2284), *A. cylindrica* var. *pauciaristata* (W 1426), *A. cylindrica* var. *rumelica* Velen. (W 5377) and two parental species i. e. *A. tauschii* (W 1973-0008961, W 1970-0016311) and *A. caudata* (W 0000441) housed in the Natural History Museum Vienna (W) and the Botanic Garden and Botanical Museum Berlin (B) were studied.

Table 1. List of Iranian *Aegilops cylindrica* accessions studied taxonomically

No.	Accession Number	Locality
1	Ag. 43	Lorestan: Khorram Abad to Sepidasht, 1900 m, Rahiminejad and Sahebi
2	Ag. 59	Fars: Dasht Arzhan, 2030 m, Rahiminejad and Sahebi
3	Ag. 108	Kurdistan: Saqez to Marivan, 1500 m, Rahiminejad and Sahebi
4	Ag. 148	Azarbaijan: 15 km remain to Ahar from Tabriz, 1550 m, Rahiminejad and Sahebi
5	Ag. 173	Kurdistan: Piranshahr to Sardasht, 1200 m, Rahiminejad and Sahebi
6	Ag. 240	Kurdistan: Boukan to Mahabad, 1490 m, Rahiminejad and Sahebi
7	Ag. 262	Azarbaijan: Urmieh, 1332 m, Rahiminejad and Sahebi
8	Ag. 84	Chaharmahal va Bakhtiari: Lordegan, Deh Soukhteh, 1770 m, Rahiminejad and Sahebi
9	Ag. 90	Kurdistan: Sanandaj to Sonqor, 1620 m, Rahiminejad and Sahebi
10	Ag. 106	Azarbaijan: Ahar to Kalibar, 1430 m, Rahiminejad and Sahebi
11	Ag. 470	Kohgiluyeh va Boyer-Ahmad: Yasouj to Babameydan, 1180 m, Hosseini and Saeedi, (HUI 17985)
12	Ag. 476	Kohgiluyeh va Boyer-Ahmad: Yasouj to Lordegan, Gharah village, 1950 m, Hosseini and Saeedi
13	Ag. 478	Alborz: Karaj to Chalous, Marzan Abad, 500 m, Hosseini and Saeedi
14	Ag. 474	Isfahan: Semirom, 2300 m, Hosseini and Saeedi
15	Ag. 479	Khorasan: Azadshaher to Bojnourd, 1450 m, Hosseini and Saeedi
16	Ag. 484	Khorasan: Shirvan to Mashhad, 1090 m, Hosseini and Saeedi
17	Ag. 488	Khorasan: Neyshabour to Sabzevar, 1080 m, Hosseini and Saeedi
18	Ag. 489	Khorasan: Sabzevar to Shahroud, Mayami, 1120 m, Hosseini and Saeedi
19	Ag. 490	Tehran: Firouzkooh to Tehran, 1810 m, Hosseini and Saeedi
20	Ag. 497	Hamedan: Hamedan to Assad Abad, 2140 m, Rahiminejad and Saeedi
21	Ag. 509	Azarbaijan: Urmieh to Sero, 1286 m, Rahiminejad and Saeedi
22	Ag. 516	Azarbaijan: Ahar to Kalibar, 35 km remain to Kalibar, 1716 m, Rahiminejad and Saeedi, (HUI 17986)
23	Ag. 576	Golestan: Golestan forest, 650 m, Hosseini and Saeedi
24	Ag. 497	Hamedan: Hamedan to Assad Abad, Assad Abad neck, 2140 m, Rahiminejad and Saeedi
25	Ag. 486	Khorasan: Ashkhaneh to Bojnourd, 850 m, Rahiminejad and Saeedi

In addition, to certify the ploidy levels of the studied materials, all the accessions were chromosomally examined from the root tips stained in aceto-orcein by squash method (unpublished data). In order to make a comparison among the materials under study, 20 morphological characters (16 quantitative and 4 qualitative) cited in the relevant literatures were evaluted (Table 2).

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Notes on *Aegilops cylindrica* (Triticeae, Poaceae) in Iran

Sayed Zabihollah Hosseini * and Mohammad Reza Rahiminejad

Department of Biology, Faculty of Sciences, University of Isfahan, Isfahan, Iran

Abstract
The present paper deals with the study of the taxonomy of one hundred accessions of *Aegilops cylindica* collected all around the country, and also Iranian materials and type specimens and related species housed in the Natural History Museum Vienna (W), the Botanic Garden and Botanical Museum Berlin (B), were studied morphologically. The results showed that morphological traits such as length of spike, length of awn, number of spikelet in each spike, presence/absence of hair on spikelets and rachis were highly variable among the studied materials. Based on the results of this study *A. cylindica* was recognized as having three varieties, all new records, for Iran.
Key words: *Aegilops cylindrica*, Triticeae, Poaceae, Taxonomy, Iran

Introduction

Aegilops cylindrica Host, an annual wild grass and almost close relative to *Triticum aestivum* L. (bread wheat), occurs throughout Mediterranean, Middle East and Asiatic regions (Linc *et al*., 1999; Slageren, 1994; Karataglis, 1989). This species with a chromosomal formula of $2n = 4x = 28$ (DDCC) has been considered as an allotetraploid species resulted from a natural hybridization between *A. caudata* L. ($2n = 2x = 14$, CC) and *A. tauschii* Coss. ($2n = 2x = 14$, DD) (Linc *et al*., 1999; Karataglis, 1989). Jaaska (1978) suggested that this species originated from east parts of Turkey, where the distribution areas of its putative parents might had been overlapped.

In his account Tzvelev (1976) recognized four varieties for *A. cylindrica*: *cylindrica, aristulata* (Zhuk.) Tzvel., *pauciaristata* Eig. and *prokhanovii* Tzvel. occurring in (the former) Soviet union (USSR); a fifth one, i. e., var. *rumelica* was added by Velenovsky for Flora Bulgarica (Velenovsky 1891). Bor (1970) recognized this species with no infra-specific subdivisions for the Flora Iranica areas (Iraq, Afghanistan and NW, W of Iran). Literature review shows that the Iranian materials of this species were the subject of taxonomic (Keshavarzi *et al*., 2006; Arzani *et al*., 2006; Kharazian, 2008), molecular cytogenetic and C-banding (Linc *et al*., 1999; Bordbar *et al*., 2011), isozyme (Jaaska, 1981), and inter-generic hybridization (Morrison *et al*., 2002) studies. *Aegilops cylindrica* is also important from the breeding point of view, due to the gene flow between this species and *T. aestivum* (Guadagnuolo *et al*., 2001).

This study aims to review the taxonomic status of *A. cylindrica* in Iran.

* Corresponding Author: zhossini@gmail.com

subsp. *spurium* (10450), *Galium verticillatum* Danthoine ex Lam. (10414), *Galium verum* L. subsp. *verum* f. *verum* (10678, 10451).
Salicaceae: **Salix excelsa* S.G.Gmelin. (10791).
Santalaceae: **Thesium kotschyanum* Boiss. (10710, 10467).
Scrophulariaceae: *Bungea trifida* C.A.Mey. (10711), *Linaria simplex* DC. (10507), *Scrophularia azerbijanica* Grau. (10475),**Verbascum orientale* L.All. (10687), *Verbascum speciosum* Schrad. (10697, 10767), *Verbascum suworowianum* K.Koch var. *suworowianum* (10424), *Verbascum suworowianum* K.Koch var. *acuminatum* (Murb.) Hub.-Mor. (10425), *Veronica microcarpa* Boiss. (10292), *Veronica persica* Poir (10501).
Thymeleaceae: *Diarthron vesiculosum* Endl. (10693).
Ulmaceae: *Celtis glabrata* Steven ex Planch. (10816, 10685).
Valerianaceae: *Valerianella oxyrhyncha* Fisch. & C.A.Mey. (10421), *Valerianella coronata* (L.) DC. (10465), *Valerianella cymbaecarpa* C.A.Mey. (10307), *Valerianella plagiostephana* Fisch. & C.A.Mey. (10708).
Verbenaceae: *Verbena officinalis* L. (10785).
Violaceae: *Viola occulta* Lehm. (10316, 10286).
Zygophyllaceae: *Peganum harmala* L. var. *harmala* (10398), *Zygophyllum atriplicoides* Fisch. & C.A.Mey. var. *atriplicoides* (10436).

Holosteum glutinosum (M.Bieb.) Fisch. & C.A.Mey. (10278), *Holosteum marginatum* C.A.Mey. (10310), *Minuartia hamata* (Hausskn.) Mattf. (10422), **Minuartia lineata* (Boiss.) Bornm. (10474), *Minuartia meyeri* Bornm. (10706, 10427),**Paronychia caespitosa* Stapf (10418), *Silene chlorifolia* Sm. (10686), *Silene commelinifolia* Boiss. var. *commelinifolia* (10682), *Silene spergulifolia* M.Bieb. (10503), *Silene stenobotrys* Boiss. & Hausskn. (10472), *Velezia rigida* L. (10416).

Cistaceae: *Helianthemum ledifolium* Mill. var. *microcarpum* Willk. (10799).

Crassulaceae: *Rosularia sempervivum* A. Berger var. *sempervivum* (10509),**Sedum subulatum* Boiss. (10679, 10680), *Sedum album* L. (10681).

Convolvulaceae: *Convolvulus lineatus* L. (10667), *Cuscuta brevistyla* A.Braun ex A.Rich. (10668).

Dipsacaceae: *Scabiosa micrantha* Desf. (10502).

Ephorbiaceae: ****Euphorbia denticulata* Lam. (10262, 10263), *Euphorbia* sp. (10400), *Euphorbia* sp. (10701, 10778).

Fabaceae: **Astragalus crispocarpus* Náběl ek (10487), *Astragalus ebenoides* Boiss. subsp. *ebenoides* (10483),**Astragalus glochidiatus* Maassoumi (10689),**Astragalus iranicus* Bunge (10279, 10841), *Astragalus latifolius* Lam. (10295), *Astragalus macropelmatus* Bunge subsp. *macropelmatus* (10351, 10305), *Astragalus rhodosemius* Boiss. & Hausskn. (10690), *Astragalus vegetus* Bunge (10314, 10350), *Astragalus wagneri* Bartle. ex Bunge (10277),**Caragana grandiflora* DC. (10303), *Medicago rigidula* (L.) All. var. *rigidula* (10428), *Onobrychis cornuta* (L.) Desv. subsp. *cornuta* (10498,10293), *Onobrychis subacaulis* Boiss. (10317), *Vicia cappadocica* Boiss. (10287).

Geraniaceae: *Biebersteinia multifida* DC. (10311),**Erodium neuradifolium* Delile ex Godron (10283, 10485),****Erodium oxyrrhynchum* M.Bieb. subsp. *bryoniifolium* Boiss.) Schönb.-Tem. (10423), *Geranium tuberosum* L. (10484, 10312).

Hypericaceae: *Hypericum scabrum* L. (10712, 10478), *Hypericum helianthemoides* (Spach) Boiss. (10771).

Lamiaceae: *Acinos graveolens* Link (10489),**Ajuga chamaecistus* Ging ex Benth. subsp. *tomentella* (Boiss.) Rech.f. (10496),****Dracocephalum thymiflorum* L. (10456, 10358, 10273), *Eremostachys macrophylla* Montbr. & Auch. (10439), *Eremostachys laciniata* Bunge (10462), *Hymenocrater bituminosus* Fisch. & C.A.Mey. (10481, 10466), *Lallemantia iberica* Fisch. & C.A.Mey. (10363, 10486), *Lamium amplexicaule* L. var. *amplexicaule* (10284), *Marrubium parviflorum* Fisch. & C.A.Mey. (10713, 10676), *Mentha longifolia* L. (10779, 10793),**Nepeta fissa* C.A.Mey. (10695), *Nepeta involucrata* Bornm. (10495, 10508), *Phlomis olivieri* Benth. (10659),**Salvia chloroleuca* Rech.f. & Aellen (10531), *Salvia pachystachya* Trautv. (10454, 10457), *Scutellaria pinnatifida* A.Ham. subsp. *alpina* (Bornm.) Rech.f. (10448), *Scutellaria platystegia* Juz. (10470), *Scutellaria* sp. (10289), *Sideritis montana* L. (10401, 10447), *Stachys inflata* Benth. (10433, 10444), *Stachys schtschegleevii* Sosn. ex Grossh (10264, 10275),****Stachys laxa* Boiss.& Buhse (10660), *Teucrium polium* L. (10797, 10663, 10432, 10443), *Thymus* sp. (10446, 10461, 10445), *Ziziphora capitata* L. subsp. *orientalis* Samuelsson ex Rech.f. (10460), *Ziziphora tenuior* L. (10403).

Linaceae: **Linum mucrunatum* Betrol. var. *mucrunatum* (10666).

Lythraceae: *Lythrum salicaria* L. (10786).

Malvaceae: **Abutilon theophrasti* Medik. (10208), *Alcea flavovirens* (Boiss. & Buhse) var. *flavovirens* (10719, 10753), **Alcea glabrata* Alef. var. *glabrata* (10796, 10812, 10205), *Hibiscus trionum* L. (10819).

Papaveraceae: *Glaucium corniculatum* (L.) Rudolph subsp. *corniculatum* (10431), *Hypecoum pendulum* L. (10282), **Papaver argemone* L. (10493), *Papaver macrostomum* Boiss. & A.Huet (10492), *Roemeria hybrida* (L.) DC. subsp. *dodecandra* (Forssk.) Maire (10270).

Plantaginaceae: *Plantago lanceolata* L. (10473).

Plumbaginaceae: *Acantholimon bracteatum* Boiss. (10482), *Plumbago europaea* L. (10815).

Polygalaceae: *Polygala hohenackeriana* Fisch. & C.A.Mey. (10285).

Polygonaceae: *Atraphaxis spinosa* L. (10696).

Primulaceae: *Androsace maxima* L. (10309, 10426).

Ranunculaceae: *Adonis aestivalis* L. (10267), *Ceratocephala falcata* (L.) Pers. (10268), *Consolida regalis* Gray subsp. *divaricata* (Ledeb.) Munz (10704, 10703, 10702), *Thalictrum isopyroides* C.A.Mey. (10313).

Resedaceae: *Reseda lutea* L. (10471, 10688), **Reseda microcarpa* Müll.Arg. (10435).

Rhamnaceae: *Rhamnus pallasii* Fisch. & C.A.Mey. (10407, 10429, 10784, 10490).

Rosaceae: *Amygdalus communis* L. (10276), *Cerasus pseudoprostrata* Pojark. (10705, 10306), *Cotoneaster nummularioides* Pojark. (10824), *Crataegus meyeri* Pojark. (10790, 10825), *Rosa canina* L. (10792, 10795, 10822), *Sanguisorba minor* Scop. subsp. *lasiocarpa* (Boiss. & Hausskn.) Nordborg (10787, 10789).

Rubiaceae: *Callipeltis cucullaria* (L.) DC. (10406),****Crucianella gilanica* Trin. subsp. *glauca* (A.Rich. ex DC.) Ehrend. (10673), *Crucianella gilanica* Trin. subsp. *trancaspica* (10674),**Gaillonia szowitzii* DC. (10665), *Galium humifusum* M.Bieb. (10675),****Galium nupercreatum* Popov (10777), *Galium spurium* L.

Appendix 1. Floristic list of AqDagh sanctuary zone in Marakan protected area and herbarium numbers. The first reported taxa from northwest of Iran have been shown with three stars and from west Azarbaijan with one star.

Gymnosperms

Ephedraceae: *Ephedra major* Host. (10672).

Angiosperms

Monocots

Amaryllidaceae: ****Allium subakaka* Razyfard & Zarre (10480), *Allium syntamanthum* K.Koch (10399)

Asparagaceae: *Ornithogalum brachystachys* K.Koch (10449), *Muscari caucasicum* (Griseb). Baker (10476, 10477), *Muscari neglectum* Guss. ex Ten. (10297).

Iridaceae: *Iris iberica* Hoffm. subsp. *lycotis* (Woron) Takth. (10499, 10301).

Ixioliriaceae: *Ixiolirion tataricum* (Pall.) Herb. subsp. *montana* (10458).

Liliaceae: *Tulipa humilis* Herb. (10241),****Tulipa lehmanniana* Mercklin (10308).

Poaceae: *Aegilops triuncialis* L. (10412), *Agropyron pectiniforme* Roem. & Schultes (10442), *Boissiera squarrosa* (Sol.) Nevski (10662, 10434),****Bromus gracilimus* Bunge (10430), ****Bromus madritensis* L. var. *madritensis* (10519, 10661), *Bromus tectorum* L. var. *hirsutus* Regel (10405, 10402), *Eremopoa persica* (Trin.) Roshev. (10438), *Eremopyrum distans* (K.Koch) Nevsk. (10404), *Melica jacquemontii* Decne. subsp. *jacquemontii* (10510, 10441), *Poa bulbosa* L. (10202, 10294), *Stipa barbata* Desf. (10415, 10420), *Taeniatherum crinitum* (Schreb.) Nevski (10408).

