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USE OF ETHNOVETERINARY MEDICINAL PLANTS TO TREAT CATTLE DISEASES BY THE OULAD HERIZ FARMERS IN THE CHAOUIA REGION, NORTHWEST OF MOROCCO

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Using medicinal plants for healthcare practices in indigenous communities presents a chance to discover natural remedies. This study aims to provide a detailed account of the ethnoveterinary knowledge of medicinal plants utilized for therapeutic purposes. From December 2020 to December 2021, a field investigation was carried out within the Oulad Heriz tribes. Two hundred local farmers were interviewed using open-ended, semi-structured discussions, free listing and focus groups. A total of one hundred medicinal plants belonging to 87 genera and 33 families were discovered to be employed for ethnoveterinary purposes. *Rosmarinus officinalis* L. was the most frequently utilized ethnoveterinary plant species in the study area (70 citations). Leaves are shown to be the most commonly employed plant parts (55%). However, digestive disorders were the most often mentioned ailment (33 medicinal plants, 599 citations), and most herbal medicines were made by infusion (46.2%). According to the current investigation findings, local farmers in Oulad Heriz have continuous knowledge of ethnoveterinary plants. This ethnoveterinary medicinal heritage needs to be incorporated with contemporary medicine to use plants and their products as potentially viable medications for various cattle illnesses.

Key words: cattle diseases, conservation, ethnoveterinary, farmers, IUCN status, medicinal plants, phytotherapy

INTRODUCTION

Worldwide, indigenous communities from many cultures use their indigenous healing knowledge to prevent and cure various diseases ~~thru~~ animal and plant-derived medicines. The connection between humans and the environment has existed since the dawn of time (Mathias-Mundy and McCorkle 1989). Natural therapeutic herbs have been available for thousands of years, and many modern medications have been derived from plant-based sources (Ansari *et al.* 2023, Cragg and Newman 2001). Indigenous peoples

have traditionally recognized plants as a means to treat common ailments in humans and animals. This is due to the longstanding socio-cultural relationship between these communities and their environment, resulting in traditional practices such as using plant-based remedies to address health issues in humans and animals (Chaachouay *et al.* 2022*a*, *b*, Mania and Mania 2005).

Ethnoveterinary medicine (EVM) is a model based on popular folk practices, techniques, expertise, ideas, philosophies, and strategies for healing different illnesses, healthy farming, and animal healthcare (Maphosa *et al.* 2010). Ethnoveterinary traditions are typically acquired through personal experience and passed down orally from one generation to the next (Chaachouay *et al.* 2019*d*, 2022*a*, Maphosa *et al.* 2010). The significance of this extensive knowledge is exemplified by the African proverb, "When a wise old man dies, a whole library vanishes". Despite this, ethnoveterinary medicine remains crucial for developing livelihoods and sustainable livestock production in many impoverished regions worldwide. In these areas, it is often the only viable option available to local farmers for treating ailments in their cattle (Mwale and Masika 2009).

This traditional approach to animal healthcare has been used for centuries by indigenous communities worldwide. The use of medicinal plants in ethnoveterinary medicine is a complex and varied practice influenced by various cultural, environmental, and economic factors. In many cases, these plants' specific ingredients and activities still need to be fully understood, but they have been observed to treat various ailments effectively. Some examples of commonly used medicinal plants in ethnoveterinary medicine include garlic, ginger, aloe vera, neem, and turmeric. For instance, in South Asia, farmers have long used neem leaves and oil as natural pesticides to control a wide range of livestock pests. In Africa, leaves from the Acacia tree treat diarrhoea in calves. In contrast, in the Amazon region, indigenous communities use the bark of the cat's claw plant to treat various ailments in their livestock (McCorkle and Mathias-Mundy 1992). Even though their ingredients and specific activities were unknown, these plants were employed to cure various ailments (Arnao 2014). Phytotherapy has various applications, such as in rural areas with limited access to healthcare facilities or in communities with low incomes and a high cost of pharmaceutical drugs. It is also used due to cultural taboos and beliefs around plant medicines and has benefited from advances in scientific assessment leading to improvements in the quality of herbal medications.

The World Health Organization (WHO) has acknowledged the role of EVM in managing animal illnesses and has estimated that in less developed nations, 80% of the population relies solely on traditional medicine for primary healthcare, including treating animal ailments (WHO 2010). More than

half of the world's population still relies only on plants as their sole source of treatment, and plants offer the majority of the active ingredients in traditional medical products (Chaachouay *et al.* 2021*a*, *b*, *c*, *d*, Van Wyk and Wink 2018).

Presently, knowledge about ethnoveterinary medicinal plants is typically safeguarded by tribal elders, making it difficult to share this information with other community members in written form. Therefore, the main objective of this study is to document the ethnoveterinary knowledge of medicinal plants used by indigenous farmers of the Oulad Heriz tribes to manage and treat various illnesses in their cattle.

METHODS

Study region – The study was conducted in the Oulad Hriz tribes of the Chaouia, province of Berrechid. This study area was created by the dismemberment of the area of Settat in 2009 (Monographie 2018). It is located at a crossroads connecting the north and south of Morocco in the plain of Chaouia. The Casablanca prefecture is limited to the north, Settat province to the south, El Jadida to the west, and Benslimane to the east. It covers an area of approximately 2,530 km^2, which represents 13% of the regional area (Fig. 1). A semi-arid, temperate climate characterizes the province. Temperatures are moderate in the coastal zone but more prevalent in the interior. Its summers are hot and humid, with temperatures ranging from 20 °C to 31 °C, and its winters are cold, from 5 °C to 18 °C. Although it fluctuates yearly, the aver-

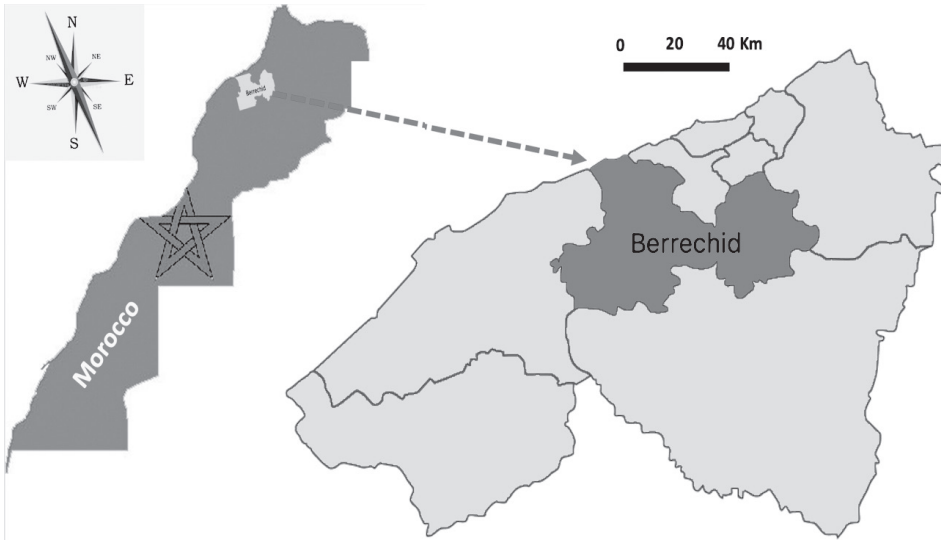

Fig. 1. Map of Morocco showing the province Berrechid

age annual precipitation received at the provincial level is approximately 380 mm (DMNM 2020). The province of Berrechid is located in the middle of the plains of the historical region of Chaouia and contains fertile soils with good agronomic ability. The territory of the province of Berrechid is a cereal and livestock area with exciting investment opportunities in the agro-industry (Monographie 2018).

Field survey – A field survey was conducted in the rural areas and tribal villages of the Berrechid province, specifically among the Oulad Heriz tribes, to gather information on the traditional use of plants in animal healthcare. This survey was carried out from December 2020 to December 2021 and involved various methods, including field observations, semi-structured questionnaire-based interviews, and open-ended interviews (Kay 1987) with cattle holders and breeders, local farmers, peasants, agricultural labourers, pastoralists, knowledgeable elders, and traditional practitioners who were given a choice to share their indigenous knowledge. The study areas were chosen based on the presence of conventional therapy practitioners and recommendations from agricultural development agents and government officials.

A total of 200 informants, consisting of 160 males and 40 females across different age groups ranging from 35 to 78 years, were purposively selected and interviewed for the study. The questionnaire used in the survey was developed and created specifically for this purpose (Appendix). The questionnaire used in the study had two sections. The first section collected sociodemographic information about the participants, such as their age, profession, gender, education level, community, and the number of animals they owned or worked with. The second section focused on documenting the medicinal plants commonly used to treat various animal health issues, including their local names, components used, preparation methods, dosage, mode and route of administration, and the specific conditions they were used for. In our investigation, our primary focus was collecting and documenting plants that possess medicinal properties and are commonly used to treat various diseases that affect animals (digestive disorders, microbial infections, pain and wounds, respiratory troubles, skin disorders, and others). Digestive disorders include a range of conditions affecting the gastrointestinal system, including enteritis, flatulence, ulcer, diarrhoea, constipation, and gastritis. Microbial infections are diseases caused by microorganisms like bacteria, viruses, fungi, and parasites. Examples include influenza, pneumonia, endoparasites, dysentery, measles, jaundice, chicken pox, rabies, anthrax, sheep pox, and goat pox. Pain and wounds encompass a variety of painful conditions and injuries, both acute and chronic, such as migraines, toothache, naval pain, earache, headache, and knee pain. Additionally, this category includes wounds, cuts, burns, and fractures, which require appropriate medical attention, wound care, and pain

management. Respiratory troubles involve disorders that affect the respiratory system, including the lungs and airways. This category includes asthma, chronic obstructive pulmonary disease, bronchitis, pneumonia, and allergies. Skin disorders include numerous conditions such as acne, eczema, psoriasis, dermatitis, albinism, eczema, ringworm, urticaria, cracked heels, vitiligo, skin oedema. The "Other" category encompasses various health conditions and concerns that do not fall into the categories above. It may include hormonal imbalances, autoimmune disorders, mental health issues, cardiovascular problems, neurological disorders, and genitourinary conditions. The "Other" category is broad and covers various health conditions requiring individual attention and treatment. The questionnaires were initially prepared in English and were later translated into Arabic for the convenience of the participants.

Plant species collection and identification – During this study, the botanical materials of 100 plant species were gathered and documented. Each plant was also assigned a local, scientific, and family name. The informants were also asked about the local names of plants used for treating livestock ailments. These plants were photographed, numbered, pressed, dried, and arranged alphabetically by vernacular name, scientific name, family name, and ethnoveterinary practices. Voucher samples were taken for each plant, likely used as reference specimens for future identification and study. This likely involved collecting plant specimens from the field, pressing and drying them, and then identifying them using floristical and taxonomic sources such as the Catalogue of vascular plants of Northern Morocco (Valdés 2002), Moroccan flora practice: vascular plant determination manual. Pteridophyte, gymnosperm, angiosperm (Fennane *et al.* 1999), Practical flora from Morocco. Vol. 2 (Fennane and Ibn-Tattou 2007), Moroccan vascular flora: inventory and chorology (Fennane and Ibn Tattou 2012), Morocco practice flora, Vol. 3 (Fennane *et al.* 2014). Additionally, taxonomic names of plant species were verified using internet databases, precisely, The Plant List (http://www.theplantlist.org).

Ethical approval and consent to participate – Before performing a field study, local authorities requested authorization to work with and gather data about helpful plants in the surrounding community. Following that, consent was gained from all Oulad Hriz farmers, who were educated about the project and its potential advantages for the whole community. At any point throughout the interviews, participants were free to terminate them.

Quantitative analyses of ethnoveterinary data – Questionnaire sheets were employed to collect ethnoveterinary data, which was then scrutinized and examined for validation or rebuttal. Subsequently, the information was entered into Microsoft Excel 2010 and IBM-SPSS Statistics Base 21 for further recording and statistical analysis.

RESULTS

Demographic data – Data were collected from 200 participants (160 males and 40 females) of ages 35 to 78 years, including shepherds, cattle holders, local farmers, knowledgeable elders, nomads, traditional practitioners, and village leaders. Among the 200 interviewed inhabitants, 15 in Oulad Ziyan, 25 in Oulad Zidane, 15 in Jacma, 20 in Lmabarkiyine, 25 in Riah, 15 in Lahsasna, 25 in Laghnimyine, 20 in Oulad Abbou, 15 in Ben Maachou, and 25 in Sahel Oulad Hriz. Based on demography, these informants were classified into various categories, as given in Table 1.

Taxonomic classification of plant species – The indigenous communities of the Oulad Hriz tribes used 100 medicinal plants for treating livestock ailments in the study. Based on their botanical characteristics, these plants were classified into 87 genera and 33 families. According to the findings of this research, the Lamiaceae family has the most significant number of plant species with 17 species (17%) followed by Apiaceae 12 species (12%), Asteraceae and Fabaceae 8 species for each family (8%), Poaceae 6 species (6%), and Solanaceae 5 species (5%). Table 2 shows the information on plant species that the local population uses for veterinary purposes.

Medicinal plants reported – The results of the floristical analysis conducted on the medicinal plants identified by the local communities of Oulad Hriz revealed the presence of 100 different plant species. The study also found that the frequency of use of these medicinal plants varied widely, with different species being used more frequently than others. In this study, the frequency of citations (FC) of the reported species ranged from 2 to 70 (Table 2). The

Table 1
Demographic information about informants in the study area

Name of the area	No. of participants	Percentage (%)	Age groups	Gender	
				Female	Male
Oulad Ziyan	15	7.5	57–78	5	10
Oulad Zidane	25	12.5	36–69	4	21
Jacma	15	7.5	36–70	5	10
Lmabarkiyine	20	10	59–67	3	17
Riah	25	12.5	35–65	7	18
Lahsasna	15	7.5	52–62	3	12
Laghnimyine	25	12.5	36–71	3	22
Oulad Abbou	20	10	54–77	2	18
Ben Maachou	15	7.5	38–65	3	12
Sahel Oulad Hriz	25	12.5	54–67	5	20

Table 2
Catalogue of medicinal herbs used by the Oulad Hriz tribes for ethnoveterinary purposes

Family and species	IS	Vernacular n.	Part	Prep.a.	Anim. tr.	Uses	Fc
Amaryllidaceae							
Allium cepa L.	NE	Lbsla	bulb	Cr, Mo	sheep	DD	20
Allium sativum L.	NE	Thouma	bulb	Ca, To	goat	PW	12
Allium porrum L.	NE	Lkerrath	bulb	Ca, To	goat, sheep	MI	8
Anacardiaceae							
Pistacia atlantica Desf.	NT	Btem	leaf	In, Or	sheep	DD	14
Pistacia lentiscus L.	LC	Drou	leaf	Fu, Na	sheep	PW	10
Apiaceae							
Ammi majus L.	LC	Bechnikha	leaf	In, Or	cow	DD	23
Ammi visnaga (L.) Lam.	LC	Kella	flower	De, Or	sheep	MI	13
Ammodaucus leucotrichus Coss.	EN	Kamon Essofi	leaf	In, Or	sheep	PW	5
Carum carvi L.	LC	Lkrwya	seed	De, Or	cow, sheep	DD	24
Cuminum cyminum L.	NE	Kamon	seed	De, Or	horse	MI	7
Daucus carota L.	LC	Khizzo	fruit	De, Or	sheep	DD	11
Foeniculum vulgare Mill.	LC	Lbesbass	seed	De, Or	cow	MI	33
Petroselinum sativum Hoffm.	NE	Maadnous	leaf	In, Or	sheep	DD	21
Pimpinella anisum L.	NE	Habbat Hlawa	leaf	In, Or	sheep	MI	8
Smyrnium olusatrum L.	LC	Lheyyar	leaf	In, Or	cow, sheep	RT	5
Ferula communis L.	LC	Aboubal	aerial	De, Or	mule	PW	4
Thapsia garganica L.	NE	Thorah	leaf	In, Or	cow, sheep	DD	6
Apocynaceae							
Nerium oleander L.	LC	Defla	leaf	Cr, Na	donkey	RT	34
Asparagaceae							
Asparagus officinalis L.	LC	Sekkoum	stem	De, Or	cow	MI	16
Drimia maritima (L.) Stearn	LC	Laansel	bulb	De, Or	goat, sheep	DD	9
Asteraceae							
Artemisia herba-alba Asso	NT	Chih	leaf	In, Or	goat, sheep	SD	48
Artemisia absinthium L.	LC	Chiba	leaf	Ca, Br	goat, sheep	DD	67
Atractylis gummifera Salzm. ex L.	LC	Addad	aerial	De, Or	goat, cow	MI	26
Calendula officinalis L.	NE	Jemra	aerial	In, Or	goat	DD	17
Centaurea maroccana Ball	NE	Tafgha	aerial	De, Or	cow	MI	6
Inula viscosa (L.) Aiton	LC	Bagraman	leaf	In, Or	cow, sheep	SD	35
Lactuca sativa L.	LC	Elkhass	leaf	In, Or	sheep	PW	20
Matricaria chamomilla L.	NE	Babounj	leaf	In, Or	goat	DD	42