Dicots

Amaranthaceae: **Amaranthus albus* L. (10814), *Amaranthus retroflexus* L. (10811), *Atriplex tatarica* L. (10810), *Caroxylon ericoides* (M.Bieb.) Akhani & E.H.Roalson (10788), *Ceratocarpus arenarius* L. (10409), *Halanthium rarifolium* K.Koch (10207), *Kraschenninnikovia ceratoides* (L.) Gueldenst. var. *ceratoides* (10669,10670), *Noaea mucronata* Asch. & Schweinf. subsp. *mucronata* (10820), *Salsola tragus* L. (10214), *Seidlitzia florida* (M.Bieb.) Bunge ex Boiss. (10220, 10212, 10213), *Suaeda altissima* Pall. (10794).

Apiaceae: *Astrodaucus orientalis* Drude (10817, 10437), *Daucus carota* L. subsp. *carota* (10781,10782, 10783),**Eryngium bungei* Boiss. (10847), *Eryngium glomeratum* Lam. (10846), *Prangos uloptera* DC. (10468), *Scandix aucheri* Boiss. (10413, 10232, 10266), *Zosimia absintifolia* (Vent.) Link (10504).

Asteraceae: *Artemisia scoparia* Waldst. & Kit. (10805), *Carduus pycnocephalus* L. subsp. *pycnocephalus* (10315), *Carthamus lanatus* L. (10801), *Centaurea phaeopappa* (DC.) Sch.Bip. (10703), *Centaurea pseudoscabiosa* Boiss. & Buhse subsp. *pseudoscabiosa* (10691), *Centaurea solstitialis* L. subsp. *solstitialis* (10800), *Centaurea virgata* Lam. subsp. *squarrosa* (Willd.) Gugler (10808, 10463), *Cichorium intybus* L. (10772), *Cirsium congestum* Fisch. & C.A.Mey. (10714), *Cousinia macroptera* C.A.Mey. ex DC. (10417, 10455), *Cymbolaena griffithii* (A.Gray) Wagenitz (10411), *Echinops orientalis* Trautv (10802), *Filago arvensis* L. (10410), *Helichrysum armenium* DC. (10694), *Jurinea pulchella* DC. (10768), *Lactuca serriola* L. (10775), *Onopordum leptolepis* DC. (10813), *Pulicaria dysenterica* (L.) Bernh. (10780),**Scorzonera mucida* Rech.f., Aellen & Esfand. (10265), *Scorzonera ramosissima* DC. (10479), *Senecio vernalis* Waldst. & Kit. (10236, 10290), *Klasea coriacea* (DC.) Holub (10798), *Tanacetum canescens* DC. (10664), *Tanacetum polycephalum* Sch. Bip. (10440), *Tanacetum uniflorum* Sch. Bip. (10459, 10683, 10684), *Taraxacum sp*. (10774, 10803), *Tragopogon coloratus* C.A.Mey. (10506), *Tragopogon graminifolius* DC. (10497), *Xanthium brasilicum* Vell. (10818), *Xeranthemum squarrosum* Boiss. (10700).

Berberidaceae: *Berberis integerrima* Bunge (10823), *Leontice armeniaca* Boiv. (10488, 10240).

Boraginaceae: ****Buglossoides tenuiflora* (L.f.) I.M.Johnst. (10298, 10296), **Heliotropium circinatum* Griseb. (10806, 10807), *Heliotropium dissitiflorum* Boiss. (10699), *Heliotropium szowitsii* Stscheg. (10698), *Lappula microcarpa* Gürke (10491, 10464, 10677, 10452), *Nonea pulla* DC. (10300), *Onosma microcarpa* DC. (10671, 10453), *Paracaryum strictum* Boiss. (10692).

Brassicaceae: **Aethionema trinervium* Boiss. var. *apterocarpum* (Rech.f. & Aellen) Hedge (10469,10304), *Alyssum inflatum* Nyàr. (10288, 10291), *Alyssum linifolium* Steph. ex Willd. var. *linifolium* (10274), *Alyssum minus* (L.) Rothm. (10204), **Alyssum muellerii* Boiss. & Buhse (10709), *Arabidopsis pumila* Busch (10269), **Arabis gerardii* Besser (10281),**Clypeola dichotoma* Boiss. (10419), *Conringia orientalis* (L.) Andrz. (10234), **Drabopsis verna* K.Koch (10233, 10203), *Goldbachia laevigata* DC. (10280), *Isatis cappadocica* Desv. subsp. *cappadocica* (10707), *Sameraria stylophora* Boiss. (10505), *Sterigmostemum incanum* M.Bieb. (10299), *Thlaspi perfoliatum* L. (10235), *Torularia torulosa* O.E.Schulz (10271).

Capparidaceae: *Capparis spinosa* L. (10769, 10770, 10804).

Caryophyllaceae: *Arenaria holostea* M.Bieb. subsp. *macrantha* (Schischk.) McNeill (10494), *Cerastium inflatum* Link ex Sweet (10500),**Dianthus orientalis* Donn var. *obtusisquameus* (Boiss.) Rech.f (10773),

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Table 5. Comparison of chorotypes in studied area in east and west Azarbaijan provinces

Marakan	IT: 65.25%	Bi- tri- or plural: 24.13%	ES: 5.31%	End: 4.25%	M: 1.06%
Munjughlu	Bi- tri- or plural: 58.51%	IT: 34.06%	End: 6.12%	ES: 1.31%	
AqDagh	Bi- tri- or plural: 59.01%	IT: 31.53%	End: 9.46%		
Arasbaran	Bi- tri- or plural: 45.6%	ES: 26.6%	IT: 23.2%	M: 4.6%	
Ghasemloo valley	IT: 61.28%	Bi- tri- or plural: 22.55%	ES: 8.33%	End: 6.86%	M: 0.98%
Mirabad	IT: 62.5%	Bi- tri- or plural: 25.52%	ES: 6.78%	End: 4.68%	M: 0.52%

Endemism

Among 227 taxa distributed in the studied area, 14 species, 4 variety and 3 subspecies were endemic. The endemic taxa in this area were: *Aethionema trinervium* Boiss. var. *apterocarpum* (Rech.f. & Aellen) Hedge, *Alyssum inflatum* Nyàr., *Dianthus orientalis* Donn var. *obtusisquameus* (Boiss.) Rech.f., *Minuartia lineata* (Boiss.) Bornm., *Paronychia caespitosa* Stapf, *Astragalus ebenoides* Boiss. subsp. *ebenoides*, *Astragalus glochidiatus* Maassoumi, *Astragalus rhodosemius* Boiss. & Hausskn., *Astragalus vegetus* Bunge, *Astragalus wagneri* Bartle. ex Bunge, *Ajuga chamaecistus* Ging ex Benth. subsp. *tomentella* (Boiss.) Rech.f., *Phlomis olivieri* Benth., *Stachys laxa* Boiss. & Buhse, *Allium subakaka* Razyfard & Zarre, *Alcea flavovirens* (Boiss. & Buhse) Iljin var. *flavovirens*, *Alcea glabrata* Alef. var. *glabrata*, *Achantholimon bracteatum* Boiss., *Rhamnus pallasii* Fisch. & C.A.Mey., *Crucianella gilanica* Trin. subsp. *glauca* (A.Rich. ex DC.) Ehrend., *Scorzonera mucida* Rech.f., Aellen & Esfand., *Heliotropium dissitiflorum* Boiss. Endemic taxa comprised 8.84% of total taxa in this area. About 6916 plant species were present in Iran out of which 1810 were endemic species (26%) (Ghahreman and Attar, 1999), this area consisted of 1.1% of Iran endemic species. Endemism frequency in families is in (Figure 5). The reason for reduction of endemism and biodiversity in this sanctuary could be due to maximum grazing that would lead to biodiversity losses and distribution of rural species. *Astragalus brachyodontus* Boiss., *Astragalus ebenoides* Boiss. subsp. *ebenoides, Astragalus vegetus* Bunge., *Astragalus wagneri* Bartle. ex Bunge., *Artemisia spicigera* K.Koch., *Echinops haussknechtii* Boiss., *Tragopogon caricifolius* Boiss., *Dianthus orientalis* Donn var. *obtusisquameus* (Boiss.) Rech,f, *Paronychia caespitosa* Stapf, *Phlomis olivieri* Benth., *Sameraria nummularia* Bornm., *Heliotropium esfandiarii* Akhani & H.Riedl, *Alcea flavovirens* (Boiss. & Buhse) Iljin var. *flavovirens*, *Acantholimon gilliatii* Turril., *Malabaila kotschyi* Boiss. were the endemic taxa in Munjughlu sanctuary that among these seven endemic taxa were common.

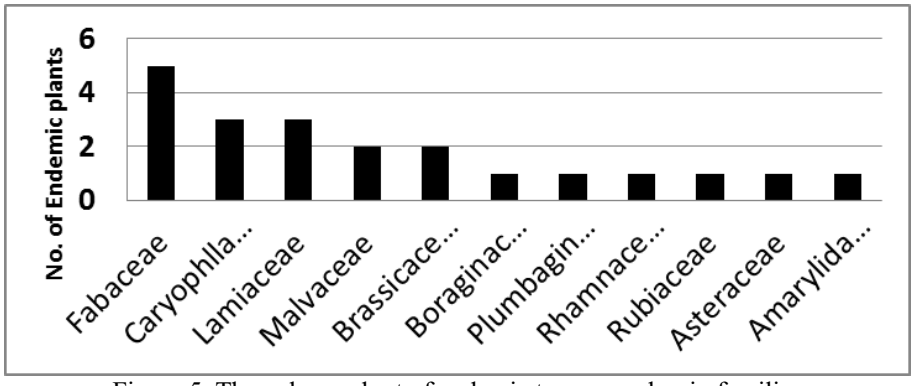

Figure 5. The column chart of endemic taxon number in families

reduction of vegetative growth to overcome difficult environmental conditions (Asri, 2003).

Comparing life forms of the studied area with other regions Assadi (1987, 1988), Manafi and Bahreiny (1997), Malekmohammadi *et al*. (2006), Hassanzadeh Gorttapeh and Panahy (2007), Hasanzadeh *et al*. (2008), and Ghahremaninejad and Nafisi (2011), maximum resemblance between AqDagh and Munjughlu followed by Marakan and Ghasemloo valley (Table 4) is revealed. In this table, Arasbaran data are from (Hamzeh'ee *et al*., 2010).

Table 4. plant life form spectrums in studied area in east and west Azarbaijan provinces

Marakan	Th: 31.91%	He: 26.95%	Ch: 16.31%	Ph: 15.06%	Cr: 9.26%
Munjughlu	Th: 40%	He: 31.92%	Ch: 15.32%	Cr: 10.21%	Ph: 1.7%
AqDagh	Th: 36.72%	He: 27.87%	Ch: 19.02%	Cr: 11.06%	Ph: 5.30%
Mishu-Dagh	He: 42.1%	Cr: 19.55%	Th: 19.24%	Ch: 14.24%	Ph: 5.3%
Arasbaran	He: 42.5%	Th: 25.8%	Cr: 18.8%	Ph: 8.1%	Ch: 4.8%
Ghasemloo valley	Th: 30.9%	He: 29.9%	Ph: 16.7%	Ch: 12.25%	Cr: 10.3%
Mirabad	He: 30.2%	Th: 28.1%	Cr: 22%	Ph: 17.7%	Ch: 12.5%

Chorology

This area is situated at junction of 2 important phytogeographic regions namely Irano-Turanian and Euro-Siberian. The vegetation of the area has been influenced by the elements of four phytogeographical regions, including Irano-Turanian, Euro-Siberian, Mediterranian, Saharo-Sindian, therefore as it is supposed rather high proportions of bi-tri or pluriregional elements occur in the area. The results showed that 59.01 % of the flora in this area belonged to bi-tri or pluriregional elements. This showed overlap of some phytogeographic areas in this region. Irano-Turanian elements (31.53%), Irano-Turanian and Euro-Siberian (27.49%), Pluriregional (13.06%), Irano-Turanian, Mediterranian and Euro-Siberian (7.66%), Irano-Turanian and Mediterranian (4.95%), Irano-Turanian, Mediterranian and Saharo-Sindian (2.25%), Irano-Turanian and Saharo-Sindian (1.8%), Irano-Turanian, Euro-Siberian and Saharo-Sindian (0.9%), Cosmopolitan (0.9%) and endemic (9.46%) were the most important Phytogeographic elements of the studied area (Figure 4). The phytochoria of the studied area that are compared with other regions (Table 5), showed the presence of similar Phytogeographical elements in AqDagh and Munjughlu and Arasbaran area. In addition, IT and IT-ES were the dominant chorotypes in all areas except in Arasbaran region.

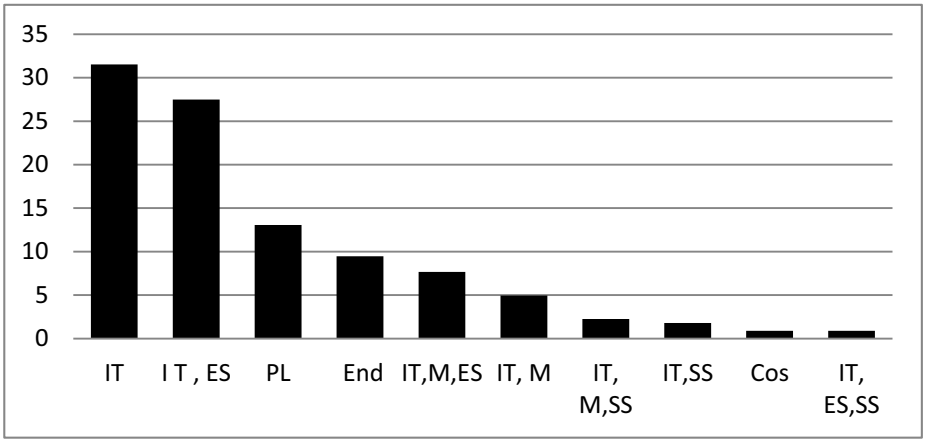

Figure 4. The column chart of percentage of phytocorya in AqDagh

Crassulaceae, Cistaceae and Capparidaceae families are present in AqDagh sanctuary but were absent in Munjughlu. In contrast, Cleomaceae, Juncaceae, Orobanchaceae, Solanaceae, Tamarixaceae, Xanthorrhoeaceae were present in Munjughlu and were absent in AqDagh region.

The Flora of AqDagh and Munjughlu had a total of 80 species, 110 genera and 36 families in common with Marakan flora. Based on this study, 59 genera and 12 families included: Violaceae, Verbenaceae, Santalaceae, Rhamnaceae, Primulaceae, Polygalaceae, Lythraceae, Ixioliriaceae, Dipsacaceae, Cistaceae and Capparidaceae, Asparagaceae are not found in study of Marakan by Hasanzadeh *et al*. (2008).

Life form

In the assessment of life form spectrum therophytes with 36.57% of flora were the most dominant, followed by hemicryptophytes (with 27.75%), chamaephytes (with 19.38%), cryptophytes (with 11.01%) and phanerophytes (with 5.29%) (Figure 3). The high presence of annual plants is the characteristic feature of this region, but with the increase in the altitude in mountainous parts hemicryptophytes became more abundant.