Table 2 (continued)

Family and species	IS	Vernacular n.	Part	Prep.a.	Anim. tr.	Uses	Fc
Brassiaceae							
Diplotaxis catholica (L.) DC.	LC	Lkerkaz	flower	De, Or	goat	MI	12
Lepidium sativum L.	NE	Hab Rchad	seed	De, Or	sheep	SD	7
Cactaceae							
Opuntia ficus-indica (L.) Mill.	DD	Sebbar	flower	Cr, Br	goat, cow	DD	36
Capparaceae							
Capparis spinosa L.	NE	Lkabbar	fruit	Ca, Na	sheep	PW	44
Caryophyllaceae							
Corrigiola telephiifolia Pourr.	LC	Thawsar-ghine	aerial	De, Or	cow, sheep	DD	25
Silene vulgaris (Moench) Garcke	LC	Thighighet	flower	Fu, Mo	goat, sheep	MI	17
Cucurbitaceae							
Citrullus colocynthis (L.) Schrad.	NE	Lhdej	seed	De, Or	cow, sheep	PW	56
Cucurbita maxima Duchesne	NE	Lguraa	fruit	Cr, Br	mule, sheep	DD	6
Cupressaceae							
Juniperus communis L.	LC	El aaraar	leaf	In, Or	sheep	MI	23
Juniperus oxycedrus L.	LC	Taga	leaf	Ca, To	sheep	RT	15
Tetraclinis articulata (Vahl) Mast.	LC	Araâr	leaf	In, Or	goat	FC	10
Euphorbiaceae							
Euphorbia helioscopia L.	NE	Hlaba	leaf	In, Or	mule, sheep	DD	6
Ricinus communis L.	NE	Lkrnek	leaf	Ca, Na	goat, horse	SD	42
Fabaceae							
Acacia arabica (Lam.) Willd.	LC	Telh	leaf	In, Or	goat, sheep	DD	12
Acacia raddiana Savi	LC	Telh	leaf	In, Or	cow, sheep	PW	10
Ceratonia siliqua L.	NE	Lkharroub	leaf	In, Or	cow, sheep	SD	49
Cicer arietinum L.	NE	Lhmes	seed	De, Or	cow	DD	8
Glycyrrhiza glabra L.	NE	Ark Sous	stem	De, Or	cow	MI	57
Medicago murex Willd.	LC	Fessa	aerial	De, Or	donkey	RT	11
Trigonella foenum-graecum L.	NE	Lhelba	seed	De, Or	sheep	DD	69
Vicia faba L.	NE	Lfoul	seed	In, Or	sheep	MI	7
Fagaceae							
Quercus suber L.	NE	Bellot Fillini	leaf	In, Br	sheep, cow	PW	26

Table 2 (continued)

Family and species	IS	Vernacular n.	Part	Prep.a.	Anim. tr.	Uses	Fc
Lamiaceae							
Ajuga iva (L.) Schreb.	NE	Chendgoura	leaf	In, Or	mule, sheep	DD	7
Calamintha officinalis Moench	NE	Manta	leaf	In, Or	sheep	SD	6
Lavandula dentata L.	NE	Lkzama	aerial	Cr, Mo	sheep	MI	44
Lavandula multifida L.	NE	Lkohila					35
Lavandula stoechas L.	NE	Lhalhal	leaf	In, Or	goat, cow	MI	33
Marrubium vulgare L.	NE	Merriwta	leaf	In, Or	sheep	RT	24
Mentha pulegium L.	LC	Fliyou	leaf	In, Or	goat, horse	DD	31
Mentha suaveolens Ehrh.	LC	Marsetta	aerial	De, Or	donkey	MI	7
Mentha viridis (L.) L.	LC	Naanaa	leaf	In, Or	cow, sheep	DD	8
Ocimum basilicum L.	NE	Lhbaq	leaf	In, Mo	sheep	MI	11
Origanum compactum Benth.	VU	Zaatar	leaf	In, Or	horse	FC	32
Origanum majorana L.	LC	Merded-douch	aerial	De, Or	mule, sheep	DD	16
Rosmarinus officinalis L.	NE	Azir	aerial	Ca, Br	horse, mule	PW	70
Salvia officinalis L.	LC	Salmya	leaf	In, Or	cow	MI	6
Teucrium polium L.	NE	Jaadiya	leaf	In, Or	cow, sheep	RT	9
Salvia verbenaca L.	DD	Khiyata	aerial	De, To	cow, sheep	DD	7
Thymus vulgaris L.	LC	Zaaitra	leaf	In, Or	cow, sheep	MI	12
Lauraceae							
Laurus nobilis L.	NE	Rend	leaf	In, Or	donkey, sheep	RT	24
Linaceae							
Linum usitatissimum L.	LC	Zriat Lkttan	seed	De, Or	cow, sheep	DD	7
Lythraceae							
Lawsonia inermis L.	NE	Lheni	leaf	In, Or	horse	SD	18
Moraceae							
Ficus carica L.	LC	Lkarous	leaf	In, Or	goat, sheep	PW	14
Myrtaceae							
Eucalyptus globulus Labill.	NE	Lkalitous	leaf	In, Or	cow, sheep	DD	53
Eugenia caryophyllata Thunb.	NE	Lqronfel	leaf	In, Or	cow, sheep	MI	24
Myrtus communis L.	LC	Rihan	leaf	In, Or	cow, sheep	RT	37
Pimenta dioica (L.) Merr.	LC	Nwiwra	leaf	In, Or	cow, sheep	FC	9
Nitrariaceae							
Peganum harmala L.	NE	Lharmel	seed	De, Or	cow, sheep	MI	6

Table 2 (continued)

Family and species	IS	Vernacular n.	Part	Prep.a.	Anim. tr.	Uses	Fc
Oleaceae							
Olea europaea L.	NE	Zitoun	leaf	In, Or	sheep	RT	8
Olea europaea var. *sylvestris* (Mill.) Lehr.	DD	Zebbouj	leaf	In, Na	cow, sheep	PW	9
Papaveraceae							
Papaver rhoeas L.	NE	Bennaamane	leaf	In, Or	cow	DD	5
Poaceae							
Hordeum vulgare L.	NE	Chaair	leaf	In, Or	cow, sheep	RT	8
Avena sativa L.	NE	Lkhortal	seed	De, Br	sheep	FC	5
Lolium rigidum Gaudin	NE	Zwan	seed	De, Or	goat, horse	RT	3
Oryza sativa L.	NE	Rouz	seed	De, To	goat, sheep	FC	6
Triticum durum Desf.	NE	Farina	seed	De, Or	cow	DD	2
Zea mays L.	LC	Draa	fruit	Cr, Br	cow, sheep	PW	6
Rhamnaceae							
Ziziphus jujuba Mill.	LC	Nbeg	leaf	In, Or	chickens, sheep	RT	5
Rubiaceae							
Rubia peregrina L.	NE	Fuwwa	root	De, Or	mule	DD	7
Rutaceae							
Citrus limon (L.) Osbeck	NE	Limon	leaf	In, Or	cow, sheep	SD	6
Ruta montana (L.) L.	LC	Lfjel	leaf	In, Or	goat	DD	11
Salicaceae							
Salix alba L.	LC	Oud lma	leaf	In, Na	cow	RT	33
Solanaceae							
Atropa belladonna L.	EN	Belladon	leaf	In, Or	goat, sheep	DD	6
Capsicum annuum L.	LC	Lflifla Ihran	fruit	Cr, Mo	horse	FC	7
Datura stramonium L.	NE	Chedcq Ejemel	leaf	De, Na	mule	DD	14
Solanum sodomaeum Dunal	NE	Maticha Dib	fruit	De, To	sheep	PW	6
Withania somnifera (L.) Dunal	NE	Ali Amlal	leaf	In, Or	sheep	DD	3
Urticaceae							
Urtica dioica L.	LC	Lhriga	aerial	De, Or	goat	RT	11
Xanthorrhoeaceae							
Aloe vera (L.) Burm. f.	NE	Sabra	aerial	Cr, Br	cow, mule	MI	6
Asphodelus microcarpus Salzm. et Viv.	LC	Lberwak	bulb	De, To	donkey, sheep	RT	25

Table 2 (continued)

Family and species	IS	Vernacular n.	Part	Prep.a.	Anim. tr.	Uses	Fc
Zingiberaceae							
Curcuma longa L.	NE	Lkharqoum	root	De, Or	cow, sheep	DD	6
Zingiber officinale Roscoe	DD	Skinjbir	root	De, Or	goat, sheep	RT	9

Abbreviations: IS = IUCN status, Prep.a. = Preparation and administration, Anim. tr. = animal treated, DD = digestive disorders, FC = fever and cough, MI = microbial infections, PW = pain and wounds, RT = respiratory troubles, SD = skin disorders
De = decoction; In = infusion; Cr = crushing; Ca = cataplasm; FC = frequency of citation; Fu = fumigation; Na = nasal; Or = oral; Mo = mouthwash; Br = brushing; To = topical; NE = not evaluated; DD = data deficient; LC = least concern; NT = near threatened; VU = vulnerable; EN = endangered

highest frequency of citations was calculated for *Rosmarinus officinalis* L. (FC = 70), *Trigonella foenum-graecum* L. (FC = 69), *Artemisia absinthium* L. (FC = 67), *Glycyrrhiza glabra* L. (FC = 57), and *Citrullus colocynthis* (L.) Schrad. (FC = 56). However, the low frequency of citations and their respective values were *Lolium rigidum* Gaudin, *Withania somnifera* (L.) Dunal (FC = 3 for each species), and *Triticum durum* Desf. (FC = 2).

Plant parts used – The local farmers of Oulad Hriz employed different parts of the medicinal plants for ethnoveterinary purposes, as shown in Table 2. The analysis of the data revealed that leaves were the most commonly used plant part (55%), followed by aerial parts (13.8%), seeds (13.2%), fruits (4.4%), flowers (4.3%), bulbs (4.1%), stem (4%), and roots (1.2%), in descending order of frequency of use.

Methods of preparation and routes of administration – Several methods of ethnoveterinary medicine preparations are employed by the local people of Oulad Hriz, the most preferred method of preparation was infusion (46.2%), followed by decoction (29.3%), cataplasm (14.2%), crushing (8.8%), and fumigation (1.5%). As shown in Table 2, the administration routes of medicinal plants were different and special. The majority of the herbal remedies (67.7%) were administered orally. A small percentage of the preparations were applied by brushing (12.2%), nasally (10.3%), or topically (4.3%), and a further 5.5% were administered locally in the mouth.

Threats to medicinal plants and associated indigenous knowledge – The 100 recorded plant species represented six IUCN Red List categories: not evaluated (NE) – 50 species; least concern (LC) – 41 species; data deficient (DD) – 4 species; near threatened (NT) – 2 species; endangered (EN) – 2 species; and vulnerable (VU) – 1 species. The dangers to medicinal plants may be classified into natural and human-induced influences. Agricultural expansion, firewood, overgrazing, chopping and burning plants to generate additional agricultural fields, lumber, charcoal, and building materials are the leading

risks to medicinal plants and indigenous knowledge, according to this study's respondents. Due to the adaptability of several medicinal plants, their extraction for medical reasons has increased the risk.

Dosage and side effects – The findings reveal that the surveyed population of Oulad Hriz indicated their utilization of medicinal plant remedies with different measurements. These measurements included 35% of plants consumed by spoonful, 27% by pinch, 21% by the handful, and 21% by cup. The absence of precise dosage awareness among the local community can potentially result in adverse health consequences, as many substances exhibit dose-dependent toxicity. Regarding the potential adverse reactions caused by plants employed in treating prevalent animal ailments, most participants lack knowledge regarding such effects. Respondents were asked a question to evaluate their understanding of the potential side effects caused by plants. The findings indicate that a large proportion of these respondents (87%) believe that plants do not have any adverse effects. However, 13% of respondents reported experiencing side effects associated with using plants to treat their livestock diseases. Notably, these participants also acknowledged the effectiveness of plants, highlighting their ability to enhance the quality of life and surpass the limitations of modern medicine.

Cattle treated – In the context of ethnoveterinary medicine in Oulad Hriz, six domestic animals were mentioned and listed for their significance to the indigenous inhabitants. These animals are sheep, cows, goats, horses, mules, and donkeys. Sheep were found to be the most commonly treated livestock, with 1122 citations indicating the importance placed on their health and well-being. Sixty-four medicinal plants treated sheep, highlighting the various treatments available. Cows were the second most commonly treated livestock, with 733 citations and 38 medicinal plants used. Goats were the third most widely treated livestock, with 482 citations and 23 medicinal plants used. Horses, mules, and donkeys were also identified as important livestock in Oulad Hriz, although they were treated less frequently than sheep, cows, and goats. Horses had 210 citations and eight medicinal plants, while mules had 135 citations and were treated with nine medicinal plants. Donkeys had 101 citations and were treated with five medicinal plants.

Ethnoveterinary disease categories – Farmers in Oulad Hriz have identified and employed 100 medicinal plants to address various animal health issues. These herbs have been used to treat livestock ailments and health conditions, including respiratory diseases, digestive disorders, skin conditions, and reproductive problems. There were 1753 use reports of these medicinal herbs, grouped into five disease categories following the International Categorization of Primary Care (ICPC) classification method (Bentsen 1986). The category with the most reports of usage was digestive diseases (599 use reports;

33 plant species). Microbial infections had the second citation value (406 use reports; 23 plant species), followed by pain and wounds (292 use reports; 14 plant species) and respiratory troubles (237 use reports; 15 plant species). The least often cited was related to skin disorders (219 use reports; 8 plant species).

DISCUSSION

The identification of 100 different medicinal plant species in a study region indicates a diverse floristical composition. This finding emphasizes the importance of biodiversity conservation, scientific research, traditional medicine practices, and community involvement to safeguard and benefit from the region's rich natural resources. The participation of males outnumbered that of females. The predominant representation of men in the research sample can be attributed to their presence in agricultural fields. The study relied heavily on male informants because female residents displayed reluctance to engage in conversations with unfamiliar men. Moreover, the local tribes' traditional customs and practices frequently discourage women from engaging in agricultural activities and livestock farming, restricting their involvement in the interviews (Chaachouay *et al.* 2023).

The results indicate that the study region has a significant diversity of ethnoveterinary medicinal plants, and the indigenous knowledge linked to commonly used species is abundant. These results are consistent with those observed in other regions of Morocco and other parts of the world (Alaoui and Laaribya 2017, Benkhnigue *et al.* 2022, Chaachouay *et al.* 2022*a*, *b*, El Khomsi *et al.* 2022, Hachi *et al.* 2015, McCorkle and Mathias-Mundy 1992, Orch *et al.* 2021, Ramana 2008). The comparison between the different studies conducted in the region shows a wide range of ethnomedicinal plants found in the area. The study has identified numerous plant species traditionally used for medicinal purposes, indicating the region's rich ethnobotanical knowledge. This diversity is likely due to the region's unique geographic location and varied climate, which supports various plant species with medicinal properties (Chaachouay *et al.* 2019*b*, *d*). The abundance of ethnomedicinal plants in the area highlights the importance of preserving this traditional knowledge and utilizing these plants sustainably.

Interestingly, almost half of the informants expressed an interest in protecting certain medicinal plants, even if they were uncommon in their area. These farmers recognize the importance of conserving these plants and are taking active steps to do so, such as cultivating the plants in their home gardens and offering advice to others in the community. On the other hand, the remaining farmers are more focused on their immediate needs and are not making any significant efforts to conserve the plants. This attitude could have

negative long-term consequences for the availability of these plants and their potential use as therapeutic agents. In general, the conservation efforts of the interested farmers are encouraging, and their actions could serve as a model for others in the community. However, it is crucial to raise awareness about the importance of conservation and to encourage more people to take action to protect these valuable plant species (Chaachouay *et al.* 2019*c*).