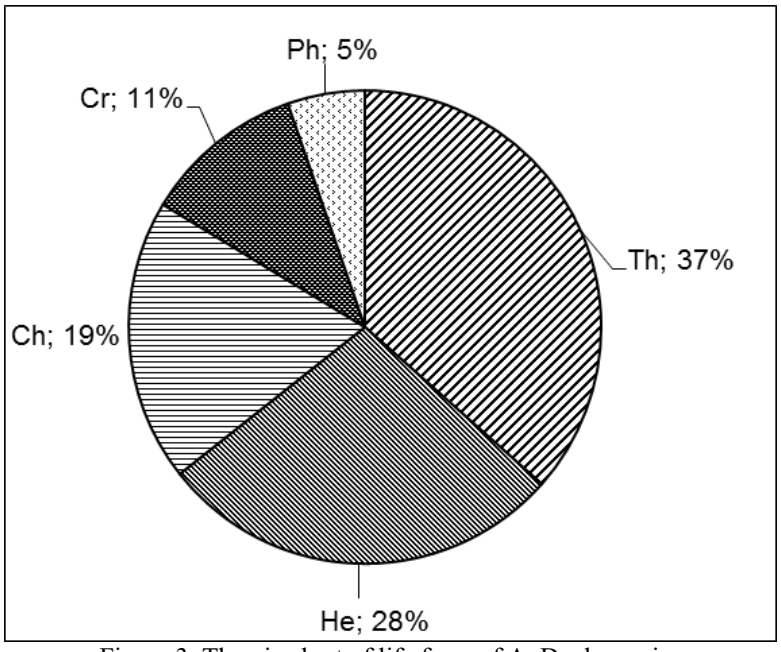

Figure 3. The pie chart of life form of AqDagh species

Life forms of plants indicated the possibility of its adaptation to environmental factors especially climatic condition. According to Mobayen (1980-1996), the frequency of therophytes was due to Mediterraniean climate and the frequency of hemicryptophytes was due to cold and temperate climate. High percentage of therophyte indicated the fact that this area had low rainfall. Dominance of therophyte and hemicryptophyte indicated the adaptation of these plants to arid and cold area. The low percentage of Cryptophyte and Phanerophyte showed that they were not adapted to existing climate and edaphical situations. In addition, based on our statististic data, this rank of frequency fitted in semi-dry and semi-dry cold condition. Therophytes adapted to drought and shortage of rainfall, because they spend their vegetative period in the form of seed and hemicryptophyte use different ways such as: reserving water, using ground water, reducing their water need by losing their leaves and

Table 2. List of rich families with more than 4 taxa in AqDagh sanctuary zone

Families	Genera	Taxa
Asteraceae	23	30
Lamiaceae	18	26
Brassicaceae	13	16
Caryophylaceae	8	14
Fabaceae	6	14
Poaceaee	10	12
Amaranthaceae	10	11
Scrophulariaceae	5	9
Rubiaceae	4	9
Boraginaceae	6	8
Apiaceae	6	7
Rosaceae	6	6
Papaveraceae	4	5

Table 3. Comparing floristic richness, taxonomic diversity and geographic characteristics of AqDagh and its adjacent studied areas

Studied area	Province	Area (hectar)	Precipitatin mm.year	Taxa	Genera
Marakan (Hasanzadeh *et al*., 2008)	east & west Azarbaijan	103986	281.1	282	167
Munjughlu (Ghahremaninejad and Nafisi, 2011)	east Azarbaijan	5270	242.88	236	166
Mishu-Dagh (Manafi and Bahreiny, 1997)	east Azarbaijan	-	736	390	232
Arasbaran (Assadi, 1987-1988; Hamzeh'ee *et al*., 2010)	east Azarbaijan	80654	316-686	1067	442
Ghasemloo Valley (Malekmohammadi *et al*., 2006)	west Azarbaijan	577	459.6	204	165
Mirabad (Hassanzadeh Gorttapeh and Panahy, 2007)	west Azarbaijan	11435	600	192	126
AqDagh (The present study)	west Azarbaijan	5184.7	360.9	227	166

Studied area	Province	Families	Taxa/ Genera	Genera/ Families
Marakan (Hasanzadeh *et al*., 2008)	east & west Azarbaijan	47	1.69	3.55
Munjughlu (Ghahremaninejad and Nafisi, 2011)	east Azarbaijan	38	1.42	4.36
Mishu-Dagh (Manafi and Bahreiny, 1997)	east Azarbaijan	60	1.68	3.86
Arasbaran (Assadi, 1987-1988; Hamzeh'ee *et al*., 2010)	east Azarbaijan	83	2.41	5.32
Ghasemloo Valley (Malekmohammadi *et al*., 2006)	west Azarbaijan	50	1.23	3.3
Mirabad (Hassanzadeh Gorttapeh and Panahy, 2007)	west Azarbaijan	41	1.52	3
AqDagh (The present study)	west Azarbaijan	47	1.37	3.53

The AqDagh sanctuary had 84 species (37%), 101 genera (60.47%) and 32 families (68%) in common with Munjughlu flora. Violaceae, Verbenaceae, Ulmaceae, Thymelaeaceae, Salicaceae, Rosaceae, Rhamnaceae, Lythraceae, Hypericaceae Ephedraceae, Convolvulaceae,

Table 1. List of taxa in Red List of IUCN. Lower risk (LR); Data deficient (DD); Vulnerable (VU)

Taxa	Rank	Taxa	Rank
Scorzonera mucida Rech.f., Aellen & Esfand.	LR	*Astragalus latifolius* Lam.	VU
Heliotropium dissitiflorum Boiss.	LR	*Astragalus crispocarpus* Nábělek	LR
Aethionema trinervium (DC.) Boiss.	LR	*Astragalus glochidiatus* Maassoumi	VU
Paronychia caespitosa Stapf	LR	*Astragalus ebenoides* Boiss. subsp. *ebenoides*	LR
Ajuga chamaecistus Ging. subsp. *tomentella* (Boiss.) Rech.f.	LR	*Astragalus iranicus* Bunge	DD
Gaillonia szovitsii DC.	DD	*Medicago rigidula* (L.) All.	LR
Stachys laxa Boiss. & Buhse	LR	*Onobrychis subacaulis* Boiss.	LR
Alcea glabrata Alef.	LR	*Acantholimon bracteatum* Boiss.	LR

Result and Discussion

Flora

In this survey from AqDagh sanctuary, 227 taxa of vascular plants consisting of 27 subspecies and 18 varieties and belonging to 47 families and 166 genera were recognized. Among these taxa, 21 taxa, 17 genera and 6 families belonged to monocotyledons, 205 taxa, 149 genera and 40 families belonged to dicotyledons and one taxon dedicated to gymnosperms. The biggest family of the region was Asteraceae with 30 taxa and 23 genera followed by Lamiaceae with 26 taxa and 18 genera, Brassicaceae with 16 taxa and 13 genera, Caryophylaceae and Fabaceae. Artemisia-Acantholimon was the phytosocioloical dominant type of Gochash habitat (Department of Environment, 2004).

In addition to the above families, there were 19 families with 1 taxa, 8 families with 2 taxa, 3 families with 3 taxa, 4 families with 4 taxa, and 13 families with more than 4 taxa (Table 2). Therefore, most of the families (72.34%) had less than 5 species frequency. Also, 25 families with 1 genus (53.19%), 6 families with 2 genera, 11 families with 3-9 genera and 5 families have 10-30 genera (Table 2); *Astragalus* with 9 species and *Gallium* with 5 species were the richest genera followed by *Centaurea, Silene, Valerianella, Verbascum* and *Alyssum* each with 4 species. High proportion of *Astragalus* in this area showed its adaptation to this mountainous area. *Astragalus* and *Silene* were the typical genera of IT phytogeographical region. 132 genera (79 %) with 1 species were also collected from the studies area. Based on Flora Iranica (Rechinger, 1963-2010), Flora of Iran (Assadi *et al*., 1988-2011) and floristic studies of east and west Azarbaijan provinces, 11 taxa from the northwest and 29 taxa from west Azarbaijan province were identified for the first time (Appendix 1). *Sedum subulatum* Boiss., *Eryngium glomeratum* Lam., *Arabis gerardii* Besser*, Astragalus wagneri* Bartle. ex Bunge, *Astragalus glochidiatus* Maassoumi, *Galium spurium* L. subsp. *spurium* were identified as rare species. *Cymbolaena griffithii* (A.Gray) Wagenitz, *Drabopsis verna* K.Koch belonged to monotypic genera, were identified in the studied area.

The flora of this region shared maximum number of common taxa (with 91 taxa) with Arasbaran protected area, but regarding number of dissimilar taxa, AqDagh showed maximum SØRENSON index comparing with Munjughlu, Marakan and Mishu-Dagh, respectively. According to this study, AqDagh sanctuary had 51 common species (22.46%), 83 genera (50%) and 34 families (72.34%) with Marakan (Hassanzadeh Gorttapeh *et al*., 2008). So based on this result some species and genera were added to Marakan flora. Considering AqDagh as a subset of Marakan, covering 5% of its area, more species diversity of Marakan flora was expected. Ratios of taxa/genus and genus/family of the studied areas are given (Table 3). Indeed a complete list for the Marakan protected area has not been reported by the previous researchers (Hassanzadeh *et al*., 2008).

AqDagh sanctuary is located in west Azarbaijan , northwest Iran (45° 09′ to 45° 16′E and 38° 51′ to 38° 56′N). This zone with 5184.7 hectares and altitude ranges between 984-1840 m, is situated in Gochash habitat (Department of Environment, 2004). Most of this sanctuary is mountainous and the rest is low alluvial lands. The average precipitation is 360/9 mm/year and the annual average temperature is 12.76°C.

According to the statistical data of Qaraziadin station, the closest meteorological station to AqDagh, and Domarton and Emberger coefficient this zone possesses respectively semi-dry and semi-dry cold climate. It is resulted from Ombrotermic curve that five months in a year is dry and the humid season continues from November to May. The maximum average precipitation occurs in May (66.3 mm) and Maximum average temperature occurs in August (27.33 $^{\circ C}$) (Figure 2).

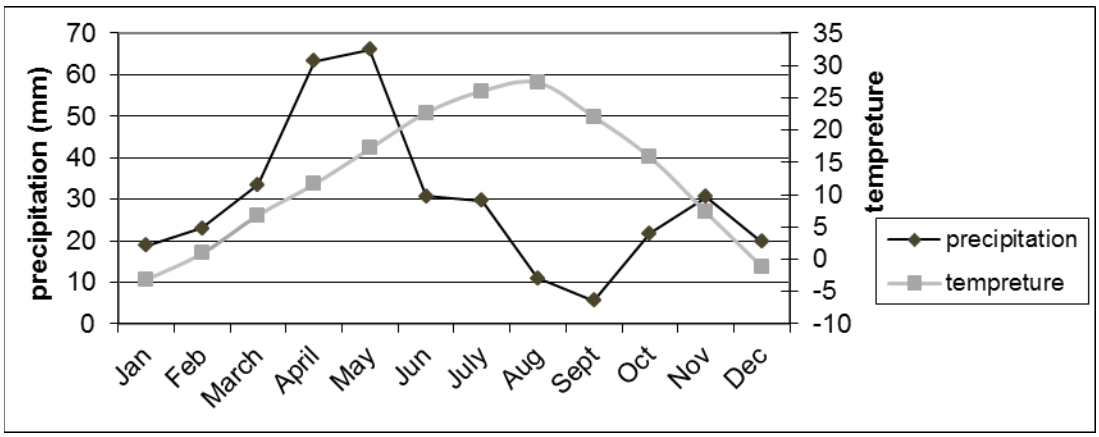

Figure 2. Omberotermic curve of Qaraziadin meteorological station

Data collection

In this investigation, the plant specimens were collected every 15 days in moist months and every 20 days in dry months, during 2008-2009. For each specimen, digital photographs were taken and the longitude, latitude and altitude of plants habitats were recorded with GPS set.

Collected specimens were pressed and transferred to the Farabi Herbarium of Kharazmi University (FAR). Some references used in plant recognition included: Komarov (1963-2001), Rechinger (1963-2010), Davis (1965-1988), and Assadi *et al.* (1988-2011). According to these Flora, plant geographical distributions were determined, and then their chorotypes were determined based on Zohary (1973) and Leonard (1988). The life forms of specimens were recognized by Raunckier's classification (Archibald, 1995). Monotypic genera and rare species were identified using "Biodiversity of plant species in Iran" (Ghahreman and Attar, 1999). Based on "Red data book of Iran" (Jalili and Jamzad, 1999) and Biodiversity of plant species in Iran, endemic taxa and red list of category (IUCN 2001) were determined (Table 1). Author names and scientific name of taxa were checked with IPNI (Macklin and Morris, 2007).

and Gulf of Oman. Nearly half of Iran is composed of arid and semi-arid high mountains (Noroozi *et al*., 2007). The alpine areas of Iran have been poorly investigated ecologically and botanically. AqDagh in Marakan protected area in northwest of Iran is a mountainous area. So, the floristic study of this area and other studies in the east and west Azarbaijan provinces were necessary.

In this research, the floristic study of AqDagh sanctuary was carried out. The most important former floristic studies includes: Ghasemloo (Shohada) Valley Forest Reservoir (Malekmohammadi *et al*., 2006), Mirabad (Hassanzadeh Gorttapeh and Panahy, 2007), Arasbaran protected area (Assadi, 1987, 1988; Hamzeh'ee *et al*., 2010), Mishu-Dagh (Manafi and Bahreiny, 1997) and Marakan (Hassanzadeh Gorttapeh *et al*., 2008) in east and west Azarbaijan provinces. The aim of this study was to achieve the following results: 1- to produce a new list of plants; 2- to compare the previous plant list of Marakan protected area with that of this study and other previous studies.

Materials and Methods

Study area

Markan protected area with 103983 hectares is located in the east and west Azarbaijan provinces. It is limited to, Qotur River plateau in the south, agricultural fields of Qaraziadin in the west, Jolfa-Khoy road in the east and Aras boundary River from north (from this point, is very close to Republic of Azerbaijan). The altitude range of the area is 720-2100 m. The Aq Chay river in the middle of area is also the approximate boundary line of east and west Azarbaijan provinces (Department of Environment, 2004). In Marakan, there are three sanctuaries consisting of Zarvin in the north slope, Munjughlu in the east and AqDagh in the west of the Aq Chay river (Figure 1).

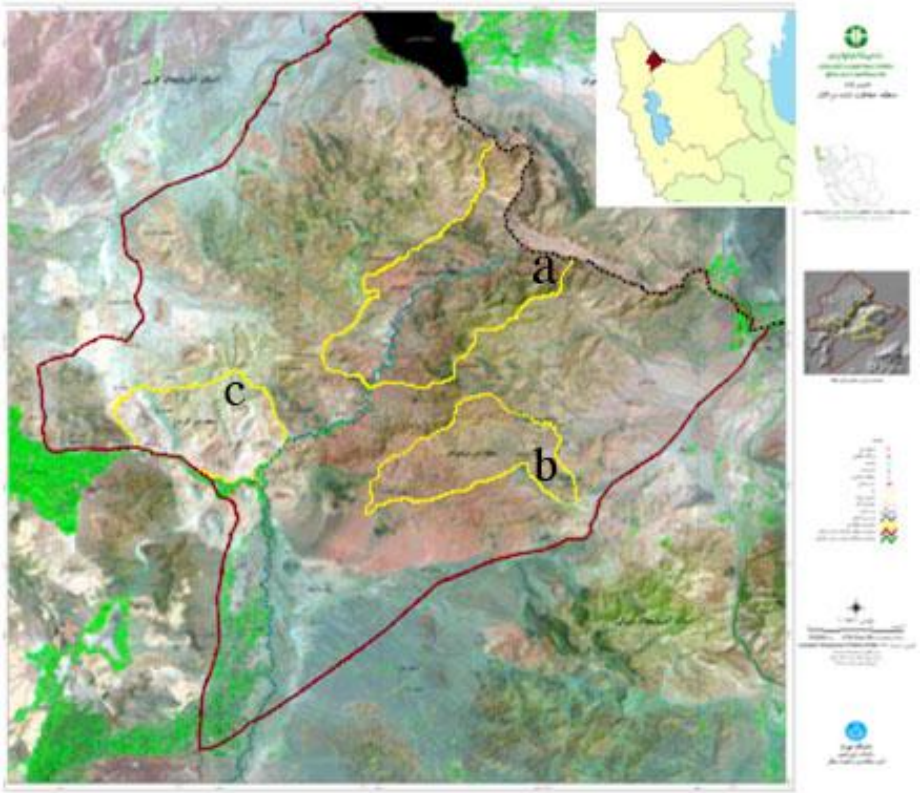

Figure 1. Marakan protected area in Iran plane and satellite picture of sanctuaries. A. Zarvin; B. Munjughlu; C. AqDagh (1:75000)

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Floristic study of AqDagh sanctuary in Marakan protected area: west Azarbaijan province, Iran

Hanieh Nafisi and Farrokh Ghahremaninejad *

Department of Plant Biology, Faculty of Biological Sciences, Kharazmi University, Tehran, Iran

Abstract
AqDagh sanctuary with the area 5184.7 hectares area is one of the three sanctuaries in the Marakan protected area, in west Azarbaijan province. It is located in the semi-dry to cold semi-dry climates. In this research, 227 taxa (species, subspecies and varieties) belonging to 47 families and 166 genera were identified during 2008 through 2009. Among the studied plants, 205 dicots and 21 monocots and one gymnosperm were recognized. Asteraceae (with 30 species) and Lamiaceae (with 26 species) were the largest families; followed by Brassicaceae, Caryophylaceae and Fabaceae. *Astragalus* (with 9 species) and *Gallium* (with 5 species) were the most diverse genera. Twenty one endemic and 6 rare taxa plus 2 monotypic genera were identified in the studied area. In addition, 11 taxa from northwest of Iran and 29 from west Azarbaijan were reported for the first time. Therophytes (with 36.57%) comprised the most dominant life form, followed by hemicryptophytes (with 27.75%) in this area. From the chorological point of the view, most of the flora has been influenced by the IT (31.53%) and IT-ES (27.49%) elements. The highest proportion of vegetation in this area belonged to bi-tri or pluriregional elements (with 59.01%).
Key words: West Azarbaijan, Aras, Flora, Chorology, Iran

Introduction

Determination of the flora of each region is a very important way to distinguish the aspects of the vital potential and varieties in the area in the specific period of time. For example the native, resistant, immigrant, invaded, medicinal plants and also new species to be determined and distinguished (Malekmohammadi *et al*., 2007). Moreover, with respect to the effects of many factors on the survival and elimination of some species the necessity of floristic studies to prevent from the destruction of habitats is justified. The knowledge of floristic composition is a prerequisite for other studies such as ecology, phytogeography, conservation management (Siadati *et al*., 2010), environment, forestry and agriculture (Malekmohammadi *et al*., 2007). Furthermore, by this way we can control the biodiversity, and implement ecosystem management for a long-term viability.

Iran has a total surface area of 1.65×10^6 km^2 (Jafari and Akhani, 2008). It is rich in plant diversity except of the interior deserts and the lowlands along the Caspian Sea, Persian Gulf

* Corresponding Author: ghahremaninejad@khu.ac.ir

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Discussion

Potentilla ghazniensis was collected from Afghanistan (Ghazni, Dasht-i-Nawar at 3000 m, 37274 LD; isotype K) (Schiman-Czeika, 1969; Soják, 1987) and later described by Soják (1987). The current species and *P. botschantzeviana* are the two representatives of the section *Lipskyanae* Czevtajbva (Faghir *et al.* 2011) in Iran. Despite of sharing common morphological traits (e.g. style somewhat thickened at base and non-widened stigma) (Soják, 2009), these two species cover different centers of distributions. *P. botschantzeviana* like several other species of the genus are distributed in north of the country (Mazandaran province, in Firuzkuh region at 2000-2500 m a.s.l.) (Faghir *et al*., 2011b). While *P. ghazniensis* is among a small number of species (e.g. *P. nuda* Boiss., *P. supina* L.) that reported from central and southern parts of the country (Faghir, 2010). *Potentilla ghazniensis* differs from its closely related species, *P. botschantzeviana* (with 1.5-2.5 mm style) (Soják, 1987), by having shorter style (1-1.3 mm long).

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the apical parts. Inflorescence subcontracted, with 3-5 flowers, pedicels long. Flowers 0.8-1 cm width; outer sepals lanceolate, 1-2 × 2-3.5 mm; inner sepals triangular, 2-4 × 8-10 mm (Figure 2E, F); stamens ± 20 in two whorls, the outer ones longer; anthers ovate, 5-9 mm long (Figure 2F), covered with straight and appressed indumentum; petals yellow, 8-1 × 4-5 mm, emarginated (Figure 2G); styles subterminal, thickened at base, 1-1.3 mm long; stigma non broadened (Figure 2H).

Figures 2: *Potentilla ghazniensis* Soják. 2. Radical (2a-2c) and cauline (2d-2e) leaves plus stipules (2e-2g); 3. Leaf upper surface; 4. Leaf lower surface; 5. Indumentum of petiole of the basal leaf; 6. External view of calyx and epicalyx; 7. Internal view of flower showing long and short stamens; 8. Petal; 9. Style and stigma. Scale Bars: 3-5 = 1 mm, 7 and 8 = 1 mm, 9 = 0.4 mm.

Potentilla ghazniensis is reported for the first time from central Iran based on the presence of some important characters especially long and short erect-subpatent hairs (0.7-1.8 mm long) of the basal leaves (Figure 2B); subterminal style thickened at the base and non broadened stigma (Figure 2H). This species was collected from Iran: Yazd province (at 31° 37' 50" N, 54° 04' 3" E), Taft city, Shirkuh Mts. at 3000-3300 m a.s.l.

Materials and Methods

The detailed study of different morphological characters were photographed by digital microscope, Dino-Lite, AN-413T model. The collected and examined specimens were deposited in the Mazandaran University Herbarium. The species identification was carried out using Flora Iranica (Schiman-Czeika, 1969), Flora of USSR (Shishkin and Yuzepchuk, 1941), Soják's recent papers (2008, 2009) and Faghir (2010).

Results

Potentilla ghazniensis Soják, Bot. Jahrb. Syst. 109 (1): 42. 1987. (Figures 1-2)

Figure 1. *Potentilla ghazniensis* Soják

Perennial herbs, caudex simple, sturdy, multicipital, covered with grayish brown relics of stipules. Stems decumbent, 4-5 cm long, covered with long or short and wavy erect hairs. Radical leaves digitate (Figure 2A), petiole 0.8-1.7 cm long, covered with long and short, erect or subpatent hairs (1-2.5 mm long) (Figure 2B); leaflets 2 × 1.5 cm, deeply pinnate, with 5-6 oblong-linear teeth on each side, covered with long appressed-erect hairs on the upper side (Figure 2C), and dense long hairs on the lower side (Figure 2D). Cauline leaves with 0.8 × 1 cm petiole, or subsessile (Figure 2A), digitate (Figures 2C, D) and ternate on

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Potentilla ghazniensis (Rosaceae), a new record from central Iran

Marzieh Beygom Faghir [1]*, Alireza Naqinezhad [2] and Abdol-Rahman Dehghani [2]

[1] Department of Biology, Faculty of Sciences, University of Guilan, Rasht, Iran
[2] Department of Biology, Faculty of Sciences, University of Mazandaran, Babolsar, Iran

Abstract
Potentilla ghazniensis is reported as a new record species from central Iran for the first time. This species is recognized by diverse indumentum (long and short erect-subpatent hairs) on the petiole of the basal leaves; and also by subterminal style, thickened at the base and non broadened stigma. *P. ghazniensis* is the second representative of the section *Lipskyanae* in Iran that differs from its closely related species, *P. botschantzeviana*, by having shorter style. The photographs of the plant and its morphological peculiarities are presented.
Key words: Flora of Iran, New report, Section *Lipskyanae*, *Potentilla*

Introduction

Potentilla L. (Rosaceae) was introduced primarily to the area covered by Flora Iranica by Schiman-Czeika (1969) (including 18 sections and 51 species). Khatamsaz (1993) classified the genus in to 4 subgenera, 14 sections and 39 species for the flora of Iran. Following Soják's extensive morphological studies (1989, 2004, 2007, 2008, 2009) and molecular phylogenetic analysis of Eriksson *et al*. (1998, 2003) and later Dobeš and Paule (2010), several subgenera and sections of the genus were treated as segregated genera (Ertter and Attar, 2007; Faghir, 2010; Soják, 2010).

In the recent years, Faghir *et al*. (2010a, 2011, 2012) took up the morphological, palynological and anatomical studies of the genus and added three new record species (*Potentilla radiata* Lehm., *P. balansae* Soják and *P. botschantzeviana* Adylov) (Faghir *et al.*, 2010b, 2011a) to the flora of Iran. Iranian species of *Potentilla* are mainly distributed in Irano-Turanian and Euro-Siberian phytogeographical regions (Schiman-Czeika, 1969; Khatamsaz, 1993; Faghir, 2010). In the framework of floristic studies in different parts of the country, *Potentilla ghazniensis* Soják was collected from steppic mountains of Shirkuh (Yazd province) and introduced for the first time for the flora of Iran.

* Corresponding Author: faghirmb@guilan.ac.ir

APPENDIX 1.	IRAN: Province, Collector, Date	Accession No.
1. *A. amardica*	Guilan: Deylaman; Shahe shahidan; Chaichi, Faghir and Shahi; 6.2012.	4872 (GUH)
2. *A. caucasica*	Mazandaran: Karaj-Chalus road, Pol-e Zanguleh, 3000 m; Nazarian; 2.8.1999.	33155 (TUH)
	Mazandaran: Kojur, Firozabad Village, 1700 m; Ghahreman and Attar; 19.6.1997.	20598 (TUH)
3. *A. citrina*	Guilan: Deylaman; Shahe shahidan; Chaichi, Faghir and Shahi; 6.2012.	4876 (GUH)
4. *A. erythropoda*	Mazandaran: Kojur, Firuzabad Village, 1700 m; Ghahreman and Attar; 19.6.1997.	20595 (TUH)
5. *A. condensa*	Guilan: Masal; Chaichi; 2012.	4871 (GUH)
	Guilan: Deylaman, Larikhani, 1500 m; Saeidi; 20.5.1993.	18845 (TUH)
6. *A. farinosa*	Ardebil: Almas pass; Chaichi, Faghir and Shahi; 8.2012.	4870 (GUH)
	Mazandaran: Ramsar; between Lapasar and Pishgah, 2600-3200 m; Maasoumi; s.d.	55188/9 (TARI)
7. *A. fluminea*	Guilan: Deylaman, Larikhani, 1530 m; Ghahreman and Attar; s.d.	18844 (TUH)
8. *A. gigantodus*	Mazandaran: Kojur, Keikuh Mountain, 2000-2300 m; Khatamsaz and Gholoizadeh; s.d.	57149 (TARI)
9. *A. hessii*	Mazandaran: Kojur, Firuzabad Village, 1700 m; Ghahreman and Attar; 19.6.1997.	20600/1 (TUH)
	Mazandaran: Kandavan; Ghahreman, Aghostin and Sheikholeslami; 6.1974.	19418 (TUH)
10. *A. hyrcana*	Guilan: Deylaman, Shahe shahidan, Chaichi; Faghir and Shahi; 6.2012.	4873 (GUH)
	Mazandaran: Kojur, Firuzabad Village, 1700 m; Ghahreman and Attar; 19.6.1997.	20597 (TUH)
11. *A. kurdica*	Guilan: Masal, Khashkhami, Chaichi; Faghir and Shahi; 6.2012.	4875 (GUH)
12. *A. meloncholica*	Guilan: Espili, Larikhani, 1530 m; Saeidi; 1993.	18841 (TUH)
13. *A. microscopica*	Ardebil: Almas pass, 2200 m; Khatamsaz and Salehnia; 6.1984.	56694 (TARI)
14. *A. persica*	Mazandaran: Tonekabon, Jannat rudbar, 1600 m; Ghahreman, Attar and Khatamsaz; 20.6.1997.	20603 (TUH)
	Mazandaran: Kojur, Firuzabad Village, 1700 m; Ghahreman and Attar; 19.6.1997.	205594 (TUH)
	Mazandaran: on the road of Karaj to Chalus, Pol-Zanguleh, 2600 m; Nazarian, 15.6.1999.	33440 (TUH)
	Tehran: Damavand, Attar and Mehdigholi; 5. 1992.	25576 (TUH)
	Azarbijan: Arasbaran, After three way to Veighan, Makidi, 1400 m; Ghahreman, Attar and Hamzehee; 2006.	35575 (TUH)
15. *A. pectinoloba*	Guilan: Deylaman, Larikhani, 1530 m; Saeidi; 5.1993.	18837 (TUH)
16. *A. plicatissima*	Ardebil: Almas pass, Chaichi, Faghir and Shahi; 8.2012.	4869 (GUH)
17. *A. pseudo-cartalinica*	Mazandaran: Kojur, Firuzabad Village, 1700 m; Ghahreman and Attar; 19.6.1997.	20602 (TUH)
18. *A. rechingeri*	Mazandaran: Kojur, Firuzabad Village, 1700 m; Ghahreman and Attar; 19.6.1997.	20601 (TUH)
19. *A. retinervis*	Mazandaran: Kojur, Firuzabad Village, 1700 m; Ghahreman and Attar; 19.6.1997.	20599 (TUH)
20. *A. sedelmeyeriana*	Mazandaran: Kojur, Firuzabad Village, 1700 m; Ghahreman and Attar; 19.6.1997.	20593 (TUH)
21. *A. sericata*	Azarbaijan: Kelaybar to Makidi, 1510 m; Ghahreman, Mozaffarian and Sheikholeslami; 5.1993.	17540 (TUH)
22. *A. surculosa*	Guilan: Masal, Chaichi; 2012.	4874 (GUH)
23. *A. rigida*	Guilan: Espili, Larikhani, 1510 m; Saeidi; 5.1993.	18842 (TUH)
	Mazandaran: Kojur, Firuzabad Village, 1700 m; Ghahreman and Attar; 19.6.1996.	20598 (TUH)
24. *A. valdehiruta*	Mazandaran: Koj, Firuzabad Village, 2200 m; Khatamsaz and Gholizadeh; 3.7.1989.	57160 (TARI)

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- Hairs 1.08-1.11 mm long ……..……..……...… 16. *A. pectinoloba*
18- Hairs 1.41-1.43 mm long ……..……..……..… 17. A. *condensa*
- Hairs 1.53-1.58 mm long ……..……..…... 18. *A. rigida*
19- Petiole covered by horizontal hairs ……..…….. 20
- Petiole glabrous or covered by erecto-patent, horizontal and declinate hairs of >1 mm long ……..…….. 22
20- Petiole densely pilose ……...……...…….…….. 21
- Petiole covered by cylindrical and ribbon shape hairs ..…. 19. *A. microscopica*
21- Petiole densely hairy; hairs 1.53-1.58 mm long ……..….. 20. *A. hyrcana*
- Petiole pubescent; hairs 1.70-1.73 mm long ……..….. 21. *A. caucasica*
22- Petiole pilose, with flat ribbon shaped hairs ...…… 23
- Petiole pilose ……..……..…… 22. *A. rechingeri*
23- Hairs 0.82-0.85 mm long ……..……..… 23. *A. hessii*
- Hairs 0.92-0.95 mm long ……..…… 24. *A. fluminea*

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hair types were recorded from all the studied populations of *A. persica* (Appendix 1), the most widely distributed (north, northwest, center and west provinces) species of the genus in Iran. However, petiole hair density of the studied taxa, varied from young to old plants and the leaves produced in early and later growing seasons (Juzepczuk, 1941).

Based on the current results, petiole indumentum data supported the previous classification of Fröhner (1969) and Khatamsaz (1993) and was reliable criterion for distinguishing the species. Based on petiole indumentum features, an identification key has been prepared as followings:

Key to species

1- Petiole covered by densely appressed-subappressed or sub-appressed-erecto-patent hairs ……..... 2
- Petiole glabrous or covered by erecto-patent, horizontal or declinate hairs 9
2- Petiole with densely appressed-subappressed hairs …….……..... 3
- Petiole with subappressed erecto-patent hairs ……..…....…….….. 5
3- Hairs argenteo-sericatae, 0.85-0.90 mm long ..…….....……. 1. *A. sericata*
- Hairs appressed-subappressed, more than 1 mm in length ….....…..... 4
4- Appressed-subappressed, pilose hairs of 1.50-158 mm long ..... 2. *A. pseudo-cartolonica*
- Appressed-subappressed, sericeo-pubscence hairs of 1.30-1.47 mm long ..... 3. *A. amardica*
5- Subappressed-erecto-patent hairs 0.85-1 mm long ..…….....……...… 6
- Subappressed-erecto-patent hairs of 1->1.50 mm long …….……....… 7
6- Densely pilose hairs of 0.94-1 mm long …..……..…… 4. *A. plicatissima*
- Pilose-subsericeo hairs of 0.85-0.90 mm long ……..…… 5. *A. citrina*
7- Hairs densely pilose, 1.58-1.64 mm long …….…….... 6. *A. valdehirsuta*
- Hairs sericeo-pilose or pilose > 1.50 mm …...……...…… 8
8- Hairs sericeo-pilose, 1.38-1.41 mm long ..…..…… 7. *A. gigantodus*
- Hairs pilose, 1.28-130 mm long ……..……..….. 8. *A. farinosa*
9- Petiole covered by only declinate hairs ……..…...…….…. 10
- Petiole glabrous or covered by erecto-patent, horizontal or declinate hairs .. 11
10- Petiole with densely pilose hairs of 1-1.1 mm long ..…… 9. *A. melancolica*
- Petiole with densely subhirsute hairs of 1.76-1.80 mm long ..... 10. *A. erythropoda*
11- Petiole covered by sparsely to ± densely erecto-patent hairs ….....…..… 12
- Petiole glabrous or covered by erecto-patent, horizontal and declinate hairs 19
12- Petiole with only sparsey to subdensely erect-patent hairs .....….…… 13
- Petiole with densely erecto-patent hairs …….....……...……..…. 16
13- Hairs shorter than 1 mm long ……..……..……..…… 14
- Hairs longer than 1 (1.35-1.40) mm long ..……...…..….....… 11. *A. kurdica*
14- Hairs 0.82-0.88 mm long ..…….……..…… 15
- Hairs 0.91-0.95 mm long …...……..…… 12. *A. retinervis*
15- Hairs 0.85-0.88 mm long ……..……...… 13. *A. sedelmeyeriana*
- Hairs 0.80-0.83 mm long ……..…….....……..……. 14. *A. persica*
16- Petiole densely hirsute ……..……..……..……..... 17
- Petioles densely sericeo-subsericeo grayish ……..…… 18
17- Hairs 1.40-1.42 mm long ……...…... 15. *A. surculosa*

Table 1. Trichome length of different studied species

Species	length (mm)
1. *A. amardica*	1.30-1.47
2. *A. hyrcana*	1.43-148
3. *A. kurdica*	1.35-1.40
4. *A. rechingeri*	0.95-0.98
5. *A. pectiniloba*	1.08-1.11
6. *A. sericata*	0.85-0.90
7. *A. citrina*	0.85-0.90
8. *A. pseudo-cartalinica*	1.50-1.58
9. *A. rigida*	1.53-1.58
10. *A. sedelmeyeriana*	0.85-0.88
11. *A. caucasica*	1.70-1.75
12. *A. surculosa*	1.40-1.43
13. *A. fluminea*	0.92-0.95
14. *A.valdehirsuta*	1.58-1.64
15. *A. condensa*	1.41-1.53
16. *A. farinosa*	1.28-1.30
17. *A. gigantodus*	1.38-1.41
18. *A. hessii*	0.82-0.85
19. *A. retinervis*	0.91-0.95
20. *A. microscopica*	1.24-1.30
21. *A. plicatissima*	0.94-1
22. *A. erythropoda*	1.76-1.80
23. *A. persica*	0.82-0.84
24. *A. meloncholica*	1-1.1

Discussion

Our observations confirmed the taxonomical usefulness of petiole trichome micromorphological characters within the studied taxa. The diagnostic application of petiole indumentum types is well known in the family Rosaceae and especially in closely related genera of the tribe *Potentilleae* (Rosaceae) (Notov and Kusnetzova, 2004; Soják 2008) like *Alchemilla* and *Potentilla* L. (Eriksen and Yurstev, 1999; Faghir *et al.*, 2010, Faghir *et al*., 2011). Juzepczuk treatment's (1941), in the Flora USSR, used petiole indumentum evidences (along with other diagnostic criteria) at different taxonomic ranks (section, series and species level). Hayirlioglu-Ayaz and Beyazoglu (1997) identified different species of Turkish *Alchemilla* (e.g. *A. plicatissima*, *A. stevenii*, *A. monticola*) using hair types of petiole and other parts (e.g. stem and hypanthium). In Flora Iranica (Fröhner, 1969) and Flora of Iran (Khatamsaz, 1993) several species of *Alchemilla* were separated based on petiole indumentum types.