The Lamiaceae family was the informants' most commonly mentioned plant family, with Apiaceae, Asteraceae, and Fabaceae following closely behind. The high frequency of these families in the Berrechid flora may have contributed to their prominence in the ethnoveterinary practices of the indigenous populations of Oulad Hriz. Additionally, these families have a long-standing history of traditional uses for medicinal purposes, which could further explain their prevalence in the reported remedies. Therefore, the knowledge and use of these plant families have been passed down through generations of the local population and have become deeply ingrained in their cultural practices. The current research is consistent with the findings given in Morocco and throughout the globe (Alaoui and Laaribya 2017, Benkhnigue *et al.* 2022, Benlarbi *et al.* 2023, Chaachouay *et al.* 2020*a*, *b*, *c*, Dilshad *et al.* 2010, Hachi *et al.* 2015, Maphosa *et al.* 2010, McGaw and Eloff 2010, Murad *et al.* 2014). Furthermore, the extensive applications of species from these families might be associated with the effectiveness of bioactive components against cattle diseases.

The study found eight different plant parts were used as restorative materials in herbal remedies preparation. The leaves and aerial parts are the most often gathered plant components for different therapeutic recipes for cattle. The healers' preference for utilizing leaves in the production of herbal remedies is likely due to their year-round availability and ease of collection, storage, processing, and handling. Leaves and seeds are the regenerative parts of the plant, making them ideal for harvesting as it does not cause the plant to wilt (Briggs and Morgan 2011). The prevailing notion is that leaves contain phytochemicals, crude medicines, and other compounds that are more easily extractable and have potential benefits in phytotherapy. The utilization of specific plant components implies that they have the most therapeutic solid characteristics, but this has to be cross-checked by biochemical analysis and pharmaceutical screening (Cieslak *et al.* 2013). Studies on ethnobotany conducted in Morocco and other countries have found that the leaves of medicinal plants are often employed to treat illnesses in humans and cattle (Ahmad *et al.* 2014, Andrade *et al.* 2017, Benkhnigue *et al.* 2022, Chaachouay *et al.* 2019*a*, *b*, *d*, 2021*b*, *c*, *d*, Chaachouay and Zidane 2021, Dilshad *et al.* 2010, Ghasemi *et al.* 2013, Majeed *et al.* 2020, McCorkle and Mathias-Mundy 1992, Orch *et al.* 2021, Van der Merwe *et al.* 2001).

The local farmers of Oulad Hriz employ medicinal herbs based on their availability in the area's vegetation, agricultural fields, and home gardens. Local usage patterns are also influenced by cultural traditions, experiences, and sickness. In our study, we identified 100 plant species used by local farmers of Oulad Hriz to treat cattle diseases. The reported plant species are native to the Oulad Hriz region and have been commonly used for treating livestock afflicted with prevalent diseases. Due to their medicinal properties, local communities have traditionally employed these plants as natural remedies. These plants are native to the Chaouia region and have been utilized by indigenous societies for generations. Although these medicinal plants have been known for centuries, they continue to be prevalent in the study area. Local farmers in Oulad Hriz possess extensive knowledge of their properties and uses. The significance of these findings lies in the fact that they establish a crucial foundation for future research into the therapeutic compounds present in these plants, potentially leading to the discovery of novel drugs. As such, this study is an essential contribution to ethnoveterinary medicine and highlights the importance of preserving traditional knowledge and practices for developing new herbal therapies.

The investigation findings indicated that the elevated frequency of citations for sheep, cows, and goats might be attributed to their widespread presence as livestock in Oulad Hriz and their increased likelihood of needing medical attention for different health issues. Nevertheless, the utilization of medicinal plants to address the health concerns of all six domestic animals underscores the significance of ethnoveterinary medicine in safeguarding the well-being and vitality of livestock within this area. The use of medicinal herbs to address animal health issues in Oulad Hriz highlights the importance of ethnoveterinary medicine in this region. The knowledge and benefit of these medicinal plants have been passed down through generations and continue to be an essential aspect of animal healthcare in this community.

A total of 100 plant species were found to cure five distinct ailments. Among these, the most often treated conditions were digestive diseases. The survey discovered that local farmers in Oulad Hriz bought herbal remedies primarily for digestive ailments. Common digestive diseases may be caused by various factors, including a lack of clear water, the stress in cattle, a shortage of grass owing to periodic droughts in the Oulad Hriz, and poisonous compounds prevalent in fields. The preponderance of digestive problems is consistent with several ethnomedicinal investigations undertaken in other areas (Benkhnigue *et al.* 2023, Chaachouay and Zidane 2019, Chaachouay *et al.* 2020*c*, 2024, Ghasemi *et al.* 2013). This statement suggests that herbal pharmaceutical medicines primarily treat moderate and chronic disorders while implying that traditional or alternative treatments may have broader appli-

cations. It is important to note that the efficacy and safety of traditional or alternative medicines may vary widely. These treatments may carry certain risks, such as the potential for adverse reactions or interactions with other medications (Edwards and Aronson 2000). Additionally, using traditional or alternative medicines may only sometimes be supported by rigorous scientific evidence, making it difficult to establish their therapeutic value. Despite these challenges, traditional or alternative drugs remain essential in many cultures and communities worldwide (Karunamoorthi *et al.* 2013). As such, it is important to promote responsible and evidence-based use of these treatments while continuing to develop and improve conventional pharmaceutical medicines to meet the needs of patients with a wide range of health conditions.

The research investigation found that many plants are obtained from the wild to treat various livestock ailments, leading to the depletion of wild plant populations and causing environmental degradation and deforestation. The ready availability and easy accessibility of these plants near homesteads are the main reasons behind their overharvesting from the wild. The plants are often used for various purposes, such as fuelwood, fodder, and agricultural implements (Chaachouay *et al.* 2023). As a result, their populations have declined significantly in the region. The overharvesting of these medicinal plant species is putting immense pressure on their growth and causing environmental degradation (Cuttelod *et al.* 2009, Singh 2000). This underscores the importance of conservation efforts to protect these species from further depletion. These species urgently need an evaluation of their current population state and reclassification by the IUCN (Butchart and Bird 2010, Locke and Dearden 2005). Culturing critically endangered medicinal plants on degraded or abandoned lands could reduce the pressure on wild populations. This approach also provides opportunities for local farmers to earn income from cultivating and selling medicinal plants. However, it is essential to ensure that cultivation practices do not harm the environment or further contribute to degradation. In addition to cultivation, urgent conservation efforts are needed to protect the rapidly dwindling populations of multifunctional medicinal plant species. This could include measures such as establishing protected areas, implementing sustainable harvesting practices, and promoting awareness and education among local communities about the importance of conservation (Bennett and Dearden 2014, Ferse *et al.* 2010). The International Union for Conservation of Nature (IUCN) could play a critical role in this effort by reclassifying critically endangered medicinal plant species and advocating for their protection.

The method of preparing medicinal drugs often differs from person to person in many cases. Different traditional veterinary healers prepared the same plant material for the same disease (Dilshad *et al.* 2010, Murad *et al.* 2014). The findings indicated that the vast majority of medicines were de-

rived from infusions. Infusion is the most effective way for active ingredients to mitigate or eliminate the harmful effects of certain substances. Therefore, the most commonly used traditional medicine technique is an infusion that combines plant components with water, tea, or soup (Benkhnigue *et al.* 2022, Chaachouay *et al.* 2019*a*, *b*, *d*, 2020*a*, *b*, Faruque *et al.* 2019, Murad *et al.* 2014, Orch *et al.* 2021). According to the findings of the study, the majority of the ethnoveterinary treatments developed by indigenous farmers are intended for oral administration. However, the high prevalence of internal illnesses in the study area may present challenges for administering oral medication. Despite this, the oral route is still considered the preferred delivery method for cure worldwide (Benkhnigue *et al.* 2022, Chaachouay and Zidane 2022, Chaachouay *et al.* 2021*b*, *c*, Douiri *et al.* 2007, Hachi *et al.* 2015, Orch *et al.* 2021). The preference for oral administration in ethnoveterinary medicine may be influenced by factors such as the ease of administration and the ability to control dosage accurately (McGaw and Eloff 2010, Van der Merwe *et al.* 2001). However, it is essential to note that using oral medication in animals may also pose certain risks, such as the potential for accidental overdose or adverse effects on the digestive system (Wen *et al.* 2015). As such, careful consideration must be given to the choice of medication and the appropriate dosing regimen for each case. Overall, the preference for oral administration in ethnoveterinary medicine underscores the importance of developing effective and safe treatments that can be easily administered in resource-limited settings.

In interviews conducted with ethnoveterinary medicine practitioners, the discussion also touched upon the potentially harmful effects of medicinal plants. In contrast to conventional veterinary medicine, which often follows standardized dosing guidelines for medications, herbal treatments in ethnoveterinary medicine lack established dosing protocols. This situation is compounded by limited research in the field, necessitating practitioners to gain more knowledge about the potential adverse effects associated with these treatments. The absence of standardization poses a risk of under-dosing and over-dosing, underscoring the importance for ethnoveterinary practitioners to proceed cautiously. Farmers practicing ethnoveterinary medicine typically determine the dosage of medicinal plants based on various factors, including the concentration of the transformed plant, the age of the animal being treated, and the specific ailment being addressed. These factors collectively influence the treatment's effectiveness and the likelihood of harmful side effects. For instance, certain plant species such as *Myrtus communis* L., *Pistacia lentiscus* L., *Eucalyptus globulus* Labill., *Trigonella foenum-graecum* L., *Origanum compactum* Benth., and *Nerium oleander* L. are prescribed in different dosages to treat similar conditions with a specific component.

Indeed, the ancient veterinary healers needed help accurately estimating dosages and establishing standardized measures for medicinal herbs. These challenges were primarily due to the need for more scientific research and systematic documentation during those times. As a result, there was a considerable risk of administering incorrect dosages or using ineffective remedies, potentially harming animals. To address these risks, practitioners of ethnoveterinary medicine today must exercise caution and conduct thorough research before utilizing medicinal plants. It is crucial to gather information on the potential effects, dosage guidelines, and safety profiles of specific plants for different animal species. This research can be based on traditional knowledge but should also incorporate scientific studies and evidence-based practices.

Moreover, it is essential to emphasize the need for further investigations into ethnoveterinary medicine. Scientific research plays a crucial role in understanding medicinal plants' efficacy, safety, and mechanisms of action. By conducting rigorous studies, researchers can fill knowledge gaps, validate traditional practices, and identify potential risks and limitations associated with specific remedies. In addition to research, establishing regulations and guidelines is vital to ensure medicinal plants' safe and effective use in animal healthcare. Regulatory frameworks can standardize dosages, quality control, labelling, and manufacturing practices of herbal products for veterinary use. Such measures can contribute to ethnoveterinary medicine's overall safety, efficacy, and quality assurance.

Collaboration between traditional healers, veterinarians, researchers, and regulatory authorities is essential in bridging the gap between traditional knowledge and modern science. This collaboration can facilitate the exchange of information, promote mutual learning, and enhance the integration of ethnoveterinary medicine into conventional veterinary practices. Consequently, the ancient veterinary healers faced challenges and knowledge gaps concerning accurate dosage estimation and standardized measures for medicinal herbs. To mitigate these risks, ethnoveterinary medicine practitioners must exercise caution and conduct thorough research on the potential effects of any medicinal plants they intend to use. Furthermore, pursuing further investigations and establishing regulations to ensure medicinal plants' safe and effective utilization in animal healthcare is essential.

CONCLUSIONS

The current research established that the study area is home to a sizable collection of ethnoveterinary medicinal plants. Local farmers in Oulad Hriz have extensive expertise in curing their livestock using these plant species. Despite modern healthcare facilities, numerous communities still rely on alternative medicine, highlighting the significance of traditional remedies based on

medicinal plants. Herbal medicines are considered practical and sustainable for rural agricultural communities due to their ease of availability, uncomplicated preparation methods, and ease of animal administration. The outcomes of this study are expected to encourage additional ethnoveterinary research aimed at developing cow disease control approaches in the study area. This includes pharmacological screening, chemical analysis for bioactive substances, and possible formulation as conventional medicinal formulations to treat various diseases. It is necessary to evaluate the effectiveness of preparations, procedures, and practices to select suitable plants for use in cattle development plans. As a result, local farmers should be made aware of the importance of biodiversity and the long-term usage of species identified as sources of ethnozoological medicine in the research region. Traditional ethnoveterinary knowledge must be conserved and disseminated to propagate and maintain it.

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APPENDIX

Questionnaire used for collection of ethno-veterinary data

Date: Survey number:

Part 1. Socio-demographic data of the informant

1. Gender:
2. Age:
3. Profession:
4. Education level:
5. Family situation:
6. Tribe:
7. How long you are living in the tribe?
8. How much livestock do you have?…..… Sheep ……. Goats …….. Cows ..…… Horses ……. Mules ..…. Donkeys ...….. Other
9. How many acres do you need for livestock?
10. Do you have a veterinarian for your livestock?
11. How many years of experience do you need to be a livestock keeper?
12. What are the problems faced by livestock farming in the Middle Atlas?
13. Informants consent for the participation in the study:

I …… hereby give my full consent and consciousness to participate in this study and declare that to the best of my knowledge the information that I have provided is true, accurate, and complete.
Signature / Thumb impression of informant:
Date:

Part 2. Ethno-veterinary information

Vernacular name:
Scientific name:
Plant type:
Source of plant:
Harvesting technique:
Plant part used:
The plant (s) associated:
Form of employment:
Method of preparation:
Mode of administration:
Dose used:
Conservation method:
Duration of the treatment:
Toxicity:
Expiration date:
Animal (s) treated:
Disease categories:
Digestive disorders:
Microbial infections:
Pain and wounds:
Respiratory troubles:
Skin disorders:
Other:

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INFLUENCE OF SPOROPHYTE EMERGENCE ON PHYTOCHEMICAL CONSTITUENTS AND ANTIMICROBIAL ACTIVITY OF *HYOPHILA INVOLUTA* (POTTIACEAE) – A BRYOPHYTE SPECIES

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Hyophila involuta (Hook) Jaeg., a sample collected from a natural population in the Biological Garden, Obafemi Awolowo University, Nigeria, was sorted into non-sporophytic and sporophytic gametophytes. This was with a view to investigating the possible influence of sporophyte emergence on the bioactive constituents and the antimicrobial potentials of the moss plant. Aqueous extracts of each of the non-sporophytic and the sporophytic gametophyte samples were prepared, and each was subjected to qualitative and quantitative phytochemical screening, gas chromatography-mass spectrometry (GC-MS) analyses, and antimicrobial potentials tests on selected bacteria and fungi following standard procedures. The results showed both extracts testing positive for alkaloids, cardiac glycosides, saponins, and steroids. Quantitatively, alkaloids and cardiac glycosides concentrations were higher in the non-sporophytic than in the sporophytic gametophytes. Nevertheless, the saponins content was higher in sporophytic gametophytes. GC-MS analyses revealed 40 and 46 bioactive compounds in the non-sporophytic and the sporophytic gametophyte samples respectively. The most prominent compound was 1, 13-tetradecadiene (13.62%) in the non-sporophytic gametophyte but cycloheptasiloxane tetradecamethyl- (13.78%) in the sporophytic gametophyte. Furthermore, the sporophytic gametophyte extract inhibited the growth of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Candida pseudotropicalis* while the non-sporophytic gametophyte extract only inhibited the growth of *P. aeruginosa* at a minimum inhibitory concentration of 40 mg/mL except for *C. pseudotropicalis* (20 mg/mL). This study therefore provided insight for investigating the medicinal values of bryophytes and concluded that the emergence of sporophytes on gametophytes of a bryophyte species can vary significantly the synthesis of its biologically active compounds and its antimicrobial activity.

Key words: antimicrobial, bryophyte, gametophyte, GC-MS, phytochemicals, sporophyte

INTRODUCTION

Plants have been noted from ancient times as important sources of herbal medicines and therapeutic phytochemical agents among other uses (Das *et al*.

2013). These phytochemical agents in plants are natural bioactive compounds that play a key role in plants' defence system and are well known for their unambiguous physiological action on the human body (Rashid *et al.* 2013). The economic importance of medicinal plants is recognised in all countries over the world, and the history of their use for medicinal purposes is probably as old as the history of mankind (Abubakar *et al*. 2016).

The systemic screening of plant extracts for antimicrobial activity is a continuous effort to find new antimicrobial compounds to halt antimicrobial resistance. Many researchers have focused on the investigation of plant extracts, essential oils, secondary metabolite isolates, and newly synthesised molecules for potent sources of antimicrobial agents (Mabona *et al*. 2013, Nazzaro *et al*. 2013, Runyoro *et al*. 2006). Disease-causing microorganisms negatively affect the economy of agricultural sectors and the health of man (Sati and Joshi 2011). Increasing public demand for drugs against emerging diseases, the development of new antimicrobial agents, and the attempts to combat microbial resistance have motivated scientists to look for new natural sources with potential pharmaceutical capabilities (Balouiri *et al*. 2016).

In bryophytes, various phytochemicals they contain have also attracted the attention of plant scientists and pharmaceutical industries. For example, the volatile phenol compounds extracted from bryophytes act as antioxidants, which can quench reactive free radicals, thus preventing the oxidation of other molecules results in the prevention of degenerative diseases as well as aging retardation (Chauhan *et al*. 2014). Furthermore, alkaloids, flavonoids, bioflavonoids, and isoflavonoids from bryophyte extracts were reported to possess effective antibacterial and antifungal activities against pathogenic microorganisms (Nantachit *et al*. 2010, Neto *et al*. 2011, Yan *et al*. 2008).

Some other species of bryophytes are also known for their ecological impacts (Shaw and Renzaglia 2004), and some have been reported for their beneficial secondary metabolites, antimicrobial potentials, and antioxidant potentials (Asakawa 2007, Singh *et al*. 2016). Other constituents in the species from this group of plants such as sesquiterpenoids, acetophenones, stilbenes, and essential oils have been found to exhibit fungicidal or fungistatic properties (Dey and De 2011). High amounts of phenols, steroids, glycosides, and flavonoids have also been reported in this group of land plants (Oyedapo *et al*. 2015).

Several factors have been reported to have significantly different impacts on the phytochemical constituents in plants (Diljkan *et al*. 2022, Iloki-Assanga *et al*. 2015). The life cycle of bryophytes is characterised by an alternation of generations between two different stages; the gametophyte and the sporophyte stages (Dziwak *et al*. 2022), however, the existing literature offered no reports on the effects associated with ~~this~~ life switches on bioactive constituents of bryophytes. Hence, this study was carried out to investigate the phytochemical constituents' status and the antimicrobial potentials of non-

sporophytic and sporophytic gametophyte extracts from *Hyophila involuta,* a bryophyte species to fill this knowledge gap.

MATERIAL AND METHODS

Plant materials – The plant material for this study *Hyophila involuta* (Hook) Jaeg. (Fig. 1), was collected avoiding weeds from a natural habitat in the Biological Garden of the Obafemi Awolowo University Campus, Ile-Ife, Nigeria, Latitude 7° 31′ 31″ N and Longitude 4° 31′ 28″ E. The collected sample was sorted into the non-sporophytic gametophytes and the sporophytic gametophytes, carefully washed in different bowls of distilled water without damaging the fragile parts and then air dried at the ambient temperature in the laboratory.

Test organisms – The test organisms for the study included Gram-negative bacteria [*Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 10145)]; Gram-positive bacteria [*Bacillus subtilis* (NCTC 8236) and *Staphylococcus aureus* (NCTC 6571)] and fungi [A*spergillus niger* (mold), and *Candida pseudotropicalis* (yeast)]. The test organisms were obtained from the Department of Pharmaceutics (Microbiology Laboratory), Faculty of Pharmacy, Obafemi Awolowo University, Ile-Ife, Nigeria.

Preparation of *Hyophila involuta* extracts – Extracts of the sporophytic and the non-sporophytic gametophyte *Hyophila involuta* were procured by soaking the air-dried milled samples (400 g each) in distilled water separately at room temperature for 72 h. The resulting suspensions were filtered using

Fig. 1. H. involuta in the natural habitat at the Biological Garden, Obafemi Awolowo University, Ile-Ife, Nigeria

Whatman No. 1 filter paper and the filtrates were evaporated to dryness on a rotatory vacuum evaporator at 40 °C to obtain the respective crude extracts.

Hyophila involuta crude extracts yield estimation – The crude extract yielded from each sample was weighed and the percentage yield was calculated using the formula:

$$\text{Percentage yield} = \frac{\text{(Weight of the extract)}}{\text{(Weight of the soaked air-dried moss sample)}} \times 100$$

Phytochemical screening of the aqueous extracts of Hyophila involuta

Qualitative phytochemical analyses – The obtained extracts were screened for the presence or absence of alkaloids, anthraquinones, cardiac glycosides, flavonoids, phlobatanins, saponins, steroids, tannins, triterpenes, and xanthoproteins following standard methods reported in the literature (Oyedapo *et al.* 1999, Sofowora 2008, Trease and Evans 2002).

Quantitative phytochemical analyses

Determination of alkaloids – The alkaloidal contents of the crude extracts were determined by adapting the method of Mythili *et al*. (2014) using dimethyl sulphoxide (DMSO), 2 N HCl, bromocresol green solution, phosphate buffer solution (pH 4.7), and chloroform. Extract (5.0 mg) was dissolved in 5.0 mL dimethyl sulphoxide (DMSO). To the solution was added 5.0 mL of 2 N HCl, filtered and the filtrate transferred to a separating funnel. About 5.0 mL of bromocresol green solution and 5 mL phosphate buffer solution (pH 4.7) were added to the filtrate. The mixture was shaken, and the complex formed was extracted separately with 1.0, 2.0, 3.0, and 4.0 mL chloroform by vigorous shaking. The extract was collected in a 10 mL volumetric flask and diluted to volume with chloroform. A set of reference standard solutions of atropine (20, 40, 60, 80, and 100 µg/mL) were also prepared in the same manner as described above. The absorbance for the tests and standard solutions was measured against the reagent blank solution at 470 nm. The concentrations of alkaloids in the extracts were extrapolated from the standard atropine calibration curve and expressed as mg/g extract atropine equivalent.

Determination of cardiac glycosides – The cardiac glycoside content of *Hyophila involuta* extracts was estimated following the method of El-Olemy *et al*. (1994) as reported by Ajiboye *et al*. (2013). One g (1.0 g) of each extract was soaked in 10 mL of 70% ethanol for 2 h and filtered. The filtrate was purified using lead acetate and Na_2HPO_4 solution and then filtered through Whatman No. 1 filter paper before the addition of freshly prepared Buljet's reagent. A blank solution was prepared simultaneously using distilled water. The absorbance of both the test and the blank (distilled water and Buljet's reagent) was taken at 495 nm.

The difference between the absorbance of the test and blank samples gives the absorbance and is proportional to the concentration of the cardiac glycosides.

Determination of saponins – The total saponin content of the *H. involuta* crude extracts was estimated using vanillin-sulphuric acid colorimetric reaction methods of Makkar *et al.* (2007) as reported by Akinseye *et al*. (2017). Extracts solution was prepared by dissolving 0.1 g of a crude extract in 20 mL of 80% aqueous methanol. An aliquot of 50 μL of extract solution was put into a test tube and 250 μL of vanillin reagent (800 mg of vanillin in 10 mL of 99.5% ethanol) was added, followed by the addition of 2.5 mL of 72% tetraoxosulphate (VI) acid. The solution was well mixed, and the tube was transferred to a water bath adjusted at 60 °C for 10 min. The solution was cooled in ice-cold water and the absorbance was read at 544 nm against the reagent blank. A stock solution (0.5 mg/mL) of standard saponin from Quilaja bark ([8047-15-2] EC No. 232-462-6) was also prepared using 80% aqueous methanol. Various dilutions of saponin (50, 100, 200, 300, 400, and 500 μg/mL) were prepared, and treated in the same manner as described above and a standard curve was plotted. The amount of saponins in the crude extracts was calculated by extrapolation from the standard saponin curve and expressed as mg of saponin equivalent per gram of dry weight (mg SE/g) of extracts.

Gas Chromatography-Mass Spectrometry (GC-MS) analyses – The crude extracts from the non-sporophytic and the sporophytic gametophytes of *H. involuta* were analysed via the Gas Chromatography-Mass Spectrometry (GC-MS) technique at the Chemical Engineering Department, University of Ilorin, Ilorin, Nigeria, on Agilent 19091S Gas Chromatograph (GC) interfaced to a Mass Spectrometer 433HP-5MS instrument following the method described by Isa *et al*. (2021). Interpretations of the spectra of the components on the mass spectrum for the GC-MS were done using the database of the National Institute Standard and Technology 11 Library (NIST11.L).

Antibacterial and antifungal activity tests

Screening for antimicrobial activity – Screening of the *H. involuta* extracts for antimicrobial activities was carried out by adapting the method of Balouiri *et al*. (2016). Duplicate rectangular filter paper strips were aseptically loaded with 20 μL of 40 mg/mL of each test extract and allowed to dry in the laminar airflow cabinet for 30 min. The impregnated filter papers were then overlaid with molten nutrient agar which had been seeded with the test organisms (bacteria) at 45 °C. For the fungi, Sarbouraud dextrose agar was used as the fungiological medium. The papers testing antibacterial activities were incubated at 37 °C, while those for antifungal screening were incubated at 25 °C in humidified Petri dishes for 24 h and 48 h, respectively. After the incubation time, the strips were sprayed with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl

tetrazolium bromide and incubated for another 2 h. The presence of clear bands around the samples on the filter paper against a purple background indicated growth inhibition.

Determination of minimum inhibitory and minimum bactericidal/fungicidal concentrations of *Hyophila involuta* aqueous extracts – Minimum inhibitory concentrations (MICs) of the moss extracts were determined using the micro-broth dilution standard method of the Clinical and Laboratory Standards Institute (2012), in a 96-well micro-plate. A volume of 100 μL of double-strength Mueller Hinton broth was dispensed into each well of the plate, thereafter, 100 μL of extract (80 mg/mL) was added into the first well to achieve a concentration of 40 mg/mL. Serial dilution was carried out by removing 100 μL of the reaction medium from the first well into the second well and so on till the tenth well which had a final concentration of 0.08 mg/mL. A volume of 100 μL was then withdrawn from the tenth well and discarded. The eleventh well (which had no test sample) served as the negative control while the positive control (twelfth well) contained 2.0 mg/mL Ciprofloxacin. This was done for all the rows of the microplate. A suspension of each test organism (5.0 μL) containing approximately 10^5 cfu/mL was then added to the respective wells leaving out the last row to serve as the uninoculated control. The experiments were carried out in duplicate and the plates were incubated at 37 °C for 24 h. For the fungi, the medium used was Sarbouraud dextrose medium and the incubation temperature was 25 °C. After the incubation period, a drop of tetrazolium salt was added to each well and inspected for a colour change to indicate the presence of viable cells in the wells. The minimum concentration that inhibited the growth of a test organism was recorded as the MIC of the test sample against the organism. However, for the minimum bactericidal/fungicidal concentration tests, the experiments from the MICs determination above were sub-cultured after the incubation period before the addition of tetrazolium salt on oven-dried sterile duplicate Mueller Hinton agar plates using a multi-inoculator. The plates were incubated at 37 °C for 72 h and observed for growth. The same procedure was repeated for the fungi using Sarbouraud dextrose agar as the recovery medium and 25 °C as the incubation temperature. The wells with the minimum concentration from which no colony or fungal growth was recoverable were taken as indicative of the minimum bactericidal and minimum fungicidal concentrations.

RESULTS

Crude extracts yield – The yield of the crude extracts obtained from the non-sporophytic and the sporophytic gametophyte *H. involuta* are presented in Table 1. The crude extract yield from the non-sporophytic sample was higher (17.84 g) than the yield from the sporophytic sample (13.16 g). Given

Table 1
Aqueous extract yield from non-sporophytic and sporophytic *H. involuta*

H. involuta (400 g)	Crude extract yield (g)	Percentage yield (%)
Non-sporophytic gametophyte	17.84	4.46
Sporophytic gametophyte	13.16	3.29

4.46% and 3.29% yields for the non-sporophytic and the sporophytic samples, respectively.

Qualitative phytochemical screening

Table 2 shows the summary of the results of the phytoconstituents screening of the non-sporophytic and sporophytic gametophyte *H. involuta* aqueous extracts. Both the non-sporophytic and the sporophytic gametophyte extracts tested positive for the presence of alkaloids, cardiac glycosides, saponins, and steroids. None of the extracts tested positive for the presence of anthraquinones, flavonoids, phlobatanins, tannins, triterpenes, and xanthoproteins.

Table 2
Phytochemical constituents of aqueous crude extracts from the non-sporophytic and the sporophytic gametophyte *H. involuta*

Phytochemicals	Crude extracts	
	Nsp	Sp
Alkaloids:		
Mayer's reagent	+	–
Picric acid	–	–
Wagner's reagent	+	+
Anthraquinones	–	–
Cardiac glycosides	+	+
Flavonoids	–	–
Phlobatanins	–	–
Saponins	+	+
Steroids	+	+
Tannins	–	–
Triterpenes	–	–
Xanthoproteins	–	–

Key: + = present; – = not detected;
Nsp = non-sporophytic gametophyte;
Sp = sporophytic gametophyte

Quantitative phytochemical analyses

The results of alkaloids, cardiac glycosides, and saponins contents estimated in the non-sporophytic and the sporophytic gametophyte *H. involuta* aqueous extracts are presented in Table 3. The non-sporophytic gametophyte contained a higher concentration of alkaloid (37.82 ± 0.001 mg AE/g) than the sporophytic gametophyte (21.45 ± 0.000 mg AE/g). Higher cardiac glycoside content was also recorded in the non-sporophytic gametophyte (25.59 ± 0.051 mg/g) than the sporophytic gametophyte (4.56 ± 0.034 mg/g). Conversely, higher saponins content (2.56 ± 0.019 mg SE/g) was recorded in *H. involuta* sporophytic gametophyte than the sporophytic gametophyte sample (1.62 ± 0.023 mg SE/g).

Table 3
Concentrations of alkaloids, cardiac glycosides, and saponins in *H. involuta*

Crude extracts	Alkaloids (mg AE/g)	Cardiac glycosides (mg/g)	Saponins (mg SE/g)
Nsp	37.82 ± 0.001[b]	25.59 ± 0.051[b]	1.62 ± 0.023[a]
Sp	21.45 ± 0.000[a]	4.56 ± 0.034[a]	2.56 ± 0.019[b]

Note: values are mean ± standard error (n = 3); means in a column with different superscripts are significantly different (P ≤ 0.05)
Key: Nsp = non-sporophytic gametophyte; Sp = sporophytic gametophyte

Gas Chromatography-Mass Spectrometry (GC-MS) analyses of the bioactive compounds in non-sporophytic and sporophytic gametophyte *H. involuta* extracts – The GC-MS analyses revealed 40 bioactive compounds present in the non-sporophytic *H. involuta* aqueous extract (Table 4) with cycloheptasiloxane, tetradecamethyl- (9.95%), n-(1-cyano-3-methyl-but-2-enyl)-acetamide (7.86%), cyclododecene, (e)- (6.79%), n-heptadecanol-1 (7.79%) and 1,13-tetradecadiene (13.62%) as the most prominent compounds. On the other hand, the sporophytic gametophyte *H. involuta* aqueous extract revealed the presence of 46 bioactive compounds (Table 5) with the following as prominent compounds - 2(3h)-furanone, 5-ethyl-dihydro- (5.71%), cyclohexasiloxane, dodecamethyl- (7.01%), 2(3h)-furanone, dihydro-5-pentyl- (9.55%), cycloheptasiloxane, tetradecamethyl- (13.78%) and cyclooctasiloxane, hexadecamethyl- (7.71%). Figures 2 and 3 showed the chromatograms of the extracts from the non-sporophytic gametophyte and the sporophytic gametophyte *H. involute*, respectively. Cyclohexasiloxane dodecamethyl-, cycloheptasiloxane tetradecamethyl-, cyclooctasiloxane hexadecamethyl- and cyclononasiloxane octadecamethyl- were identified to be common in the two extracts. However, higher concentrations of these compounds were recorded in the extract from

Table 4
Bioactive compounds in the aqueous extract of the non-sporophytic gametophyte of *H. involuta* using GC-MS

S/n	RT (min)	Area (%)	Identified compounds
1	6.782	0.98	1,3-Oxathiolane, 2-ethyl-2-methyl-
2	8.214	0.59	4-Pyridinecarbonitrile
3	11.092	0.98	2(3H)-Furanone, dihydro-5-methyl-
4	11.423	0.45	(2,2,2-Trifluoroethoxy)ethene
5	13.125	0.81	1,3a-Epoxy(3aH)indene, 1,4,5,6,7,7a-hexahydro-1,3,4,4,7a-pentamethyl
6	13.369	0.41	4-Bromo-3-chloroacetanilide
7	15.126	1.63	2H-Pyran-2-one, tetrahydro-