This study deals with the micromorphological details of petiole indumentum types and according to our findings, two main types, seven subtypes of petiole hairs were recorded in the genus *Alchemilla*. However, presence of flat ribbon shape trichome has not been reported earlier (Fröhner, 1969; Khatamsaz, 1993). The cylindrical hair was the most dominent type and erecto-patent was the most aboundent subtype in the studied species. The erecto-patent indumentum was found either alone or mixed with sub-appressed trichome of the subtype II and horizontal and declinate hairs of the subtype IV. In contrast, densely appressed-subappressed of the subtype I and declinate pilose of the subtype VII had minimum occurrence within the studied species.

In the current analysis, the petiole hairs of several populations of the same species were examined. The petiole indumentum type was constant within the populations of the same species (e.g. *A. caucasica*, *A. farinosa*, *A. hessii*, *A. hyrcana* and *A. persica*). Erecto-patent

Subtype VI: Glabri-horizental-erecto-patent-declinate: this subtype was common in *A. fluminea*, *A. hessii* and *A. rechingeri* (Figures 3C and3D).
Subtype VII: Declinate pilose: this subtype was found in *A. erythropoda* (Figures 3C and 3D) and *A. meloncholica*.

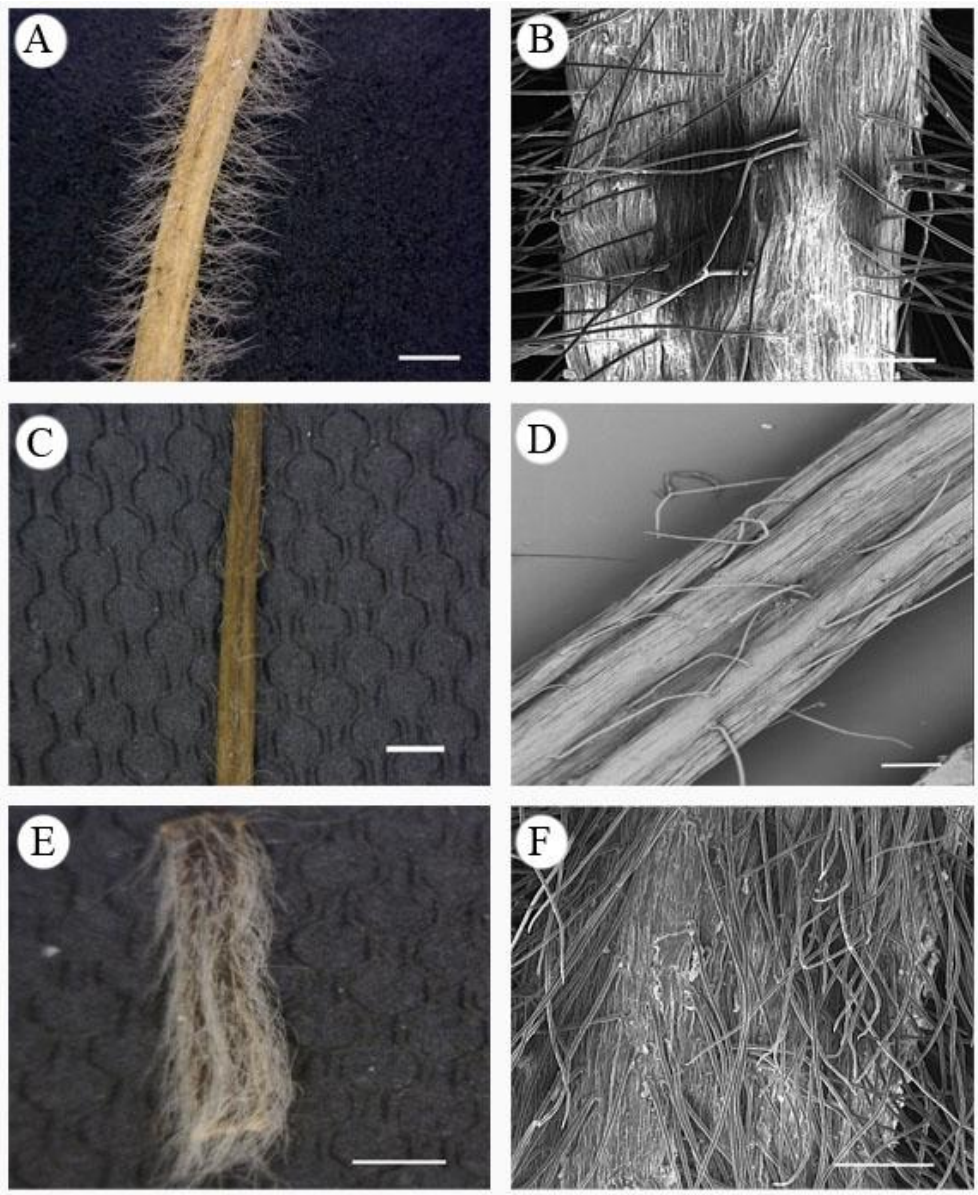

Figure 3. A and B. *A. caucasica*; C and D. *A. rechingeri*; E and F. *A. erythropoda.* Scale Bars: A, C and E = 1 mm, B, D and F =200 µm

The indumentum length was measured (Table 1). *A. erythropoda* with 1.76-1.80 mm and *A. persica* with 0.82-0.84 mm had the maximum and minimum hair length, respectively. In many species scattered secretory glands, wax granules and platelets were visible on the petiole surfaces.

The first type is found in all the 24 species but, the second type is observed only in the five studied species (*A. kurdica*, *A. hessii*, *A. fluminea*, *A. pseudo-cartalinica* and *A. sericata*). Seven subtypes were identified as following:

Subtype I: Densely appressed-subappressed: this subtype was recorded in *A. amardica* (Figures 2A and 2B), *A. pseudo-cartalinica* and *A. sericata*.

Subtype II: Subappressed-erecto-patent: this subtype was identified in *A. citrina*, *A. farinosa*, *A. plicatissima*, *A. valdehirsuta* and *A. gigantadus* (Figures 2C and 2D).

Subtype III: Erecto-patent: this subtype was recognized in *A. kurdica*, *A. persica*, *A. retinervis* and *A. sedelmeyeriana* (Figures 2E and 2F).

Subtype IV: Densely erecto-patent: this subtype was found in *A. rigida*, *A. condensa*, *A. surculosa* (Figures 2G and 2H) and *A. pectiniloba.*

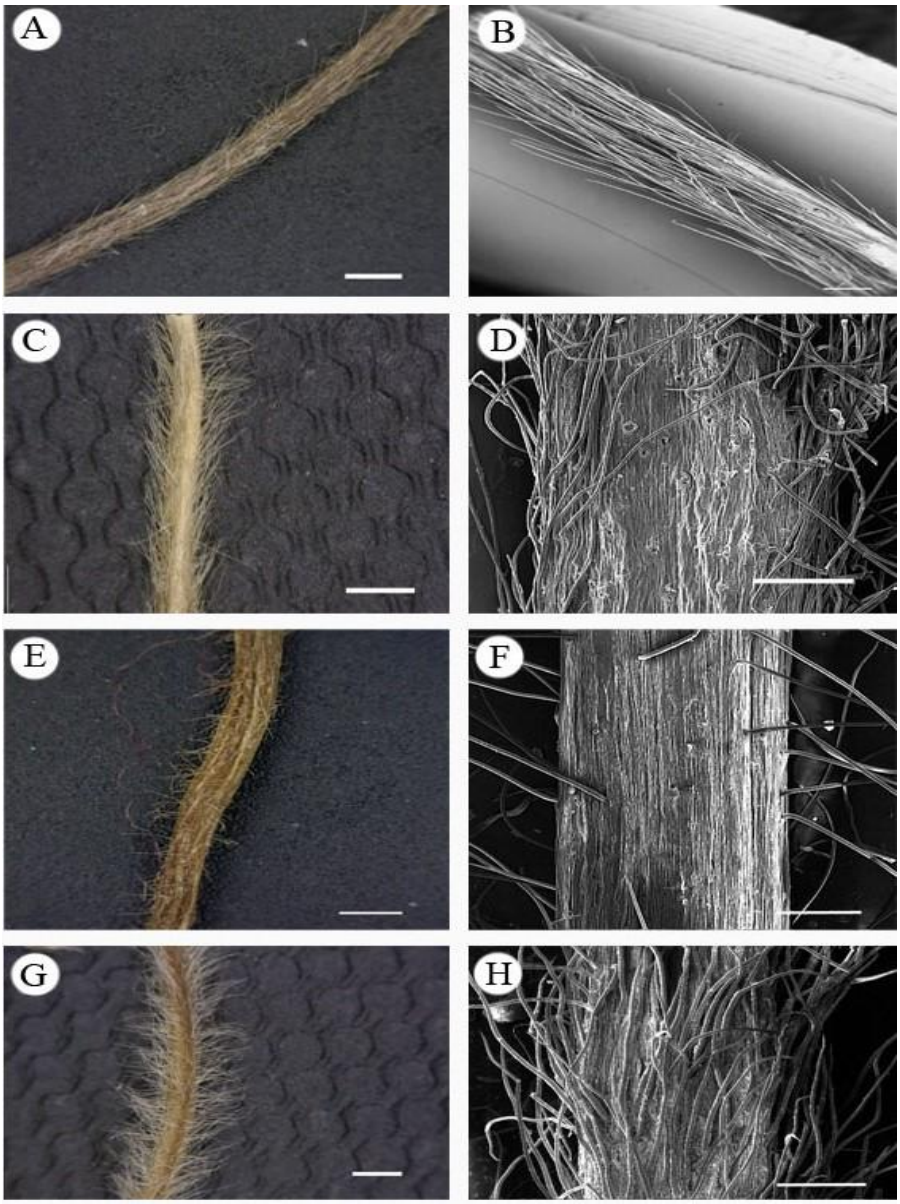

Figure 2. A and B. *A. amardica*; C and D. *A. gigantodus*; E and F. *A. sedelmeyeriana*; G and H. *A. surculosa*, Scale Bars: A, C, E and G = 1 mm, B, D, F and H = 200 µm

Subtype V: Densely horizontal: this subtype was present in *A. caucasica* (Figures 3A and 3B), *A. hyrcana* and *A. microscopica*.

Azerbaijan and Turkmenistan, but Khatamsaz (1993) considered 24 species of the genus for Flora of Iran.

Alchemilla is a taxonomically difficult group (Izmailow, 1981) and a well-known example of polyploidy, autonomous apomixis (in the sense of agamospermy), in the Rosaceae (Czapik, 1996). Many representatives of this genus display heteroblastic plasticity, variability and instability in morphological characters (e.g. flowers, leaves and indumentum). This causes great difficulties in their identification and results in formation of several species complexes and micro-species (Asker and Jerling, 1992; Horandl, 2004). The present micromorphologic research, aims to find new consistent diagnostic characters (e.g. petiole micromorphological) for a precise delimitation of various species and to evaluate the extent to which the petiole indumentum micromorphology can be used for classification of the species of *Alchemilla* in Iran.

Materials and Methods

In this study, petiole morphology of 24 species (including different taxa from several populations) of Iranian species of *Alchemilla* was examined. We used both freshly collected samples (from 2010 to 2012, during spring and late summer) and dried herbarium specimens of the Guilan University Herbarium (GUH), the Tehran University Herbarium (TUH) and the Herbarium of the Research Institute of Forests and Rangelands of Iran (TARI). All the examined petioles were belonged to well-developed basal leaves. The voucher specimen of each newly collected species deposited in the Guilan University Herbarium (GUH). Species sampled are listed in Appendix 1. For scanning electron microscopy (SEM), the petioles (a portion of them, constantly taken from distal end) were selected, washed and then kept for drying (Eriksen and Yurstev, 1999; Faghir *et al*, 2010). Dried materials were mounted on SEM stubs by double-sided adhesive tape of silver paint and finally coated with gold in a sputter coater. Scanning electron microscopy was carried out using a Vega Tescan Razi instrument. The petiole photograph also were taken by digital microscope, Dino-Lite, AN-413T model. Flora Iranica (Frohner, 1969), Flora of Iran (Khatamsaz, 1993) and Flora of USSR (Juzepczuk, 1941), were the principal references for identification and terminology of indumentum in this paper.

Result

Our findings revealed two main types of petiole indumentum including: the straight cylindrical and flat ribbon shape hairs (Figure 1).

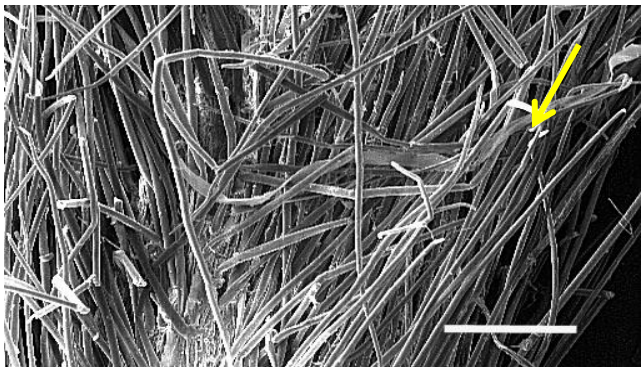

Figure 1. Cylindrical and flat ribbon shape trichome of *A. microscopica* (yellow arrow indicates the ribbon shape hair). Scale Bar = 200 µm

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Petiole indumentum types of the genus *Alchemilla* (Rosaceae) from Iran

Marzieh Beygom Faghir *, Kobra Chaichi Khairkhah and Robabeh Shahi Shavvon

Department of Biology, Faculty of Sciences, University of Guilan, Rasht, Iran

Abstract
Petiole indumentum types of 24 species of the genus *Alchemilla* were studied using scanning electron microscope (SEM) and digital microscope. All the examined petioles were belonged to the well-developed basal leaves**.** Two main indumentum types (cylindrical and flat ribbon shape) and seven subtypes including: dense appressed-subappressed; subappressed-erecto-patent; erecto-patent; dense erecto-patent; dense horizontal; glabri-horizontal-erecto-patent-declinate and declinate trichome were identified. The current results revealed that petiole micromorphological characters were taxonomically informative criteria and could be used in species classification. A key for species identification is presented.
Key words: *Alchemilla*, Indumentum, Petiole, Iran

Introduction

Alchemilla L. (Rosaceae) was primarily introduced by Linnaeus (1753) and classified as *Eualchemilla* by Fock (1988), Lagerheim (1894) and Haumann and Balle (1936). The genus thought to be related to the subtribe *Sanguisorbinae* (Hutchinson, 1964) because of its inconspicuous flowers. However, its relation to the tribe *Potentilleae* (Notov and Kusnetzova, 2004) was confirmed by DNA sequence data (Eriksson *et al.*, 1998, 2003) and based on anther structure (anthers with one elliptic theca on the ventral side of the connective) it has been placed in the subtribe *Alchemillinae* (Soják, 2008).

The genus *Alchemilla* comprises more than 1000 species (Fröhnerm, 1995) and has Holarctic distribution, with a main center of species richness in west of Eurasia, but they also may grow in south India, Sri Lanka, Java, China, Japan and on the mountains of Africa and Madagascar (Gehrke *et al.*, 2008). Species of *Alchemilla* are distributed mainly in north and northwest of Iran. However, some species may occur in west and center of the country (Frohner, 1969; Khatamsaz, 1993). They have woody rhizome (Pawlowski and Walters, 1972), commonly growing in open meadows, stony slopes, shady places, river banks and forest edges of alpine and subalpine regions, from 1700 to 3300 m altitudes (Juzepczuk, 1941; Frohner, 1969; Khatamsaz, 1993). Frohner treatment's (1969) of *Alchemilla*, in Flora Iranica recognized 31 species from Iran, Afghanistan, parts of west Pakistan, north Iraq,

* Corresponding Author: faghirmb@guilan.ac.ir

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In the UPGMA dendrogram (Figure 3), the five *Triticum* species studied were divided into three groups: (1) A: (*T. monococcum* and *T. urartu*), (2) B: (*T. boeoticum* subsp. *thaodar* and *T. boeoticum* subsp. *boeoticum*), and (3) C: (*T. durum, T. turgidum* and *T. aestivum*) (Figure 3) (Akhavan and Saeidi, 2010; Jalilian and Rahiminejad, 2011).

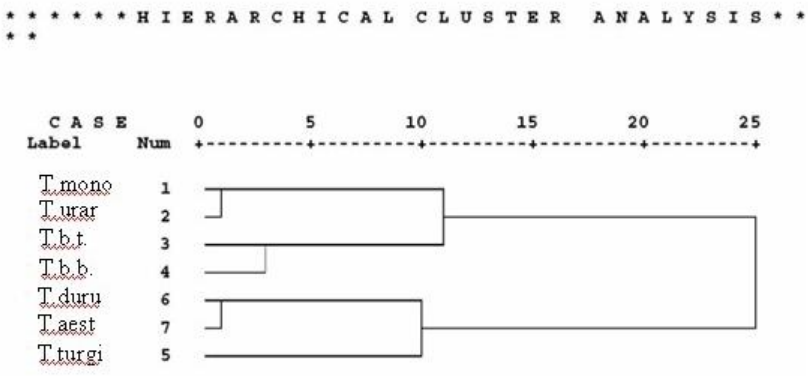

Figure 3. The UPGMA dendrogram (used Euclidian Distance) shows relationships between *Triticum* species based on chromosomal characters (see Table 2). T.mono. *T. monococcum*; T.urar. *T. urartu*; T.b.t. *T. boeoticum* subsp. *thaodar*; T.b.b. *T. boeoticum* subsp.*boeoticum*; T.duru *T. durum*; T.turgi. *T. turgidum*; T.aest. *T. aestivum*.

According to the results, it might be suggested that *T. durum* was more primitive than *T. turgidum* and *T. monococcum* could be considered as a donor of A genome to *T. durum* and *T. aestivum*. This hypothesis verified SSR analysis (Ehtemam *et al*., 2010; Keshavarzi *et al.*, 2012).

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TCL value ranged between 156.210 µm (*T. durum*) and 130.133 (*T. turgidum*). Based on the result of this study, it could be concluded that generally, the chromosomal length in the *T. urartu* was shorter than other species of the genus *Triticum*. In this species, minimum value of TF%, CI and A1 belonged to *T. boeoticum* subsp. *thaodar* and maximum showed in *T. monococcum*. Also among this group (diploids) arm ratio (r) and AsI% in *T. monococcum* were the lowest and in *T. boeoticum* subsp. *thaodar* was the highest. Results showed that coefficient of variability for intra-specific chromosome length variation of short and long arms (Table 3) was higher for short arms and that it can be concluded that short arms were more effective in TCL than long arms. Karyotype studies were principally based on the idea that symmetrical karyotypes were more primitive than asymmetrical ones; and this holds true for longer chromosomes compared to shorter ones; median centromeres with arms of equal length are more primitive than chromosomes with arms of unequal length; low basic numbers give rise to higher ones. These features are based on the comparison between karyotypes of known relative antiquity, as determined through classical taxonomy (Sharma, 1990).

Table 3. Coefficient of variability for intra-specific chromosome length variation of short and long arms for *Triticum* species used in this study

Chromosome arms	***T. monococcum***	***T. urartu***	***T. boeoticum* subsp. *thaodar***	***T. boeoticum* subsp. *boeoticum***	***T. turgidum***	***T. durum***	***T. aestivum***
Short arm (in%)	9.88 – 18.64	9.52 – 19.09	13.57 – 16.93	7.85 – 15.75	17.25 – 21.82	9.46 – 24.51	17.38 – 24.77
Long arm (in%)	7.60 – 13.46	8.88 – 15.48	10.70 – 13.01	11.64 – 14.06	11.63 – 16.82	8.19 – 18.32	12.93 – 19.09

The scatter diagram based on A1 and A2 (Romero-Zarco, 1986) constructed three groups of karyotype asymmetry in the accessions studied: 1- *T. aestivum* (a) with the highest asymmetrical karyotype, 2- *T. monococcum*, *T. boeoticum* subsp. *thaodar* and *T. boeoticum* subsp. *boeoticum* with the lowest asymmetrical karyotype and 3- *T. urartu*, *T. turgidum* and *T. durum* with an intermediate between two previous groups (Figure 2) (Jalilian and Rahiminejad, 2011).

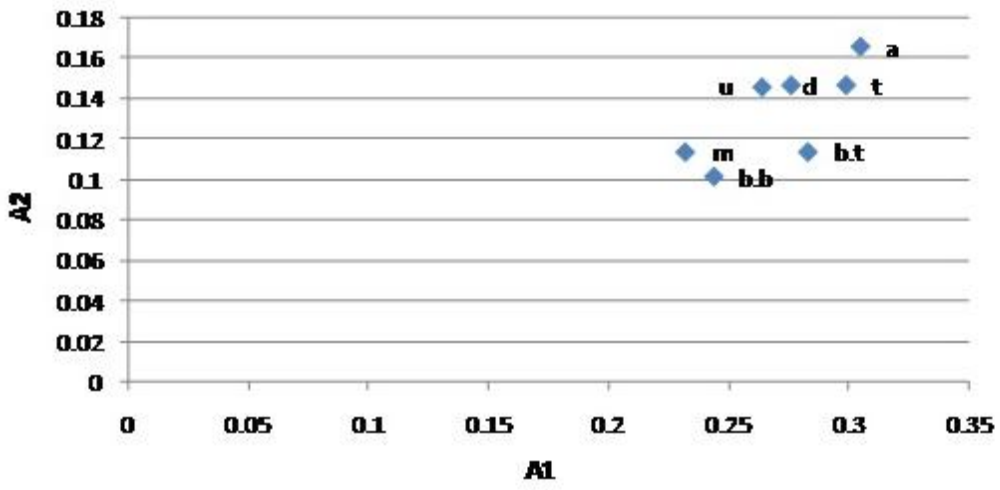

Figure 2. Scatter diagram show the relationships between the *Triticum* species based on the intrachromosomal (A1) and interchromosomal (A2) asymmetry indices. Values of A1 and A2 are summarized in Table 2. (m. *T. monococcum*; u. *T. uratu*; b.b. *T. boeoticum* subsp. *boeoticum*; b.t. *T. boeoticum* subsp. *thaodar*; d. *T. durum*; t. *T. turgidum*; a. *T. aestivum*)

sub-metacentric (sm) chromosomes in their Karyotype formulae (Table 1).

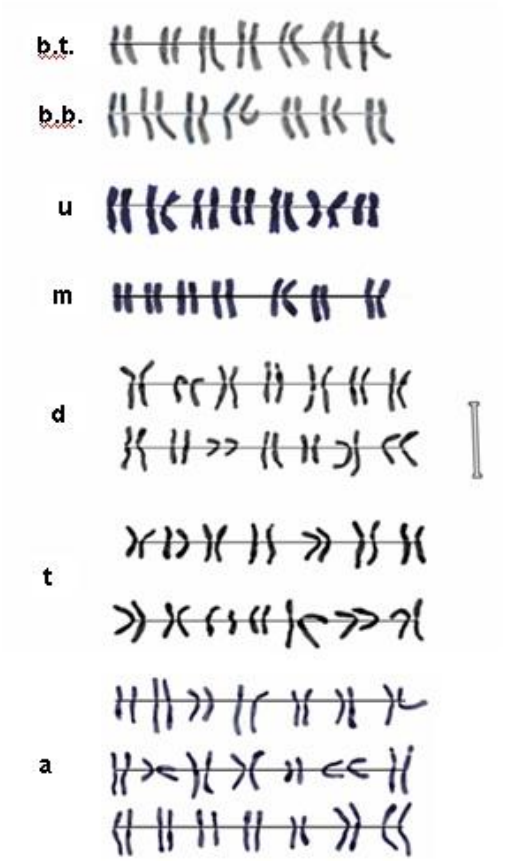

Figure 1. Mitotic methaphase in *Triticum* speies studied. b.t. *T. boeoticum* subsp. *thaodar*; b.b. *T. boeoticum* subsp.*boeoticum*; u. *T. urartu*; m. *T. monococcum*; d. *T. durum*; t. *T. turgidum*; a. *T. aestivum*. Scale Bar = 20 µm. Arrow shows the satellite chromosome

All the accessions without T.duru-1, T.aest-73 and T.aest-47 (2A category), were placed in 1A category of Stebbins (1971) asymmetry categories (not showed in these Tables). Total chromosome length depended on the ploidy levels and chromosome numbers and consequently this parameter was too variable (Table 2).

TCL values varied between genotypes (Table 2). The TCL value ranged from 48.316 µm (*T. urartu*) with MCL of 7.606 ± 0.63 µm to (*T. aestivum*) with MCL of 11.475 ± 0.88 µm. The most variable chromosomes in length SE of MCL were found in *T. turgidum* (SE of MCL = 1.94 µm) whereas the most similar chromosomes were scored in *T. boeoticum* subsp. *thaodar* (SE of MCL = 0.13 µm) (Table 2). The TF% value ranged from 43.29 (*T. monococcum*) to 40.66 (*T. turgidum*) (Table 2). The CI ranged between 0.431 (*T. monococcum*) and 0.404 (*T. turgidum*). The longest and the shortest chromosome length were found in *T. aestivum*. The highest mean length arm was in *T. aestivum*. and the lowest mean was in *T. urartu*. The arm ratio ranged from 1.27 in *T. monococcum* to 1.47 in *T. turgidum*. The ASI% varied from 56.69 (*T. monococcum*) to 59.35 (*T. turgidum*). The SI% ranged from 75.41 (*T. boeoticum* subsp. *thaodar*) to 50.418 (*T. aestivum*). DRL in *T. monococcum* was the highest (4.201) and in *T. aestivum* was the lowest (3.099). In general, A1 and A2 values showed the high degree of karyotype symmetry in the majority of the genotype studied (Razik Kamel, 2006). A1 ranged from 0.305 (*T. aestivum*) to 0.232 (*T. monococcum*) and A2 ranged from 0.102 (*T. boeoticum* subsp. *boeoticum*) to 0.166 (*T. aestivum*) (Table 2). Among the diploid species, the TCL value varied from 74.758 µm (*T. boeoticum* subsp. *boeoticum*) to 48.316 µm (*T. urartu*). Between tetraploid accessions the

Species	Accession	2n	Ploidy level	Genome	Locality and Altitude(m)
	T.turgi- 43	28	4x	AB	Chaharmahal and Bakhtiari: Bazoft Morez valley (2000)
	T.turgi- 8	28	4x	AB	Chaharmahal and Bakhtiari: Borojen to Izeh (2190)
	T.turgi- 10	28	4x	AB	Khuzistan: Izeh (900)
	T.turgi- 194	28	4x	AB	Kurdistan: between Sanandaj and Saghez (1595)
	T.turgi- 80	28	4x	AB	Kermanshah: Mahi Dasht (1290)
	T.turgi- 25	28	4x	AB	Lorestan: Malavi toward Khorram Abad (1200)
	T.turgi-120	28	4x	AB	East Azarbaijan: Ahar (1320)
T. aestivum	T.aest-47	42	6x	ABD	Chaharmahal and Bakhtiari (2000)
	T.aest-74	42	6x	ABD	Ilam: Do Rahe (1410)
	T.aest-129	42	6x	ABD	Booshehr: Bandargah to Deilam (17)
	T.aest-73	42	6x	ABD	Khuzistan: Karkheh (13)
	T.aest-97	42	6x	ABD	Malayer 50 km to Arak (2010)
	T.aest-96	42	6x	ABD	Tehran: Firooz Kuh (1700)
	T.aest-107	42	6x	ABD	West Azarbaijan: Boukan to Mahabad (1290)
	T.aest-49	42	6x	ABD	Isfahan: Daran (2190)
	T.aest-82	42	6x	ABD	Kermanshah: Mahi Dasht (1290)
Chinese spring	C.S.	42	6x	ABD	Provided by the Institute of Plant Biology, University of Zurich, Switzerland

*All samples are kept in the herbarium of Isfahan University

Table 2. The means of karyotypic characters of forty-six mitotic chromosomes in diplo, tetra and hexaploids species of the genus *Triticum* used in this study (n = Chromosome number, TCL = Total haploid chromatid length, MCL = Mean of chromosomes length, TF% = Total form percent, Cent.Index = Centromeric Index (= S/(L+S)), Lon.Ch. = Longest chromosome length, Sho.Ch. = Shortest chromosome length, ML = Mean of large arms, MS = Mean of short arms, r = Arms ratio, AsI% = Asymmetry index, S% = Ratio between the shortest and longest of the chromosomes percent, DRL = Difference of range relative length, A1 = Intrachromosomal asymmetry, A2 = Interchromosomal asymmetry (C.V.) and RL = Relative length of chromosomes)

Species	2n	TCL	MCL±SE	TF%	Cent.Index	Lon. Ch.	Sho.Ch.	M.L. arm
T. monococcum	14	59.610	8.515 (±1.02)	43.29	0.431	9.775	7.349	4.816
T. urartu	14	48.316	7.606 (±0.36)	42.35	0.423	10.161	7.518	4.433
T. boeoticum subsp. *thaodar*	14	70.277	8.482 (±0.13)	41.60	0.415	11.457	8.589	5.871
T. boeoticum subsp. *boeoticum*	14	74.758	10.679 (±1.55)	42.72	0.427	12.233	9.186	6.105
T. turgidum	28	130.133	9.294 (±1.94)	40.66	0.404	13.007	7.81	5.475
T. durum	28	156.210	11.157 (±1.54)	41.437	0.41	13.35	8.151	6.545
T. aestivum	42	241.002	11.475 (±0.88)	40.831	0.406	14.834	7.528	6.777

Species	M.S. arm	r	AsI%	SI%	DRL	A1	A2	RL
T. monococcum	3.698	1.27	56.69	74.55	4.201	0.232	0.114	14.28
T. urartu	3.256	1.36	57.67	74.16	3.767	0.264	0.146	14.28
T. boeoticum subsp. *thaodar*	4.168	1.41	58.39	75.41	4.002	0.283	0.114	14.28
T. boeoticum subsp. *boeoticum*	4.554	1.33	57.11	71.25	3.715	0.244	0.102	14.28
T. turgidum	3.819	1.47	59.35	62.425	3.409	0.299	0.147	7.14
T. durum	4.615	1.43	58.63	60.277	3.707	0.276	0.147	7.14
T. aestivum	4.698	1.45	59.085	50.418	3.099	0.305	0.166	4.76

Results and Discussion

The results showed that about 33% of diploid accessions had sub-metacentric (sm) chromosomes in their Karyotype formula and other ones had only metacentric chromosomes. Tetraploid accessions had 2-4 sub-metacentrics (sm) and hexaploid accessions possessed 2-5

the short arm/total length of the chromosome X 100) (Arano and Sattio, 1980), total form percentage (TF%) (Huziwara, 1962), centromeric index mean of long and short arms (CI), asymmetry index (AsI%) (Arano and Sattio, 1980), S% (The shortest chromosome length/the longest chromosome length), DRL (difference of range relative length), intrachromosomal asymmetry index (A1) (Romero-Zarko, 1986), interchromosomal asymmetry index (A2) (Romero-Zarko, 1986), Stebbin's classification (Stebbins, 1971) and karyotype formula (Levan *et al.*, 1964) were calculated (Table 2). The data analyzed with Excel, SAS (version 14) and SPSS (version 16) statistical softwares.