Table 4 (continued)

S/n	RT (min)	Area (%)	Identified compounds
8	15.345	0.46	4H-Furo[3,2-b]pyrrole-5-carboxylic acid, 4-(2-oxopropyl)-
9	15.489	0.53	2 (3H)-Furanone, 5-butyl-dihydro-4-methyl-
10	16.772	0.52	Silacyclohexan-4-one, 1,1-dimethyl
11	17.247	0.46	Phytol
12	18.536	0.67	Morpholine, 2,6-dimethyl-
13	19.643	0.57	2,6-Difluoroaniline
14	21.006	5.49	Decanal
15	22.064	0.41	1,3-Dioxolane, 4-pentyl-5-propyl-2,2-bis(trifluoromethyl)-, cis-
16	24.115	2.43	2-Nonanone
17	24.822	0.86	3-Cyclohexen-1-ol, 4-methyl-1-(1-methylethyl)-, (R)-
18	25.423	4.24	Cyclohexasiloxane, dodecamethyl-
19	26.380	0.42	2,7-Octadien-4-ol, 2-methyl-6-methylene-, (S)-
20	27.331	1.14	2-Octadecyl-propane-1,3-diol
21	27.881	1.75	2-Decen-1-ol, (E)-
22	30.596	0.73	2-Decanone
23	30.827	9.95	Cycloheptasiloxane, tetradecamethyl-
24	33.473	1.51	Fumaric acid, pent-4-en-2-yl tridecyl ester
25	35.369	0.80	Propane, 3,3-dichloro-1,1,1,2,2-pentafluoro-
26	35.769	4.93	Cyclooctasiloxane, hexadecamethyl-
27	35.857	2.28	Carbonic acid, 2,2,2-trichloroethyl undec-10-enyl ester
28	36.038	2.69	Fumaric acid, 2-formyl phenyl hexyl ester
29	36.570	7.86	N-(1-Cyano-3-methyl-but-2-enyl)-acetamide
30	36.807	1.74	2,6,10-Dodecatrienoic acid, 3,7,11-trimethyl-, ethyl ester, (Z,Z)-
31	37.308	0.66	1,2-Dihydropyrido(3,2,1-kl)phenothiazin-3-one
32	37.739	1.49	Cyclononasiloxane, octadecamethyl-
33	37.946	1.29	Acetamide, 2-(4-methyl piperazine-1-yl)-N-(2-cyclopropanoyl-benzofuran-3-yl)-
34	38.002	6.79	Cyclododecene, (E)-
35	38.171	7.79	n-Heptadecanol-1
36	38.352	0.61	cis-10-Heptadecenoic acid, methyl ester
37	38.509	2.15	Methyl palmitate
38	39.378	13.62	1,13-Tetradecadiene
39	39.510	4.33	Cyclotetradecane
40	39.610	2.96	9-Octadecenoic acid, methyl ester, (E)-

Key: RT = retention time

the sporophytic *H. involuta* (Fig. 4). Cycloheptasiloxane tetradecamethyl- was the most abundant of the bioactive compounds identified in both the non-sporophytic and the sporophytic *H. involuta* extracts.

Antibacterial and antifungal activities of the aqueous extracts of H. involuta

The results of the preliminary antimicrobial activities of the studied moss extracts are shown in Table 6. It was recorded that sporophytic gameto-

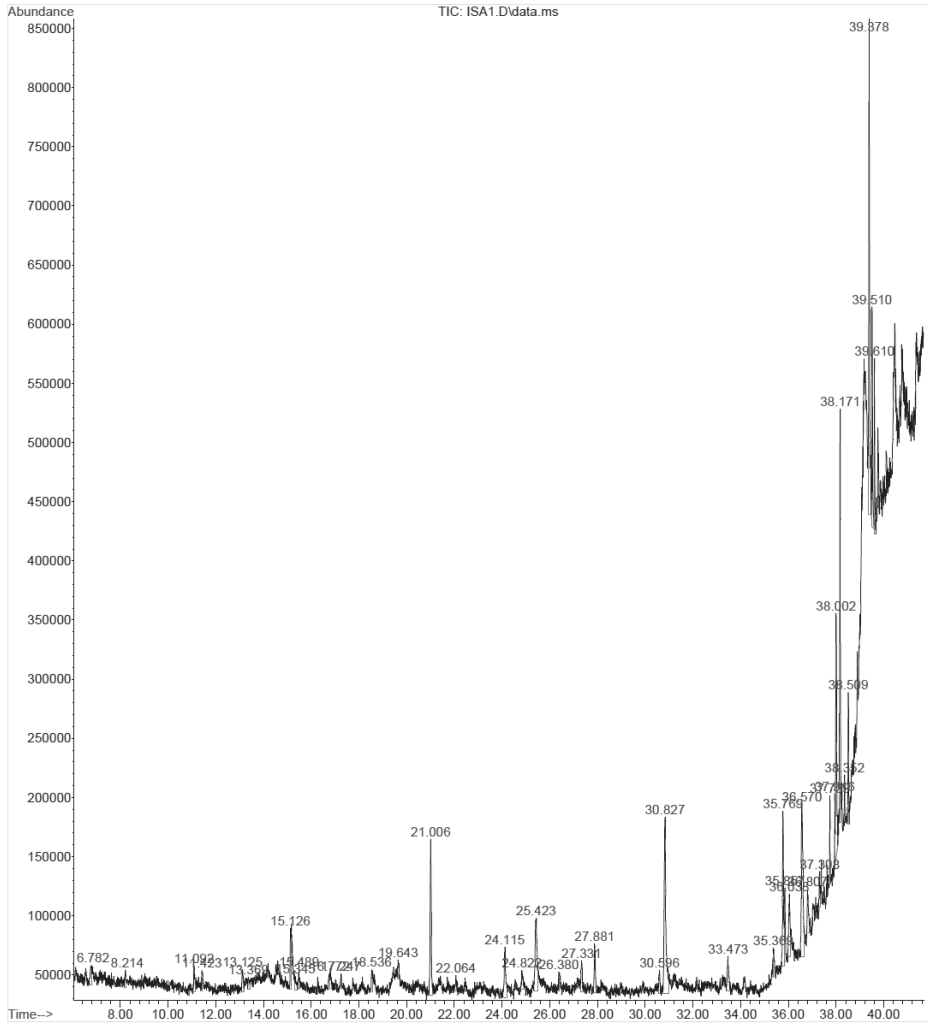

Fig. 2. GC-MS chromatogram of aqueous extract of the non-sporophytic gametophyte *H. involuta*

phyte *H. involuta* extract inhibited the growth of *E. coli*, *P. aeruginosa,* and *C. pseudotropicalis* while the non-sporophytic gametophyte extract inhibited only the growth of *P. aeruginosa*. None of the extracts showed growth inhibition against *B. subtilis*, *S. aureus*, and *A. niger*. Furthermore, the minimum inhibitory concentration recorded for each of extract was 40 mg/mL except for *C. pseudotropicalis* where the sporophytic gametophyte *H. involuta* extract gave a minimum inhibitory concentration of 20 mg/mL (Table 7).

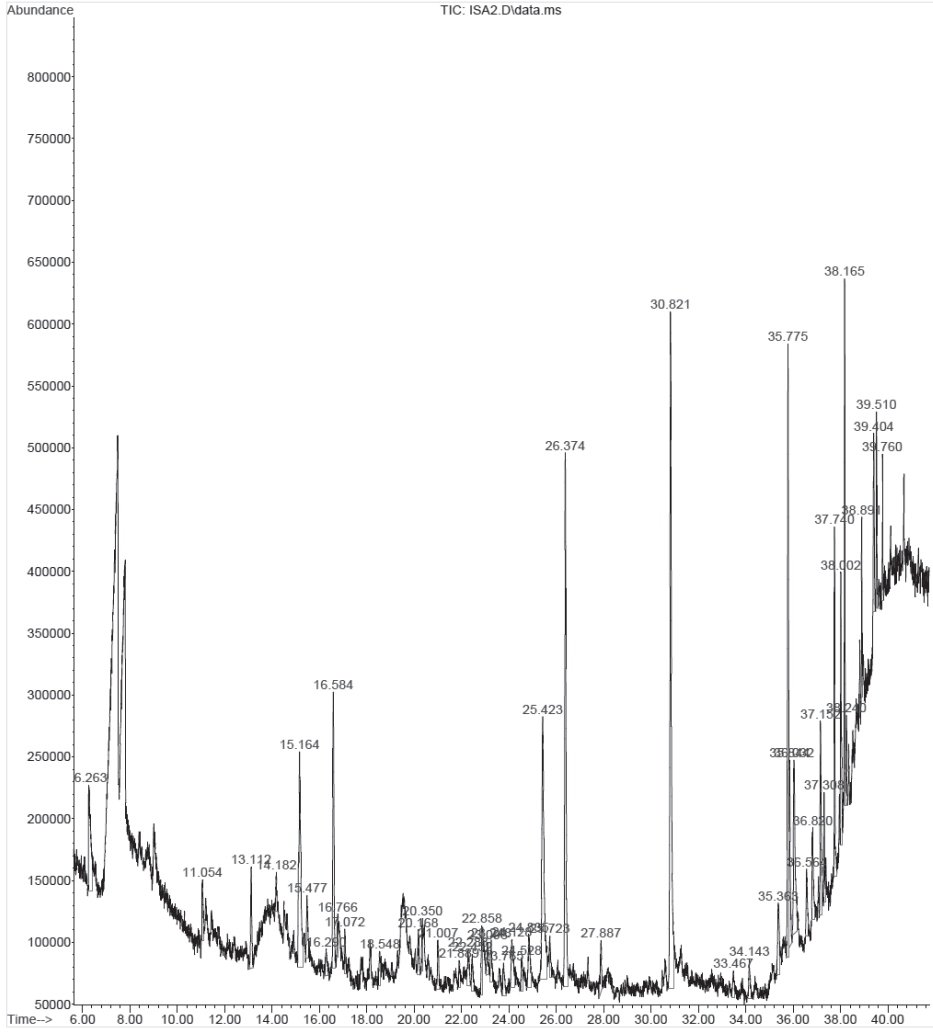

Fig. 3. GC-MS chromatogram of the aqueous extract of the sporophytic gametophyte *H. involuta*

Table 5
Bioactive compounds in the aqueous extract of the sporophytic gametophyte of *H. involuta* using GC-MS

S/n	RT (min)	Area (%)	Identified compounds
1	6.263	2.84	2-amino-1-(3,4-methylenedioxyphenyl)-butane
2	11.054	0.98	Furan, tetrahydro-2,5-dimethyl-
3	13.112	1.49	cis-9-Tetradecen-1-ol
4	14.182	0.44	5-Hydantoinacetic acid
5	15.164	5.71	2(3H)-Furanone, 5-ethyl-dihydro-
6	15.477	1.01	Thiazole, 5-methyl-
7	16.290	0.53	4-Isopropylcyclohexanone
8	16.584	4.24	Ethyl 4-(ethyloxy)-2-oxobut-3-enoate
9	16.766	0.57	Carbonic acid, butyl 2-methylbutyl ester
10	17.072	0.61	1-Isopropylcyclohex-1-ene
11	18.548	0.55	Octane, 4-methyl-
12	20.168	0.70	9-Octadecenamide, (Z)-
13	20.350	0.72	2,5-Dimethyl-1,4-diacetyl-1,2,4,5-tetraazacyclohexane
14	21.007	0.76	2-Methyl-Z,Z-3,13-octadecadienol
15	21.889	0.43	2-Coumaranone
16	22.283	0.78	2-Butenal, 3-methyl-
17	22.452	0.67	2H-Thiopyran, 3,4-dihydro-
18	22.88	0.70	2,5-Dioxabicyclo[2.2.2]octane-3,6-diol, 1,3,4,6-tetramethyl-
19	23.096	0.49	Octahydronaphthalen-4a-ol
20	23.246	0.65	Thiophene-2-carboxamide, N-decyl-N-methyl-
21	23.765	0.70	ortho-Hydroxypropiophenone
22	24.128	1.26	Indole
23	24.528	0.55	N-(2-Methyl-2H-tetrazol-5-yl)-acetamide
24	24.835	1.31	Butanoic acid, tridec-2-ynyl ester
25	25.423	7.01	Cyclohexasiloxane, dodecamethyl-
26	25.723	0.59	1,1'-(4-Methyl-1,3-phenylene)bis[3-(5-isopropyl-1,3,4- thiadiazol-2-yl)urea]
27	26.374	9.55	2(3H)-Furanone, dihydro-5-pentyl-
28	27.887	0.86	Cyclononanone, 2-hydroxy-
29	30.821	13.78	Cycloheptasiloxane, tetradecamethyl-
30	33.467	0.50	Cyclohexane, 1,2-dimethyl-3-pentyl-4-propyl-
31	34.143	0.69	Hexanoic acid, 6-cyano-

Table 5 (continued)

S/n	RT (min)	Area (%)	Identified compounds
32	35.363	1.47	13-Octadecenal
33	35.775	7.71	Cyclooctasiloxane, hexadecamethyl-
34	35.844	2.32	Oxirane, hexadecyl-
35	36.032	3.91	Dodecanoic acid, pentafluorophenyl ester
36	36.564	1.13	Caryophyllene oxide
37	36.820	1.45	6-tert-Butyl-2,4-dimethylphenol
38	37.152	2.83	2-Butyl-5-methyl-3-(2-methyl prop-2-enyl)cyclohexanone
39	37.308	0.95	Ethyl 4-(5-methyl-1,1-dioxido-4-oxo-2-phenyl-1,3-thiazolidin-3-yl)-benzoate
40	37.740	2.86	Cyclononasiloxane, octadecamethyl-
41	38.002	3.14	E-12-Tetradecen-1-ol
42	38.165	4.07	n-Nonadecanol-1
43	38.240	0.62	Aspidospermidine-20,21-diol, 1-acetyl-17-methoxy-
44	38.891	2.21	Cyclodecasiloxane, eicosamethyl-
45	39.404	2.03	9-Eicosyne
46	39.510	1.61	Cyclohexane, 1,2,4,5-tetraethyl-, (1.alpha.,2.alpha.,4.alpha.,5.alpha.)-

Key: RT = retention time

Table 6
Antimicrobial activities of *H. involuta* aqueous extracts

H. involuta (40 mg/mL)	Organisms					
	Escherichia coli ATCC25922	*Bacillus subtilis* NCTC8236	*Pseudomonas aeruginosa* ATCC10145	*Staphylococcus aureus* NCTC6571	*Candida pseudotropicalis*	*Aspergillus niger*
Nsp	–	–	+	–	–	–
Sp	+	–	+	–	+	–

Key: – = absence of inhibition; + = presence of inhibition; Nsp = non-sporophytic gametophyte extract; Sp = sporophytic gametophyte extract; ATCC = American Type Culture Collection; NCTC = National Collection of Type Culture

The minimum bactericidal/fungicidal concentration of the sporophytic gametophyte *H. involuta* extract on *E. coli* and *C. pseudotropicalis* was 40 mg/mL. On the other hand, both extracts from the non-sporophytic and the sporophytic gametophytes *H. involuta* showed a minimum bactericidal concentration of above 40 mg/mL against *P. aeruginosa* (Table 8).