Table1. List of scientific name, accessions codes*, ploidy level, kind of genomes and collecting localityies used for comparative cytological study of Iranian *Triticum* species

Species	Accession	2n	Ploidy level	Genome	Locality and Altitude(m)
T. monococcum	T. mono-30	14	2x	A	Kermanshah: Gardaneh Reno(1480)
	T. mono -10	14	2x	A	Kurdistan: 3 km to Saghez (1620)
	T. mono -41	14	2x	A	Isfahan: Semirom to Yasouj (2100)
	T. mono -39	14	2x	A	Arak toward Malayer (2020)
	T. mono -40	14	2x	A	Tehran: Taleghan valley (1850)
T. boeoticum subsp. *thaodar*	T.b.t.-37	14	2x	A	Kurdistan: 5 km after Jenan to Saqez (1770)
	T.b.t.-8	14	2x	A	Chaharmahal va Bakhtiari: Shahr-e-Kord, Shapoorabad to Jooneghan (2090)
	T.b.t.-34	14	2x	A	Arak: 15 km to Malayer (1840)
T. boeoticum subsp. *boeoticum*	T.b.b.-19	14	2x	A	Ilam toward Kermanshah: Gardaneh Reno (1370)
	T.b.b.-5	14	2x	A	Lorestan: 35 km to Khorram Abad from Malavi (1100)
	T.b.b.-20	14	2x	A	Kermanshah: 10 km to Harsin (1330)
	T.b.b.-86	14	2x	A	Kermanshah toward Kamyaran (1340)
	T.b.b.-3	14	2x	A	Kohgiluyeh va Boyer-Ahmad near Yasouj
T. urartu	T.ura-156	14	2x	A	West Azarbaijan: Maku (1580)
	T.ura-84	14	2x	A	Kermashah: 10 km to Saqez from Asadabad (1320)
	T.ura-2	14	2x	A	Ardabil: 10 km to Kaghazkanan (1349)
	T.ura-8	14	2x	A	Chaharmahal va Bakhtiari: between Gandoman and Lordegan (2080)
	T.ura-59	14	2x	A	Kurdistan: Saqez (1770)
T. durum	T.duru-86	28	4x	AB	Kermanshah: Kamyaran (1440)
	T.duru-24	28	4x	AB	Lorestan: Malavi toward Khorram Abad (1200)
	T.duru-166	28	4x	AB	Chaharmahal va Bakhtiari: DoAb Samsami (2000)
	T.duru-1	28	4x	AB	Kohgiluyeh va Boyer-Ahmad (990)
	T.duru-165	28	4x	AB	Chaharmahal va Bakhtiari: near Chaghakhor lake (2190)
	T.duru-109	28	4x	AB	West Azarbaijan: Sardasht to Baneh (1050)
	T.duru-15	28	4x	AB	Khuzistan: Haftgel to Masjed Soleiman (550)
	T.duru-126	28	4x	AB	Kurdistan: 6 Km to Alamoot (1660)
	T.duru-7	28	4x	AB	Chaharmahal and Bakhtiari: Borojen to Izeh (2190)
T. turgidum	T.turgi-211	28	4x	AB	West Azarbaijan: Khoy (1110)
	T.turgi- 45	28	4x	AB	Chaharmahal va Bakhtiari: Bazoft (2190)
	T.turgi- 2	28	4x	AB	Kohgiluyeh va Boyer-Ahmad: Yasouj (2880)

Because of different workers had to do without standardization of techniques in different laboratories, their reports showed inconsistencies in the standard karyotype analysis based on chromosome length and arm ratio still holds its merit (Jahan and Vahidy, 1989). The analysis of plant genomes has provided insight into how these evolutionary events have occurred and the rate at which evolution could have taken place. Wheat has played a major role in the development of the world civilization. The domestication of wheat was a major event in the world civilization because it allowed humans to change from nomadic hunter gathers to permanent residents of specific locations. Many useful genes from wild species are available to be used in breeding Programmers as source of genes for disease resistance applying different chromosomal engineering techniques involving backcrosses, "bridges" crosses and selection of cytological as well as desired agronomic characteristics (Stalker, 1980; Dhalival *et al.*, 1986; Gale and Miller, 1987). By 1951, the work of Lilienfeld and colleagues had established many of the genomic relationships of the diploid and polyploid *Triticum* L. and *Aegilops* L. species on the basis of chromosome pairing in hybrids (Lilienfeld and Kihara, 1951). These studies have continued to the present and have accurately classified chromosome translocations (Naranjo, 1995; Naranjo *et al.*, 1988; Maestra and Naranjo, 1999; Jauhar *et al*., 1991). Archaeological evidence has shown that *Triticum turgidum* L. (AABB) has been grown in both Mesopotamia (Tigris and Euphrates River Valley) and in the Nile River Valley 10,000 years ago. Because wild *T. tauschii* (Coss.) Schmalh. is found only in the mountain region of south Russia, west Iran and north Iraq, it is thought that the hybridization that produced *T. aestivum* L. have occurred in these regions. It has been suggested that this occurred as recently as 8,000 years ago which coincides with the development of collective settlements by man (Wazuddin and Driscollt, 1986).

The goal of the present study was to find relationships between *Triticum* species using karyotype analysis and molecular markers.

Materials and Methods

Plant materials

A total of 46 wheat accessions were collected for this study. The materials were taxonomically identified based on Rahiminejad and Kharrazyan (2005). The Karyotype analysis was performed on the accessions belonging to five species and two subspecies carrying A genome (*Triticum monococcum* L., *T. boeoticum* Boiss. subsp. *thaodar* (Reut.) Schiemann, *T. boeoticum* subsp. *boeoticum*, *T. urartu* Tumanian ex Gandilyan, *T. durum* Desf., *T. turgidum* and *T. aestivum*) (Table 1).

Chromosome spread preparation

Triticum seeds were germinated at 25ºC on moist filter paper in Petri dishes. Actively-growing root tips, 1 cm in length, were excised from the germinating seeds and pretreated with α-bromonaphthalene for 4-6 hours at 4ºC in refrigerator and fixed in chromic acid-formaldehyde mixture (1:1 of 1% chromic acid + 10% formaldehyde) at about 4ºC for 24 hours. The root tips were transferred into 70% ethanol solution and kept refrigerated till staining. Subsequently, the root tips were hydrolyzed in 1 N NaOH at 60ºC for 10 minutes, stained for 24 hours in hematoxylin stain at 30ºC, and squashed in 45% glacial acetic acid. Before squashing, the root tips were treated with cellulase-pectinase enzyme solutions at 37ºC for 10-15 minutes. The selected cells were photographed under an Olympus AX-40 light microscope. Karyotypes were obtained from well-spread metaphase plates.

All chromosomal sizes were measured with computer-aided program Micro Measure 3.3. Software (Reeves, 2001). Relative length in proportion to total genome length (RL), total chromosome length (TCL), mean chromosome length (MCL), arm ratio (short arm length/long arm length) with their respective standard errors and centromeric index (length of

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Phylogenetic comparison of the A genome using karyotype analysis in some *Triticum* species

Mohammad Hosein Ehtemam [1]*, Mohammad Reza Rahiminejad [2], Hojatollah Saeidi [2] and Fatemeh Ebrahim [1]

[1] Department of Agronomy and Plant Breeding, College of Agriculture, Isfahan University of Technology, Isfahan, Iran
[2] Department of Biology, Faculty of Sciences, University of Isfahan, Isfahan, Iran

Abstract
The Karyotype analysis was performed on 46 wheat accessions belonging to five species (*Triticum monococcum*, *T. urartu*, *T. durum*, *T. turgidum* and *T. aestivum*) and two subspecies (*T. boeoticum* subsp. *thaodar* and *T. boeoticum* subsp. *boeoticum*) carrying A genome. All chromosomal sizes were measured with computer-aided program Micro Measure 3.3. Software. RL, TCL, MCL, arm ratio, centromeric index, TF%, mean of long and short arms, AsI%, S%, DRL, A1, A2 and karyotype formula were calculated for each chromosome. All the accessions were placed in 1A category of stebbines asymmetry categories. The scatter diagram based on A1 and A2 constructed three groups of karyotype asymmetry in the accessions studied: 1- *T. aestivum* with the highest asymmetrical karyotype, 2- *T. monococcum*, *T. boeoticum* subsp. *thaodar* and *T. boeoticum* subsp. *boeoticum* with the lowest asymmetrical karyotype and 3- *T. urartu*, *T. turgidum* and *T. durum* being with an intermediate between the two previous groups. *T. monococcum* based on the A1 and A2 index (asymmetric index) had the oldest and the most primitive karyotype among diploid species. According to the results, it might be suggested that *T. durum* is more primitive than *T. turgidum* and *T. monococcum* could be considered as a donor of A genome to *T. durum* and *T. aestivum*.
Key words: A genome, Karyotype analysis, Phylogeny, *Triticum*

Introduction

Plant nuclear genomes are enormously variable. Chromosome number, the degree of gene clustering, and chromosome size can all differ considerably, even between closely related species (Kellogg and Bennetzen, 2004).

The earliest work on wheat chromosomes relied on reconstructions made from microtomal serial sectioning of root tips. The development of squash technique reduced the possibilities of misinterpretations. Studies of the morphology of chromosomes in *Triticum* species have been made by many workers, including Schulz-Schaeffer and Haun (1961), Khan (1963), Kimber (1971), Johnson and Dhaliwal (1978), Kerby and Kuspira (1987), Miller (1987) and Khan (2005).

* Corresponding Author: hehtemam@cc.iut.ac.ir

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different stocks in this area. However, it is necessary to do some other extensive studies to find out the main parameters affecting heterozygosity deficiency in this area.

In this study, all F_{ST} values showed significant difference between each two populations ($P<0.01$), suggesting that the four populations are genetically differentiated and don't represent a single panmictic population, meaning that there is some mating restrictions, either genetic or behavioural, upon the population (Freeland, 2007). The effects of different barriers such as hydroelectric dams or even agricultural weirs have been studied on different freshwater fish species mainly migratory forms such as bull trout, *Salvelinus confluentus* (Neraas and Spruell, 2001), white-spotted charr, *Salvelinus leucomaenis* (Yamamoto *et al*., 2004), asper, *Zingel asper* (Laroche and Durand, 2004), brown trout, *Salmo trutta* (Hansen *et al*., 2014) and some cyprinids such as chub, *Leuciscus cephalus* (Laroche *et al*., 1999) and roach, *Rutilus rutilus* (Hanfling *et al*., 2004). In most cases, it has been clearly stated that landscape fragmentation has caused a significant genetic differentiation and population isolations in freshwater fish. Recently, Blanchest *et al*. (2010) showed that response to fragmentation was highly species-specific and depended on different factors such as dispersal ability (body size) and effective population size. They stated that although, smaller fish such as *Phoxinus phoxinus* has lower dispersal ability, they are slightly affected by fragmentation because of the larger effective population size. On the contrary, fish species of intermediate body size such as *Leuciscus leuciscus* was highly affected, whereas the largest fish species, *Leuciscus cephalus* was moderately affected by fragmentation mainly because of their dispersal ability. Based on our finding, *P. esfahani* can be categorized as intermediate body size fish. So, its population structure, at least from genetic point of view, can be affected seriously by fragmentation.

The findings reported in this study nevertheless reveal important implications for stock conservation of *P. esfahani* in the Zayandeh Rood River. The scenario, however, is not complete and further samplings from additional sites, taken during different seasons of the year, and using more specific molecular markers should be taken into account.

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respectively (Table 6). So, the highest amount of genetic variations was because of differences within individuals and only a small portion of the variations was assigned to the difference between populations (Table 6).

Table 6. AMOVA of data microsatellite for four populations. F_{IS}. 0.189**; F_{ST}. 0.025*; F_{IT}. 0.159**. **. P<0.01; *. P<0.05

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among population	3	14.258	0.052	2.52
Among individuals within population	116	189.300	0.381	18.49
Within individuals	119	287.500	2.39	78.99
Total	238	491.058	2.82	100

Gene flow was estimated at 4.49, 7.63, 12.14, 17.83 and 7.94 for CtoF-172, Bl1-2b, CnaB-030, Llea-071 and Ca3, respectively. The mean value of gene flow was as high as 8.18 for all population at all loci.

Discussion

The long-term persistence of a fish species can be investigated by allelic diversity, gene diversity, effective population size and population structure (Liu, 2007). Despite the importance of *Petroleuciscus esfahani* as an endemic fish in the Zayandeh Rood River, very little information regarding to this species is available.

In this study, we have employed 5 polymorphic microsatellite loci to assess the genetic relationship among populations of *P. esfahani* from four stations in the Zayandeh Rood River before and after the dam. According to the results, all stations had high number of alleles where KH station showed the highest (9.6 in average). Based on the expected hetrozygosity and number of alleles, the results of this study revealed that genetic diversity of four populations of *P. esfahani* was high. It is well-documented that artificial propagation and heavy fishing activities are two main reasons for reduction of aquatic genetic diversity (Beaumont and Hoare, 2003). Based on the available information, there have been no or very limited activities done on this species in the Zayandeh Rood basin. So, it could be expected that the populations have not been touched showing high level of genetic diversity in each station because of the high population effective size.

The deviations from Hardy-Weinberg equilibrium in most loci were observed. Some factors such as genetic drift, selection and small size of population can affect deviations from Hardy-Weinberg equilibrium (Birgitte *et al.*, 2005; Zhao *et al.*, 2005). Hardy-Weinberg equilibrium theory is based on distribution of genes and different genotypes in a population and thus, the accuracy of the theory is related to fulfilling some conditions such as no migration, large number of the population, no genetic drift, random reproduction, no selection and no mutation (Freeland, 2007). Among all 15 meaningful deviations from Hardy-Weinberg most of them showed heterozygosity excess maybe because of population mixing and sampling bias (Rousset and Raymond, 1995; Liu, 2007). Heterozygosity deficiency which was observed in CH population could arise from existence of substructured population of this species in this area. It is well-documented that an overall deficiency of heterozygosity is obvious in substructured stocks, the proportional magnitude depends on the nature of substructuring, i. e., the number of subpopulations, the time of divergence and the rate of gene flow amongst them (Chakraborty and Jin, 1992). The CH samples were collected from a relatively large man-made lake which may provide a pool of

effective and observed allele were 6.13 and 8.82 respectively (Table 2). Across the five loci and within the four populations, only six private (specific) alleles were observed, CtoF-172 and Ca3 showed 2 specific alleles for CHD and KH and LleA-071 and Ca3 showed only one specific allele for SB and CH, while, there were no specific alleles for Bl-12b and CnaB-030 loci at any populations.

Significant deviations from Hardy-Weinberg equilibrium at the locus level are shown in Table 3. From all combinations (station × locus) only CHD and CH station at LleA-071 and CHD, KH and SB at Ca3 locus were in Hardy-Weinberg equilibrium (Table 3). The other combinations showed significant deviation from Hardy-Weinberg equilibrium most of which due to heterozygosity excess except for CH at Ca3 locus which showed heterozygosity deficiency (Table 3).

Table 3. Deviation of Hardy-Weinberg equilibrium for five loci in four populations of *Petroleuciscus esfahani* (P<0.05). n.s. In Hardy-Weinberg equilibrium (P>0.05); HE. Heterozygosity excess; HD. Heterozygosity deficiency (P<0.001); CHD. Cheshmeh Dimeh; KH. Khersoonak; CH. Chamgordan; SB. Safaeye Bridge

Population/locus	**CtoF-172**	**CnaB-030**	**BL1-2b**	**LleA-071**	**Ca3**
CHD	HE	HE	HE	n.s	n.s
KH	HE	HE	HE	HE	n.s
CH	HE	HE	HE	n.s	HD
SB	HE	HE	HE	HE	n.s

Genetic Variation among Sampling Regions

Pairwise F_{ST} values were ranging from 0.013 (between CHD and KH station) to 0.032 (between CHD and CH station) (Table 4). All values showed significant difference between each two populations, probabilities of F_{ST} determined by AMOVA tests, at P≤0.0001.

Table 4: Pairwise F_{ST} values between four populations based on five microsatellite loci, all combination between two populations were significant (P<0.01). CHD. Cheshmeh Dimeh; KH. Khersoonak; CH. Chamgordan; SB. Safaeye Bridge

Stations*	CHD	KH	CH	SB
CHD	-----			
KH	0.013	-----		
CH	0.032	0.025	-----	
SB	0.019	0.014	0.026	-----

Genetic distance calculated between each pair of stations ranged from 0.141 (between CHD and KH) to 0.244 (between CHD and CH), while the genetic identity ranged from 0.756 (between CHD and CH) to 0.859 (between KH and CHD, Table 5).

Table 5. Pairwise population of genetic distance (below diagonal) and genetic identity (above diagonal) (Nei, 1972) detected at 5 loci in *Petroleuciscus esfahani* samples. CHD. Cheshmeh Dimeh; KH. Khersoonak; CH. Chamgordan; SB. Safaeye Bridge

	Stations	**Genetic Identity**			
		CHD	**KH**	**CH**	**SB***
Genetic Distance	CHD	-	0.859	0.756	0.818
	KH	0.141	-	0.958	0.966
	CH	0.244	0.194	-	0.922
	SB	0.182	0.200	0.151	-

The genetic variations of all samples were separated into 3 different parts using molecular analysis of variance (AMOVA) including among populations, among individuals within population and within individuals which measured as 2.52, 18.49 and 78.99 %

Table 2. Genetic variabilities of five microsatellite loci in four populations of *Petroleuciscus esfahani* in Zayandeh Rood River in Iran. N. Sample size; Ho. Observed heterozygosity; He. Expected heterozygosity; No. Observed allele; Ne. Effective allele; PHW. Hardy-Weinberg probability test: *P<0.05; **P<0.01; F_{IS}. Fixation indices; n.s. Not-significant; CHD. Cheshmeh Dimeh; KH. Khersoonak; CH. Chamgordan; SB. Safaeye Bridge

Locus	Parameter	CHD	KH	CH	S
CtoF-172	N	30	30	30	30
	Ho	1.00	1.00	1.00	1.00
	He	0.82	0.75	0.77	0.79
	No	8	5	5	6
	Ne	5.34	3.92	4.27	4.50
	PHW	**	**	**	**
	F_{IS}	0.230	0.342	0.305	0.285
BL1-2b	N	30	30	30	30
	Ho	1.00	1.00	1.00	1.00
	He	0.87	0.80	0.82	0.78
	No	9	8	9	6
	Ne	7.14	4.73	5.14	4.32
	PHW	**	*	**	*
	F_{IS}	0.162	0.267	0.241	0.300
CnaB-030	N	30	30	30	30
	Ho	1.00	1.00	1.00	1.00
	He	0.78	0.79	0.75	0.79
	No	6	6	5	6
	Ne	4.39	4.56	3.82	4.50
	PHW	**	**	**	**
	F_{IS}	0.294	0.280	0.354	0.285
LleA-071	N	30	30	30	30
	Ho	1.00	1.00	1.00	1.00
	He	0.89	0.86	0.89	0.89
	No	12	10	10	11
	Ne	8.14	6.81	8.28	8.07
	PHW	n.s	**	n.s	**
	F_{IS}	0.140	0.171	0.137	0.141
Ca3	N	30	30	30	30
	Ho	0.96	1.00	0.86	0.90
	He	0.90	0.91	0.62	0.90
	No	13	14	14	14
	Ne	9.18	9.77	6.78	9.09
	PHW	n.s	n.s	**	n.s
	F_{IS}	0.084	0.113	0.272	0.011
Mean (all loci)	Ho	1.00	0.99	0.98	0.92
	He	0.82	0.85	0.83	0.82
	No	8.6	9.6	8.6	8.6
	Ne	5.96	6.84	6.9	5.66
	F_{IS}	0.234	0.182	0.204	0.153

CnaB-030 locus showed the lowest polymorphism regarding the number of observed allele (5.7), expected heterozygosity, He (0.777), polymorphism information content (PIC) up to 0.749 and the number of effective allele for each population was 4.31. On the other hand, Ca3 locus showed the highest polymorphism by calculating the number of observed allele, expected heterozygosity, PIC and the number of effective allele as 13.7, 0.892, 0.903 and 8.70, respectively. Genetic variations of all stations were high. The average of expected and observed heterozygosity measured as 0.849 and 0.978 respectively and the number of

followed by 35 cycles of 30 second at 94ºC, Annealing temperature 56ºC for all loci except Ca3 which annealed at 58 ºC for 30 s, 30 s at 72 ºC and the final extension of 72 ºC for 5 min. PCR products were separated on a polyacrylamide 12% gel and then stained by silver nitrate method (May *et al.*, 1997). Alleles were sized using an allelic ladder each gel contained (100 bp, Fermentase, Germany) to assist in consistent scoring of alleles.

Table 1. Characteristics of *Petroleuciscus esfahani* microsatellite loci used at present study* (*- Annealing temperature was 56ºC for all loci except Ca3 which annealed at 58 ºC)

Locus	Primer sequence 5'3'	GenBank Accession	Motif	Predicted Size
BL1-2b	F:TTTGCACTAGTAACGAGCATCA R:CAGCACAGTTTCTCCATCCA	FJ468347	$(TG)_{12}$	140-170
LleA-071	F:GTCTTAGATTGTGTAGCGGG R:ACTTCAGTTACTAAGAGATTAGTGA	FJ601719	(CA)6T(AC)10	340-350
CnaB-030	F:ACGAATGAGAAGCTCGTG R:TCGTCATGCAGTTCATCCT	GU254028	(AC)6	120-130
CtoF-172	F:ACCAAGGTGAAAGCCTGTAA R:GGACACGATGACAACGG	GU254034	(GT)13N14(TG)3	110-124
Ca3	F:GGACAGTGAGGGACGCAGAC R:TCTAGCCCCCAAATTTTACGG	AF277575	(TAGA)14	150-200

DNA Analysis

Effective number of alleles (Ne), gene flow (Nm), genetic distance and allelic frequency were determined as number of alleles per locus (A) and heterozygosity (H) directly from microsatellite phenotypes using GENEPOP version 3.2 (Raymond and Rousset, 1995). To test for deviation from Hardy-Weinberg Equilibrium (HWE) comparisons were made between observed heterozygosity (Ho), and expected heterozygosity (He) using exact tests as implemented by Power Marker version 3.0 (Liu and Muse, 2005) and GENEPOP 1.2 (Raymond and Rousset, 1995). This software employed the Markov chain method to estimate the probability of significant deviation from HWE. Genetic differences between populations were evaluated by calculating pairwise F_{ST} values and testing their significance by boot strapping analysis (1000 replicates) using ARLEQUIN 3.1 (Schneider *et al.*, 2000). This program was also used to partition variation within and between populations using by analysis of molecular variance AMOVA version 4 procedures (Excoffier and Schneider, 2005). Genetic differentiations among four populations (KH, CHD, SB and BA) were also evaluated using ARLEQUIN 3.1 (Schneider *et al.*, 2000).

Results

Within population variation

All five loci were polymorphic and variable in all populations (Table 2). A total of 54 alleles ranging in size from 103 to 350 bp were found over the five loci. The number of alleles ranged from 6 in CnaB-030 to 17 in Ca3 locus. Within populations, the lowest mean number of alleles per locus (8.6) was observed in three populations including KH, SB and CH. While the highest mean number of alleles per locus (9.6) was found in CHD population. Average observed heterozygosity was calculated from 0.92 in CH to 1.00 in KH population (Table 2).

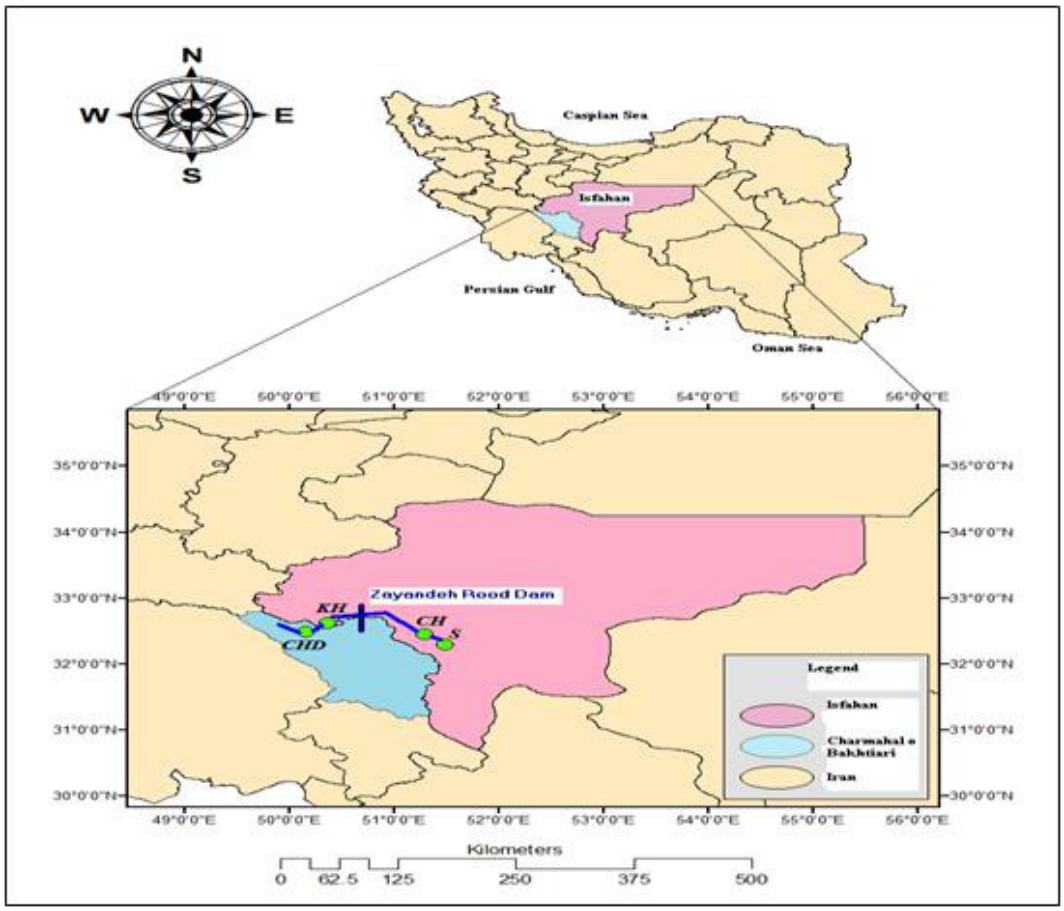

Figure 1. Sampling stations for *Petroleuciscus esfahani* along with Zayandeh Rood River. CHD. Cheshmeh Dimeh; KH. Khersoonak; CH. Chamgordan; SB. Safaeye Bridge

E

Total genomic DNA was extracted from dorsal fin tissue following the Bio Basic Kit of Genet Bio Company (Canada) based on manufacturers manual provided in the kit. The quality and concentration of DNA were assessed using 1% agarose gel electrophoresis and spectrophotometry. Finally, DNA was stored at -20˚C until use.

PCR Profiles and Primer Sequences

Five pairs of microsatellite primers (CtoF-172, Bl1-2b-CnaB-030, LleA-071 and Ca3) were selected over 41 microsatellite loci designed for European Cyprinids (Dubut *et al.*, 2010; Table 1). These loci have been selected based on the PIC (polymorphic information content, number of observed alleles in different cyprinid species such as *Telestes souffia* and *Telestes muticellus* (Dubut *et al*., 2009a), *Leuciscus leuciscus* (Dubut *et al*., 2009b), *Alburnus alburnus* (Dubut *et al.*, 2010) as well as *Alburnus mossulensis* (Shafee *et al.*, 2013).

The polymerase chain reaction (PCR) conditions, especially annealing temperatures, were optimized for the microsatellite loci as a necessity to produce scorable amplification products. Polymerase chain reaction was performed in a 10 µL reaction volume containing 100-150 ng of template DNA, 10 pmol of each primer, 200 µM each of the dNTPs, 1 U of *Taq* DNA polymerase (Cinagene, Tehran, Iran), 1.5 mM $MgCl_2$ and 1X PCR buffer. Thermal cycling condition for five loci were: an initial denaturation at 94ºC for 2 minutes

human activities such as water pollution (Nadig *et al.*, 1997) and dam or weir construction (Hansen *et al.*, 2014). However, the ecological response of the aquatics is very species-specific dependent on dispersal ability (body size), effective population size, and intensity of fragmentation (Blanchet *et al.*, 2010).

The Zayandeh Rood chub, *Petroleuciscus esfahani* is a native species in the Zayandeh Rood and Karoon (Tigris) basins in Iran (Coad and Bogutskaya, 2010). The species has wide distribution in the river both in up and down stream regions. The average body size of the fish is about 120 mm and it mainly feed on aquatic invertebrates and insects (Keivany *et al.*, in press). Despite of the importance of the Zayandeh Rood chub with respect to ecological view, the effects of different man-made or natural destructive phenomenon on the population structure of the species is unknown. The understanding of genetic diversity is one of the most important steps toward stable ecological management of fish stocks (Ward and Grewe, 1995).

Several approaches such as allozyme and DNA markers have been used for population structure studies. Among which, short simple tandem repeat (SSR or microsatellite) received much more attention during last decade mainly, because of high level of polymorphisms, co-dominant nature, uniform spreading all over the genome, reproducibility as well as neutral modality (Okumuş and Ciftci, 2003; Liu and Cordes, 2004). It has several practical applications in fisheries and aquaculture management such as population genetic structure analysis (Rezaei *et al.*, 2010; Ghasabshiran *et al.*, 2013), stock, strain or individual identification, gene mapping and parentage and pedigree analysis (Beaumont and Hoare, 2003; Liu, 2007).

Currently, there is no information available about genetic structure of Zayandeh Rood chub in different regions (up and downstream) of the River. So, the purpose of this research was to study the population genetic structure of the species as a native and common fish species in the one of the most important river ecosystem in the central part of Iran. The results of this study can help managers to find out more about the impact of the different anthropogenic activities on the river ecosystem as a whole.

Materials and Methods

Sample collection

One hundred and twenty (120) individuals of *Petroleuciscus esfahani* were collected from four stations in October 2011 including Cheshmeh Dimeh (CHD), Khersoonak (KH), Chamgordan (CH) and Safaeye Bridge (SB) (Figure 1), the two first stations as an upstream location, located before Zayandeh Rood Dam and the others as a downstream station after the dam. All stations located in Isfahan province except for CHD which is located in Charmahal va Bakhtiari province (Figure 1). For each sample, 2-3 g dorsal fin tissue was collected and conserved in absolute ethanol for subsequent DNA extraction and amplification.

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Population genetic of *Petroleuciscus esfahani* (Teleostei: Cyprinidae) in Zayandeh Rood River, Iran

Neda Shojaee [1], Salar Dorafshan [1]*, Nourollah Mirghaffari [1] and Majid Talebi [2]
[1] Department of Natural Resources, Isfahan University of Technology, Isfahan, 84156-83111, Iran
[2] Department of Biotechnology, College of Agriculture, Isfahan University of Technology, Isfahan, Iran

Abstract
Population genetics of *Petroleuciscus esfahani* in Zayandeh Rood River, Iran were analysed using 120 samples of adult fish from four stations of the river Cheshmeh Dimeh (CHD), Khersoonak (KH), Chamgordan (CH) and Safaeye Bridge (SB) and 5 pairs of microsatellite primers. All loci showed polymorphism. A total number of 54 alleles were recorded across loci ranging from 6 at CnaB-030 to 17 at Ca3. The mean number of alleles per populations ranged from 9.6 in CHD to 8.6 in others. Mean observed heterozygosity at the five loci detected ranged from 0.92 to 1.00 which showed high level of genetic diversity in each population. Deviation from Hardy-Weinberg equilibrium was obvious in most combinations (locus × population), mainly due to heterozygosity excess. The lowest and highest genetic distances were calculated between CHD-KH and CHD-CH populations, respectively. The results showed low but significant F_{ST} values between each pair of the populations. This investigation represented at least four separate populations of *P. esfahani* in the river which showed the effects of river landscape fragmentation on population genetic structure of *P. esfahani*.
Key words: Microsatellite, *Petroleuciscus esfahani*, Population, Zayandeh Rood River

Introduction

All around the world, civilization has arisen near to the constant high quality water resources. In the Middle East, the role of freshwater resources such as rivers has been very critical for human civilization because of the historical low availability of water. The Zayandeh Rood River as the only main surface water in the central part of Iran has different ecological, social and economical key roles. In recent decades, several anthropogenic activities such as dam and weir construction, water pollution, introduction of non-native fish species such as common carp, *Cyprinus carpio* or semi-natural phenomenon like several years of drought and climate changes have significantly altered its ecological structure. The Zayandeh Rood dam was built in 1971 is one of the highest dam in the Middle East (about 100 m height) which was built to provide hydroelectric power as well as constant water supply for agriculture (more than 100000 ha) for many years. Indeed, several non-continual weirs have been made in the river mainly in downstream to regulate water level and water direction mainly for agriculture purposes. It is well-documented that

* Corresponding Author: sdorafshan@cc.iut.ac.ir

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Email: TBJ@ui.ac.ir
Website: http://uijs.ui.ac.ir/tbj
Tel: +98(31)37934255
Fax: +98(31)37932177

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