Table 7
The minimum inhibitory concentrations (MICs) of *H. involuta* aqueous extracts

Extracts	Organisms / Minimum inhibitory concentrations (mg/mL)					
	Escherichia coli ATCC25922	*Bacillus subtilis* NCTC8236	*Pseudomonas aeruginosa* ATCC10145	*Staphylococcus aureus* NCTC6571	*Candida pseudotropicalis*	*Aspergillus niger*
Nsp	–	–	40	–	–	–
Sp	40	–	40	–	20	–

Key: – = not determined; Nsp = non-sporophytic gametophyte extract; Sp = sporophytic gametophyte extract; ATCC = American Type Culture Collection; NCTC = National Collection of Type Culture

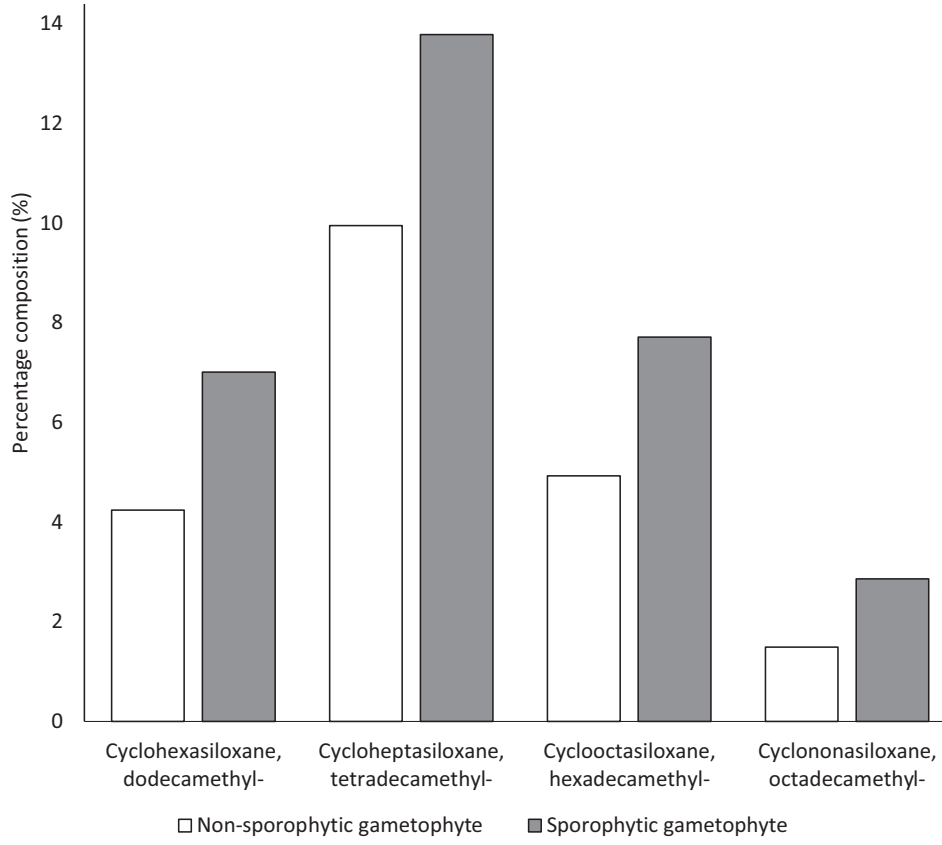

Fig. 4. Common bioactive compounds in the non-sporophytic and sporophytic gametophytes of *H. involuta* aqueous extracts

Table 8
Minimum bactericidal/fungicidal concentrations of *H. involuta* aqueous extracts

H. involuta extracts	Organisms / Minimum inhibitory concentrations (mg/mL)					
	Escherichia coli ATCC25922	*Bacillus subtilis* NCTC8236	*Pseudomonas aeruginosa* ATCC10145	*Staphylococcus aureus* NCTC6571	*Candida pseudotropicalis*	*Aspergillus niger*
Nsp	–	–	>40	–	–	–
Sp	40	–	>40	–	40	–

Key: – = not determined; Nsp = non-sporophytic gametophyte; Sp = sporophytic gametophyte; ATCC = American Type Culture Collection; NCTC = National Collection of Type Culture

DISCUSSION

Different factors have been noted to influence the synthesis of plants' specialised metabolites, thereby causing significant differences in their yields of bioactive compounds (Radojević *et al.* 2021, Radušienė *et al*. 2012, Yuan *et al*. 2020). This study explored the bioactive compounds contained in the aqueous extracts of the non-sporophytic and sporophytic gametophytes of *H. involuta*. Extraction and analysis of plant materials play important roles in the development of qualitative herbal formulation (Sasidharan *et al*. 2011). Thus, monitoring the production of specialised metabolites in plants is necessary considering the occurring global climatic changes on one hand and the continual development of resistance by disease-causing organisms against some of the existing antibiotics on the other hand (Oladipo *et al*. 2022, Pant *et al*. 2021). Screening of both the non-sporophytic gametophytes and the sporophytic gametophytes of *H. involuta* in this study could be regarded as one of the ways of obtaining essential information regarding chemical constituents of bryophytes (Talukdar *et al*. 2010).

The observed variations between the crude extract yields and the biochemical constituent compositions of the non-sporophytic gametophyte and the sporophytic gametophyte of *H. involuta* were in agreement with the findings of Ashraf and Harris (2013) and Friščić *et al*. (2018) who reported variations in the amount of specialised metabolites among different plant parts of species from the same genus. The yielded aqueous crude extract from the non-sporophytic gametophyte was higher (17.84 g) than the yield from the sporophytic gametophyte (13.16 g). Phytochemically, both extracts tested positive for the presence of alkaloids, cardiac glycosides, saponins, and steroids, but, the concentrations of each in each of the extracts differed. Alkaloids

(37.82 ± 0.001 mg AE/g) and cardiac glycosides (25.59 ± 0.051 mg/g) in the non-sporophytic gametophyte were higher than in the sporophytic gametophyte where 21.45 ± 0.000 mg AE/g and 4.56 ± 0.034 mg/g were recorded for alkaloids and cardiac glycosides respectively. Similarly, the saponins contents of the samples also differed. However, contrary to alkaloids and cardiac glycosides, the saponins content (2.56 ± 0.019 mg SE/g) was higher in the sporophytic gametophyte sample, than in the non-sporophytic gametophyte sample (1.62 ± 0.023 mg SE/g).

The variations observed in the bioactive compounds' composition of the extracts as revealed by the GC-MS analyses where 40 bioactive compounds were identified in the non-sporophytic gametophyte as against the 46 bioactive compounds identified in sporophytic gametophyte further indicated that the emergence of sporophyte on a bryophyte could bring about variations in the biochemical compound compositions of a bryophyte species. Cycloheptasiloxane tetradecamethyl- was identified as the most prominent compound present in each of the two extracts. This compound was found higher in concentration in the sporophytic gametophyte than in the non-sporophytic gametophyte. Compounds such as cyclohexasiloxane dodecamethyl-, cyclooctasiloxane hexadecamethyl-, and cyclononasiloxane octadecamethyl- were also commonly identified present in the two extracts and were also higher in concentrations in the extract from the sporophytic gametophyte of *H. involuta* than in the non-sporophytic gametophyte. However, these observations disagreed with Hartmann (1996), who reported higher rates of metabolites' biosynthesis in young tissues. The results from this study showed that, while some secondary metabolites were higher in the non-sporophytic gametophyte extract which could be likened to younger tissue in bryophyte, others were higher in the sporophytic gametophyte extract. Although the influence of maturity stages on the bioactive constituents of some selected mosses and some other plants was reported (Blum-Silva *et al*. 2015, Isa *et al*. 2021), there has been no general conclusion applicable to all plant species. Mazzafera *et al*. (1994) reported higher caffeine contents in younger leaves of *Coffea arabica* L. Similarly, Esmelindro *et al*. (2004) reported a significantly higher content of caffeine and theobromine in leaves at six months than in older leaves. In the evaluation of the methylxanthine content in *Ilex paraguariensis* samples under different growth conditions, treatment, and age, Dartora *et al*. (2011) reported that the results obtained did not show significant differences in methylxanthine content in leaves between one and six months. It has also been reported that medicinal plants grown under semi-arid conditions do reveal higher concentrations of relevant natural products than identical plants of the same species which are cultivated in moderate climates (Sasidharan *et al*. 2011).

In this study, both extracts of *H. involuta* inhibited the growth of *E. coli*, *P. aeruginosa*, and *C. pseudotropicalis*. The recorded activities against the tested disease-causing microbes could be attributed to the presence of the detected and identified phytochemicals as established by various researchers (Chandra *et al*. 2013, Olasoji *et al*. 2019). Both extracts from the studied bryophyte species showed the presence of important bioactive compounds that can be considered for use in the preparation of useful drugs. Bioactive compounds occur naturally in plants as a defence mechanism to protect them from various diseases and are responsible for the medicinal activities of plants (Frahm 2004). The findings from this study showed that the non-sporophytic gametophyte extract of *H. involuta* inhibited only the growth of *P. aeruginosa* (a Gram-negative bacterium) while the sporophytic gametophyte extract in addition to being inhibitory to the growth of *P. aeruginosa* also inhibited the growth of *E. coli, and C. pseudotropicalis*. The difference in the pattern with which the *H. involuta* extracts inhibited the growth of the test microorganisms may be attributed to the variations in the bioactive compounds' composition of the extracts occasioned by the emergence of the sporophyte in the extract sporophytic gametophyte. This observation is in agreement with the report of Guo *et al*. (2013) that different factors do have different extents of effects on different plants, thus resulting in differences in the contents of the plants' accumulated secondary metabolites which would in turn influence the potency of plants' extracts.

Generally, the synthesis of plant metabolites is a complex one that could differ between tissues or organs and thereby affected by different factors. Among these factors are the plants' need to adapt to prevailing conditions in their environment (Friščić *et al*. 2018) and the protection of plants against different types of pathogens as well as their effects (Deora and Deora 2018). The accumulation of secondary metabolites in plants is one of the wide spectra of acclimatisation responses evolved to cope with rapid changes in their surroundings (Hartmann 2007, Wink 2003), and perhaps in the case of *H. involuta* in this study, the change in body physiology leads to the emergence of the sporophytes.

CONCLUSIONS

Given the observed differences in the constituents of the non-sporophytic and the sporophytic gametophytes of *H. involuta*, this study concluded that the emergence of sporophyte on the gametophyte of a bryophyte species could bring about significant variations in the synthesis and concentrations of its bioactive compound constituents as well as that of its antimicrobial activities. However, further research work should be carried out on the sporophyte

of *H. involuta* to have a good idea of its bioactive constituents relative to the whole shoot, the rhizoids, and the gametophyte of the moss. This would give further information and a better understanding of the bioactive constituents and beneficial potentials in this group of plants.

*

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NEW AND NOTEWORTHY LICHENFORMING AND LICHENICOLOUS FUNGI 13. A REVISION OF THE *XANTHORIA ECTANEOIDES* COMPLEX (XANTHORIOIDEAE, TELOSCHISTACEAE) INCLUDING THE NEW SPECIES *XANTHORIA PYLYPORLYKII*

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Xanthoria ectaneoides, usually recognised by its secondary sublobules, is circumscribed in a strict sense using spore size and a molecular phylogeny based on ITS-sequences. The species, earlier considered a synonym of *X. aureola,* forms a subclade with *X. coomae* and the new species *X. pylyporlykii*, described here, whereas *X. aureola* is positioned in the *Xanthoria calcicola* subclade. The new species *X. pylyporlykii* is characterised by a combination of characters typical either for *Xanthoria ectaneoides* or *X. coomae*.

Kew words: ascospore, ascospore septum, Denmark, Germany, lichen-forming fungi, Sweden, *Xanthoria aureola, X. ectaneoides, X. pylyporlykii*

INTRODUCTION

The genus *Xanthoria* has, in similarity with several other well-known lichen genera, been heavily re-evaluated in the molecular era. Morphological characters used for delimitations of species and genera showed good correlation with molecular data. Seventeen lichen groups, earlier included in *Xanthoria,* have been segregated as separate genera (Kondratyuk *et al.* 2022*b*). Of approximately 50 *Xanthoria* species in the pre-molecular era (Kärnefelt 1989), only 13 species remain in the genus in its strict sense (Kondratyuk *et al.* 2022*b*), although many new species have been described both in *Xanthoria* s. str. and its segregates.

The present work is limited to *Xanthoria* in its strict sense, where several subclades can be discerned, one of which is the *Xanthoria ectaneoides* subclade. The basionym for *Xanthoria ectaneoides* (Nyl.) Zahlbr. is *Physcia ectaneoides*

Nyl., described in the 19th century (Nylander 1883). The species is revised using a recent collection from areas around the southwestern part of the Baltic Sea. The phylogeny is based on ITS-sequences. Non-molecular characters were investigated for all specimens including the type specimen of *X. ectaneoides* from France. *Xanthoria ectaneoides* has been considered as a synonym of *X. aureola* (Ach.) Erichsen (Gaya *et al.* 2012, 2015, Lindblom and Ekman 2005). The wide species concept for *Xanthoria parietina* and *X. aureola* does not have support from molecular data.

Already in the 1990s, the senior author was aware of an undescribed taxon in Scandinavia with very long ascospores, to 20 μm long, and very wide ascospores septa, 11–12 μm wide. The undescribed taxon was recognised by its spore size since the ascospores of *Xanthoria parietina* are smaller, i.e. 10–15 × 6–8 μm with 6–8 μm wide septa. In 2022–2023 an extensive material of *Xanthoria* s. str. was collected in the southwestern Baltic Sea area, in connection with a project on lichenicolous fungi growing on *Xanthoria* (Kondratyuk *et al.* 2023). The undescribed taxon was found to be frequent in the investigated area.

Prior to describing a new species, a revision of all available types of old taxa of the genus *Xanthoria* was performed. Long ascospores (to 20 μm long) with wide septa (*ca* 11–12 μm) revealed here as ~~species~~ specific for *Xanthoria ectaneoides* (Nyl.) Zahlbr. (Nylander 1883), the second species to be described in *Xanthoria* after *X. parietina* (Linnaeus 1753).

The aim of this paper was to revise the *Xanthoria ectaneoides* complex, based on material from the area around the southwestern part of the Baltic Sea, using morphology, anatomy and molecular phylogeny. This integrative approach in taxonomy of the genus *Xanthoria*, i.e. correlation between morphology and anatomy, particularly details of ascospores and their septa, was elaborated by the Austrian lichenologist Josef Poelt and his colleagues in the premolecular era (Giralt *et al.* 1993, Kondratyuk and Poelt 1997, Poelt and Petutschnig 1992*a*, *b*, etc.). Several species described later were confirmed by molecular phylogeny (Arup *et al.* 2013, Kondratyuk *et al.* 2013, 2017, 2020).

MATERIAL AND METHODS

Lichen-forming fungi of the *Xanthoria calcicola* and the *X. parietina* complexes occurring on hard substrates, i.e., rocks, bricks, tiles and metal roofs were collected at 65 localities in southern Scandinavia, i.e. Skåne, the southernmost province of Sweden, southern Denmark and northern Mecklenburg-Vorpommern and Schleswig-Holstein in Germany (Table 1). The specimens will preferably be deposited at C, GB, KW-L, LD, some of them will be distributed in the exsiccate Plantae Graecenses. The revision was mainly based on our own collections and type specimens of *Xanthoria aureola*, *X. calcicola* and *X. ectaneoides*.

Table 1
List of localities for the *Xanthoria ectaneoides* complex (* = SK, ** = AT and NT, *** = US)

No	Locality	Date / collector(s)	Position	Number of specimens		
				Total	with *X. ectaneoides*	with *X. pylyporlykii*
1	Denmark, Amager, Tårnby par., the church, on tiles on the cemetery wall	13.08.2023*	55.6280° N, 12.6028° E	35	27	3
2	Bornholm, Nexø, on rock wall	28.10.2022*	55.0629° N, 15.1250° E	56	11	3
3	Nylars par., the church, on rock wall	28.10.2022*	55.0724° N, 14.8100° E	11	2	1
4	Nyker par., the church, on rock wall	29.10.2022*	55.1396° N, 14.7595° E	11	2	1
5	Østerlars par., the church, on rock wall	29.10.2022*	55.1648° N, 14.9656° E	2	–	2
6	Rønne par., the church, on tiles on the cemetery wall	29.10.2023*	55.0935° N, 14.7007° E			
7	Svaneke par., on tiles on the church and cemetery wall	29.10.2023*	55.1343° N, 14.1412° E			
8	Fyn, Svendborg Landevej, on concrete	28.05.2023*	55.1860° N, 10.7330° E	44	–	6
9	Jutland, Haderslev, the old church, on tiles on the cemetery wall	1.07.2023*	55.2501° N, 9.4891° E	33	5	
10	Skagen par., the church, on tiles on the northern wall at the church	16.07.2023**	57.7214° N, 10.5847°E			1
11	Møn, Borre par., the church, on tiles on the cemetery wall	22.09.2022*	54.9959° N, 12.4432° E	6	–	1
12	Fanefjord par., the church, on tiles on the cemetery wall	11.10.2022*	54.9013° N, 12.1511° E	23	2	2
13	Zealand, Lillerød par., the church, on tiles on the cemetery wall	11.06.2023*	55.8734° N, 12.3460° E	7		4
14	Bjernede par., the church, on tiles on the cemetery wall	26.05.2023*	55.462° N, 11.625° E	22	–	2
15	Farum par., the church, on tiles on the cemetery wall	4.02.2023*	55.8070° N, 12.3573° E	54	9	18
16	Fjenneslev par., the church, on tiles on the cemetery wall	26.05.2023*	55.4336° N, 11.6875° E	22		1
17	Gørløse par., the church, on tiles on the cemetery wall	3.12.2022*	55.8853° N, 12.1991° E	22	1	4
18	Helsinge, the church yard, on tiles on the cemetery wall	26.03.2023*	56.0208° N, 12.1969° E	170		14
19	Højby par., the church, on tiles on the cemetery wall	25.06.2023**	55.9128° N, 11.5996° E	15		3
20	Slangerup, SE edge of Lystrup forest, on tile roof	3.12.2022*	56.2316° N, 10.2303° E	4	–	3

Table 1 (continued)

No	Locality	Date / collector(s)	Position	Number of specimens		
				Total	with *X. ectaneoides*	with *X. pylyporlykii*
21	Søborg par., the castle ruins, on modern brick inclusions	16.04.2023*	55.0877° N, 12.3055° E	45		13
22	Søborg par., the church, on tile roof	16.04.2023*	55.7352° N, 12.5120° E	70		1
23	Ærø, Søby par., the church, on tiles on the cemetery wall	26.05.2023*	54.9386° N, 10.2568° E	55		2
24	Marstal, the church, on tiles on the cemetery wall	26.05.2023*	54.8550° N, 10.5170° E	59	1	9
25	Marstal, Ommel church, on tile roof	27.05.2023*	54.8646° N, 10.4891° E	17		3
26	Tranderup, the church	27.05.2023*	54.8941° N, 10.3101° E			2
27	Ærøskøbing, the church, on tiles on the cemetery wall	26.05.2023*	54.8879° N, 10.4122° E	71		2
28	Germany, Mecklenburg-Vorpommern, Rostock district, Cammin	1.10.2023*	53.967° N, 12.3333° E		1	3
29	Rostock district, Alt Bukow, the church, on tiles on the cemetery wall	2.10.2023*	53.9963° N, 11.6077° E		6	1
30	Rostock district, N of the nature reserve Heiligensee, branches on the beach	28.10.2023***	54.2297° N, 12.1769° E		1	1
31	Rostock district, Rostock, opposite Kanonsberg	2.10.2023*	54.0914° N, 12.1300° E		5	1
32	Rostock district, Russow	2.10.2023*	54.0605° N, 11.6490° E		7	2
33	Nordwestmecklenburg district, Blowatz-Dreveskirchen	2.10.2023*	53.9939° N, 11.5385° E			1
34	Poel island, dirt road between Neuhof and Seedorf, transformer station, roof of the transformer station	7037, 4.11.2023***	53.9972° N, 11.4156° E		2	2
35	Poel island, Timmendorf, northern harbour pier, south exposed side of the pier, gneiss	7042, 4.11.2023***	53.9925° N, 11.3997° E	4		3
36	Poel island, Kirchdorf, church, southern side, brick	7051, 4.11.2023***	53.9944° N, 11.0381° E	3		3
37	Vorpommern-Rügen district, Darß peninsula, coast between light house and Ahrenshoop, branches from a tree fallen on the beach	6998, 3.10.2023***	54.4525° N, 12.4858° E	3		1

Table 1 (continued)

No	Locality	Date / collector(s)	Position	Number of specimens		
				Total	with *X. ectaneoides*	with *X. pylyporlykii*
38	Vorpommern-Rügen district, Darß peninsula, coast between light house and Ahrenshoop, branches from a tree fallen on the beach	7000***	54.4489° N, 12.4836° E			
39	Schleswig-Holstein, Nordfriesland, Ockholm, churchyard, church, western side, brick	7005, 6.10.2023***	54.6652° N, 8.8275° E	4		1
40	Nordfriesland, Fahretoft, the church, western side, small annex, north exposed, brick	7009, 7.10.2023***	54.7055° N, 8.7908° E	3	2	
41	Nordfriesland, Nordmarsch-Langeneß, Kirchwarf, fence post, wood	7014, 7.10.2023***	54.6411° N, 8.6169° E	7	1	
42	Nordmarsch-Langeneß, harbour near Peterswarf, wooden bench, wood	7018, 7.10.2023***	54.6375° N, 8.6328° E	10	9	
43	Nordmarsch-Langeneß, harbour near Peterswarf, protection wall, xeric-supralittoral, sunny place, concrete, siliceous rock	7021, 7.10.2023***	54.6375° N, 8.6328° E	5	1	
44	Nordmarsch-Langeneß, Neuwarf, dyke, xeric supralittoral, sunny place, gneiss	7026, 7.10.2023***	54.6392° N, 8.645° E	5	5	1
45	Nordmarsch-Langeneß, harbour W of Mayenswarf, xeric supralittoral, sunny place, wood	7032, 7.10.2023***	54.6336° N, 8.5389° E	4		1
46	Sweden, Skåne, Bromma par., the church, on rocky wall	28.09.2022*	55.4707° N, 13.8001° E	34		2
47	Brönnestad par., Hovdala castle, on granitic rocks	26.08.2022*	56.1040° N, 13.7138° E	10		1
48	Bunkeflo par., Lernacken, on granitic rocks	12.07.2022*	55.5541° N, 12.9191° E	13	–	1
49	Everöd par., the church, on tile roof	4.03.2023*	55.9018° N, 14.0730° E	64	2	22
50	Gislöv par., Gislövsläge, on coastal granitic wall	6.09.2022*	55.3567° N, 13.2369° E	20	3	7
51	Hofterup par., Järavallen, on wooden substrate	9.06.2022*	55.6895° N, 12.9418° E			1
52	Husie par., the former LV4 military area, on cement columns	27.08.2022*	55.5773° N, 13.0840° E	6	1	2
53	Lund, Biologihuset	22.10.2022*	55.7118° N, 13.2066° E		3	

Table 1 (continued)

No	Locality	Date / collector(s)	Position	Number of specimens		
				Total	with X. *ectaneoides*	with X. *pylypovlykii*
54	Malmö, Västra Hamnen, on granitic rocks	16.08.2022*	55.6133° N, 12.9813° E	14	1	2
55	Stehag par., Stehag, Rapsvägen 3, on tile roof	30.06.2022–25.07.2023*, **	55.9009° N, 13.3948° E	45	23	19
56	Stehag par., NW Stehag, on rocks near roadside trees	5.02.2023*	55.9113° N, 13.3896° E			1
57	Norra Vram par., the church, on tiles on the cemetery wall	12.11.2022*, **	56.0870° N, 12.9734° E	15	3	18
58	Tofta par., the church, on tiles on the cemetery wall	2.04.2023*	56.8669° N, 12.9262° E	75	1	5
59	Igelösa par., the church, on tiles on the cemetery wall	11.05.2023*	55.7631° N, 13.2744° E	16	2	1
60	Mölleberga par., the church, on tiles on the cemetery wall	11.05.2023*	55.6085° N, 13.1770° E	14	1	
61	Ramlösa (S of Helsinborg), on roadside rocks near parking area	12.08.2022*	55.8056° N, 12.7333° E		2	1
62	Skanör par., the church, on vertical surfaces of thumbs at the cemetery	23.08.2022*	55.4195° N, 12.8497° E	1	1	
63	Svedala par., the church, on tiles on the cemetery wall	*	55.5122° N, 13.2256° E			1
Total				1269	150	214

The specimens were sprayed with water preferably from ten minutes to half an hour before they were removed from the substrate. Mature apothecia were cut by hand. Fifteen sections of each apothecium were mounted in the same water droplet to contain a sufficient amount of ascospores, at least 50 in light field of the microscope, for statistic measurements. Ascospores were exclusively measured outside of asci and sections. At least 50 measurements of adult ascospores were performed and included in the further statistical analysis.

The specimens were studied and determined microscopically and vouchers for DNA-analyses prepared at the unit of Molecular Cell Biology, Department of Biology, Lund University.

DNA extraction, PCR amplification and sequencing

Genomic DNA was extracted directly from a portion of thallus with apothecia from each specimen using a modi-

fied 2% CTAB method (Gardes and Bruns 1993). The ITS-nrDNA region was amplified using the primer pair ITS1F (Gardes and Bruns 1993) and ITS4 (White *et al.* 1990). PCR products were visualised on 1% agarose gel with ethidium bromide through Gel documentation system (Sambrook and Russel 2001). PCR products were sent for sequencing to Tartu, Estonia. However, molecular data for *Xanthoria ibizaensis* are obtained in the Molecular Cell Biology unit of Lund University (Sweden).

Phylogenetic analysis

The newly generated sequences were compared to GenBank database sequences using BLAST search (http://www.ncbi.nlm. nih.gov/BLAST/). All sequences were aligned with sequences of selected representatives of Teloschistaceae obtained from GenBank (see Table 2 for voucher details). Maximum likelihood (RAxML) analyses were performed for the representatives of the Teloschistoideae at first using RAxMLHPC v.8 on XSEDE (Stamatakis 2014) under the GTRGAMMA model on CIPRES Science Gateway (Miller *et al.* 2010). Rapid bootstrap analyses were performed with 1,000 bootstrap replicates. Matrix of the whole genus *Xanthoria* including 56 voucher specimens of the 13 species belonging to this genus and the outgroup *Martinjahnsia resendei* were analysed with Maximum Parsimony (MP), Minimum Evolution (ME) and Maximum Likelihood (ML) methods. The MP tree was obtained using the Tree-Bisection-Regrafting (TBR) algorithm, within the ME method the evolutionary distances were computed using the Maximum Composite Likelihood method (Tamura *et al.* 2004), and the ML analysis was conducted with the lowest BIC scores (Bayesian Information Criterion) model. The bootstrap consensus trees inferred from 1,000 replicates each. The analyses involved 59 nucleotide sequences, there were a total 580 positions in the final dataset. All three analyses were conducted in MEGA11 (Tamura *et al.* 2021).

RESULTS AND DISCUSSION

Results from statistical analysis of data on ascospores

The results from the statistical treatment of ascospore data are based on investigations of more than 900 specimens and data from about 45,000 ascospores. Results of measurements of minimum 50 ascospores from each specimen excluding the most extreme measurements.

Several types of ascospores were observed in *Xanthoria* species. However, in the present study we discuss spore-data exclusively from the *Xanthoria parietina, X. ectaneoides* and *X. coomae* types. Spore type and septum type may be different within a species (Table 3).

Table 2

Sequences used in the phylogenetic analyses (sequences generated for this study as well as new names are in **bold**). Abbreviations: Ref = references, * = sub *Xanthoria parietina*, ** = sub *Xanthoria* sp., *** = *Xanthoria ectaneoides*

Species, voucher number in the phylogenetic tree	Isolate	Country	nrITS	Ref
Martinjahnsia resendei	BCC-Lich 13176	Spain	AF101284	Martin and Winka 2000
Martinjahnsia resendei	Xres233b	Spain	EU639641	Gaya *et al.* 2008
Martinjahnsia resendei	BCC-Lich 13259	Spain	AF101285	Martin and Winka 2000
Martinjahnsia resendei	BCC-Lich 13175	Spain	AF101283	Martin and Winka 2000
Xanthoria aureola	LIQ109XAAU-2	Spain	whole genome	Llewellyn *et al.* 2023
Xanthoria aureola	Gaya 9	Sweden	JQ301690	Gaya *et al.* 2012
Xanthoria cf. *aureola*	SS0065	UK	ON437600	Brown unpubl.
Xanthoria calcicola	Voucher 105/1	Switzerland	AJ320152	Scherrer and Honegger 2003
Xanthoria calcicola	FNM-088	UK	EU681295	Fedorenko *et al.* 2009
Xanthoria calcicola	Voucher A6	France	AJ320130***	Scherrer and Honegger 2003
Xanthoria calcicola	Voucher 80	UK	AJ320150	Scherrer and Honegger 2003
Xanthoria coomae	2001 Lindblom BH19 (BG)	Norway	AY438298*	Lindblom and Ekman 2005
Xanthoria coomae	M-0102316	Germany	JF831894*	Beck and Mayr 2012
Xanthoria coomae	CANB Kondratyuk 20494, holotype	Australia	KC179410	Arup *et al.* 2013
Xanthoria coomae	Millanes 849(s) AM553	Spain	OQ249845*	Freire Rallo *et al.* 2023
Xanthoria coomae	ALV16819	South Africa	MH714517*	Wirth *et al.* 2018
Xanthoria ectaneoides	LD-M51	Denmark	LD-M51	this paper
Xanthoria ectaneoides	LD-M52	Denmark	LD-M52	this paper
Xanthoria ectaneoides	LD-M61	Denmark	LD-M61	this paper
Xanthoria ectaneoides	LD-M69	Denmark	LD-M69	this paper

Table 2 (continued)

Species, voucher number in the phylogenetic tree	Isolate	Country	nrITS	Ref
Xanthoria ectaneoides	LD-M70	Denmark	LD-M70	this paper
Xanthoria ectaneoides	LD-M73	Denmark	LD-M73	this paper
Xanthoria sp. 2	117(75.8) R. Honegger 379t1	France	AM408403***	Eichenberger 2007
Xanthoria sp. 2	FNM-087	UK	EU681299***	Fedorenko *et al.* 2009
Xanthoria sp. 2	Voucher B5	France	AJ320131***	Scherrer and Honegger 2003
Xanthoria sp. 2	Voucher 83, 84	UK	AJ320135***	Scherrer and Honegger 2003
Xanthoria sp. 2	Voucher 90/1, 90/2	France	AJ320149***	Scherrer and Honegger 2003
Xanthoria sp. 2	M158t5a1 929.12) R. Honegger 158t5	France	AM292821	Eichenberger 2007
Xanthoria ibizaensis	M12a, holotype	Spain	M12a	this paper
Xanthoria ibizaensis	M12b, holotype	Spain	M12b	this paper
Xanthoria mediterranea	705(117.15) R. Honegger 427t1	Italy	AM408410	Eichenberger 2007
Xanthoria mediterranea	LIQ75XAME-2	Israel	whole genome	Llewellyn *et al.* 2023
Xanthoria cf. *mediterranea*	SH85-2001	Greece	AJ320140**	Scherrer and Honegger 2003
Xanthoria cf. *mediterranea*	Voucher 43	Italy	AJ320134***	Scherrer and Honegger 2003
Xanthoria cf. *mediterranea*	L174t1 (100.3)	Tunisia	AM292822***	Scherrer and Honegger 2003
Xanthoria monofoliosa	Voucher 4	Italy	AJ320147****	Scherrer and Honegger 2003
Xanthoria monofoliosa	L104	Spain	AM292818****	Scherrer and Honegger 2003
Xanthoria monofoliosa	M282t1ad (97.19)	Italy	AM292842****	Scherrer and Honegger 2003
Xanthoria monofoliosa		Souch Africa	EU681817	Fedorenko *et al.* 2009
Xanthoria monofoliosa		South Africa	AM697817**	Eichenberger 2007
Xanthoria parietina	Honegger 56t8	USA	AM697845	Eichenberger 2007

Table 2 (continued)

Species, voucher number in the phylogenetic tree	Isolate	Country	nrITS	Ref
Xanthoria parietina	Honegger 271t1	Spain	AM697841	Eichenberger 2007
Xanthoria parietina	Honegger 320t2	Switzerland	AM697848	Eichenberger 2007
Xanthoria parietina	Honegger 265t1	Spain	AM697847	Eichenberger 2007
Xanthoria parietina	Honegger 347	USA	AM697842	Eichenberger 2007
Xanthoria parietina	Honegger 348t4	USA	AM697838	Eichenberger 2007
Xanthoria polessica	U3115	Belarus	MT928333	Tsurykau *et al.* 2020
Xanthoria polessica	U3114	Belarus	MT928332	Tsurykau *et al.* 2020
Xanthoria pylyporlykii	LD-M49	Denmark	LD-M49	this paper
Xanthoria pylyporlykii	LD-M56	Denmark	LD-M56	this paper
Xanthoria pylyporlykii	LD-M59	Denmark	LD-M59	this paper
Xanthoria pylyporlykii	LD-M77	Denmark	LD-M77	this paper
Xanthoria pylyporlykii	LD-M78	Denmark	LD-M78	this paper
Xanthoria sp. 1	TBL-2021 LIQ80XSP	Italy	whole genome	Llewellyn *et al.* 2023
Xanthoria steineri	LIQ73XASTE-2	Israel	whole genome	Llewellyn *et al.* 2023
Xanthoria steineri	SH5-2001	Cyprus	AJ320142**	Scherrer and Honegger 2003
Xanthoria tendraensis	KHER 12109a	Ukraine	MZ196456	Khodosovtsev *et al.* 2023
Xanthoria tendraensis	KHER 12109	Ukraine	MZ196457	Khodosovtsev *et al.* 2023
Xanthoria tendraensis	KHER 11232	Ukraine	MZ303030	Khodosovtsev *et al.* 2023

Table 3
Type of ascospores of some *Xanthoria* species

Species name	Spore type	Spore size (μm)	Septum type	Septum width (μm)	Approximate number of ascospores measured
X. parietina	'*parietina*'	10–15 × 6–8	'*parietina*'	6–8	500
X. ectaneoides	'*ectaneoides*'	15–18 × 5–7	'*ectaneoides*'	10–13	8,000
X. coomae	'*ectaneoides*'	15–17 × 6–8	'*coomae*'	7–10	1,000
X. pylyporlykii	'*parietina*'	12–15 × 6–8	'*coomae*'	7–10	10,000

Table 4
Molecular data on members of the genus *Xanthoria* (data on vouchers '*Xanthoria* sp.' available in GenBank are not included here)

	parietina	*aureola*	*steineri*	*mediterranea*	*ectaneoides*	*calcicola*	*coomae*	*polessica*	*monofoliosa*
nrITS	257	3		2	13	12	4	2	3
18S nrSSU	10	1			1	1			
28S nrLSU	22	1				2			
12S mtSSU	7	1		1	2	5	2		1
23S mtLSU	12				3	1			
hydrophobin	57				12	3			
beta-tubulin	30			1	5	1			
RPB2	1	1				1			
RPB1	1	1				1			
polyketide synthase gene	2								
28S-18S intergenic space	35	2				3			
putative non-ribosomal peptide synthase-like gene	1								
SLA2 gene, DNA lyase gene and MAT 1-2-1 gene	1								
whole genome	1	1	1						
Total	436	11	1	3	36	30	3	2	1

Xanthoria parietina is characterised by medium sized ascospores, 10–15 × 6–8 μm, – the *parietina* spore type – and a medium wide septum, 6–8 μm wide – the *parietina* septum type.

Xanthoria ectaneoides is a unique species with the longest ascospores – the *ectaneoides* type, and the widest (10–13 μm wide) septa – the *ectaneoides* type. These data come from measurements of the type specimen of *X. ectaneoides* and confirmed by measurements of more than 8,000 ascospores from collections in areas around the southwestern Baltic Sea. The spore types and septum types of *Xanthoria parietina* and *X. ectaneoides* are unique and not overlapping.

On the contrary, *X. coomae,* described in 2008 (Kondratyuk *et al.* 2008), is characterised by having the *Xanthoria ectaneoides* spore type and the *X. coomae* type of septum.

Xanthoria pylyporlykii, described here, is distinguished after the combination of *parietina* spore type and *coomae* septum type. These data are confirmed by measurements of more of 10,000 ascospores of specimens from southwestern Baltic region.

Molecular data on Xanthoria species

To check positions of the newly collected specimens, ITS-sequences from all *Xanthoria* species available in the GenBank were included in the analysis (Table 2). The highest number of *Xanthoria* sequences in the GenBank are submitted under the name *Xanthoria parietina*, in the pre-molecular era considered to be one of the most thoroughly studied of all lichen species (Honegger 1996), however, today it is clear that many of these sequences represent other species. Whole genomes of several *Xanthoria aureola, X. mediterranea, X. steineri* as well as *Xanthoria* sp. 1 and *Xanthoria* sp. 2 are now available (Tables 2 and 4) (Llewellyn *et al.* 2023). The nrITS sequences of these species mentioned, except for *Xanthoria* sp. 2 (data on nrITS of which are still not available via BLAST) were extracted from the whole genome and used in the present phylogeny analysis.

The phylogenetic tree based on ITS-sequences is divided in two main clades, the *X. calcicola* and *X. parietina* clades, and *Xanthoria monofoliosa* positioned on a separate branch (Arup *et al.* 2013, Fedorenko *et al.* 2009, 2012, Gaya *et al.* 2012, 2015, Kondratyuk *et al.* 2014, 2017, 2020).

The matrix of nrITS sequences contains more than 330 specimens of the genus *Xanthoria,* including also data on specimens named as *Xanthoria* sp. However, vouchers incorrectly named *Xanthoria parietina* are nested among other species, i.e. *Xanthoria coomae, X. monofoliosa, X. polessica*, and even true *Xanthoria ectaneoides* s. str. In the same way, sequences labelled *X. calcicola* and *X. ectaneoides* in the GenBank are also spread on several branches. Some separate subclades probably represent undescribed species.

To illustrate the tree in the best way, only a selection of the sequences available in the GenBank were included. Thus, for *Xanthoria calcicola, X. coomae, X. monofoliosa* and *X. parietina*, the most frequently represented species in the GenBank, only 5–7 sequences were selected, whereas all sequences for *Xanthoria polessica, X. ibizaensis, X. mediterranea* where included. Species names are set according to positions in the tree, whereas their names in the GenBank submissions, if different, are indicated within brackets references / footnotes (Fig. 1 tree, and Table 4).

An ITS-sequence extracted from the whole genome of *Xanthoria steineri* (Llewyllen *et al.* 2023) helps to confirm additional specimens of this species from Israel.

NEW MOLECULAR DATA

Sequences for some *Xanthoria* species are produced within this study for the first time. These are *Xanthoria ibizaensis* S. Y. Kondr et A. S. Kondratiuk, the holotype, described from the Balearic Islands (Kondratyuk *et al.* 2020), position in the *Xanthoria monofoliosa* subclade (Fig. 1), *X. streineri* I. M. Lamb, described from Iran, is confirmed from Israel in this study using a voucher by Scherrer and Honegger (2003) mentioned as *Xanthoria* sp. SH5-2001, *X. ectaneoides*, six vouchers, the new species *X. pylyporlykii* (described below), five vouchers, and *Xanthoria* aff. *aureola*, one voucher).

Xanthoria ectaneoides (Nyl.) Zahlbr.
(= *Physcia ectaneoides* Nyl.)

France: 'Monspelii, Lavalette' [Herault, Montpellier], lectotype, H-NYL 32723.

Thallus from almost undeveloped or network-like with a very narrow thalline main lobes where mostly apothecia make this lichen or '*anularis*' type i.e. similar to the Euroasian species *Kudratoviella anularis* (Clauzade et Poelt) S. Y. Kondr., L. Lőkös, Kärnefelt et A. Thell). The central portion of the thallus is degenerate, thus only peripheral parts are present (Kondratyuk *et al.* 2022*a*), to an almost single-level or more or less film-like, rosette-formed thallus, i.e. the *X. coomae* type of thallus, where separate lobes are not seen in the centre. Comparatively distinctly developed in the peripheral zone; small secondary sublobules present in the centre, often originating from remains of overmature apothecia as narrow overlapping parts.

Apothecia small, 1(–2) mm diam., numerous, usually widely dispersed, rarely crowded, in the centre of the thallus, lecanorine with more or less plane disc, usually with a smooth, rarely crenulate, thalline margin; ascospores nar-

row and rather long, 15–18(–20) × 5–7 μm with very wide ascospore septum 10–13 μm.

Xanthoria ectaneoides and *X. pylyporlykii* form sister branches in the *Xanthoria ectaneoides* subclade (Fig. 1). *Xanthoria ectaneoides* used to be recognised exclusively by its secondary sublobules. In this study, species delimiting characters of the ascospores are added. Its closest relative, *Xanthoria pylyporlykii* is

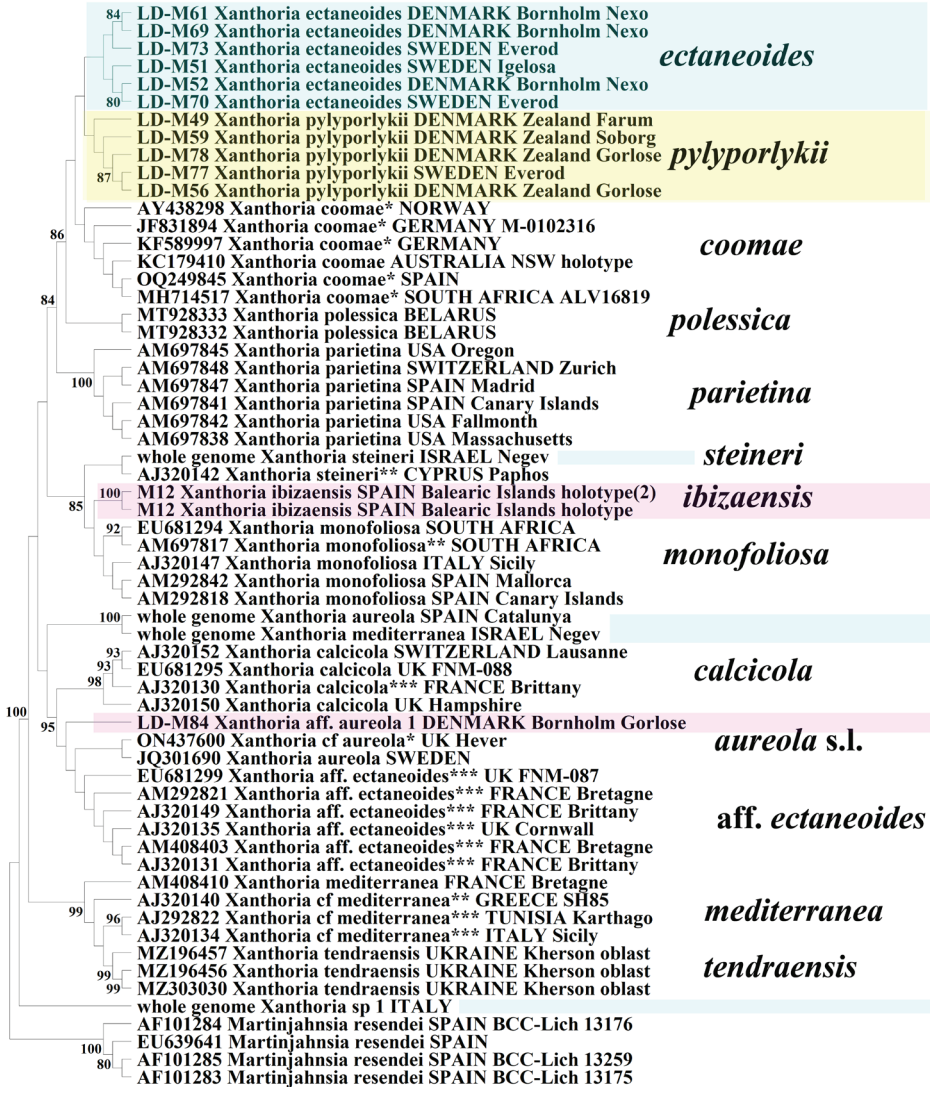

Fig. 1. Consensus MP tree after nrITS of the members of the genus *Xanthoria*. Abbreviations: * = data are submitted to GenBank under *Xanthoria parietina*, ** = data provided under *Xanthoria* sp., *** = data are provided under *Xanthoria ectaneoides*

lacking secondary sublobules and differ by shorter ascospores and narrower ascospore septa, the *X. coomae* type.

Ecology: This species was described from limestone. From our study it is rather common in the southwestern Baltic area on hard substrates: tiles, concrete, metal and granite, as well as rarely collected on bark of trees.

Distribution: Since this species was considered a synonym of *X. aureola*, data on ecology and distribution are incomplete during the latest decades and therefore not considered. *Xanthoria ectaneoides* is found at more than 30 localities in Sweden, Denmark and Germany (Fig. 1, Table 1), however, is probably widely distributed in the European continent.

Taxonomic notes: *Xanthoria ectaneoides* has a characteristic thallus with a smooth central part, numerous secondary sublobules, lecanorine apothecia usually distantly spread, emarginate or with subconvex disc at overmature, the ascospores are the longest observed in the genus and the septum is the widest in the genus.

Material referred to as '*Xanthoria ectaneoides* sensu German lichenologists' was typically used for sterile thalli densely covered by secondary lobules. Although we believe that most of these thalli really was true *X. ectaneoides*, this

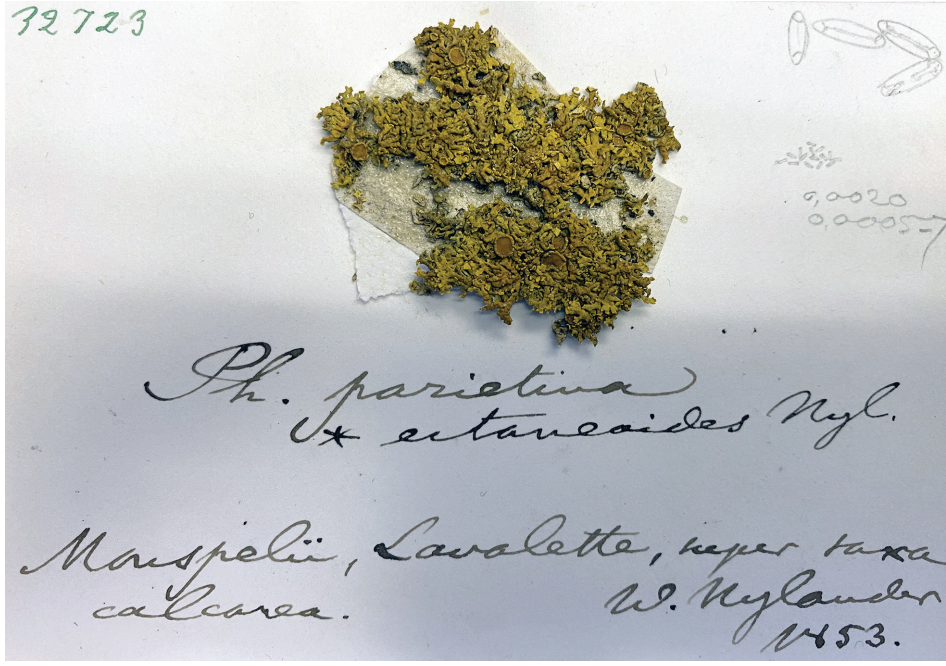

Fig. 2. The type specimen of *Xanthoria ectaneoides* (Nyl.) Zahlbr. (lectotype, H-NYL 32723), with Nylander's line drawings and handwritten measurements of 'conidia'. These, however, were in fact ascospores

Fig. 3. *Xanthoria ectaneoides* (Nyl.) Zahlbr. (photo in field condition, Lund 25.04.2023)

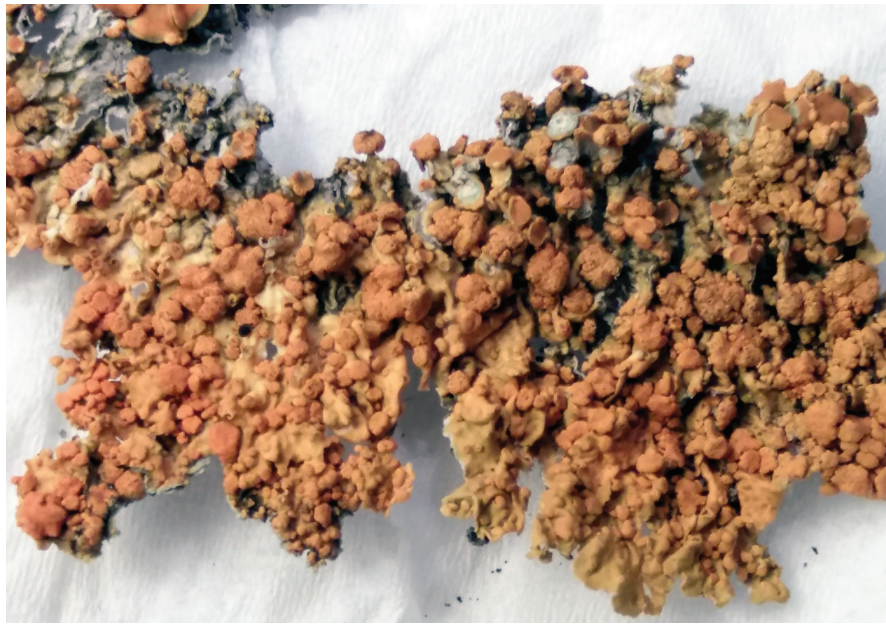

Fig. 4a. *Xanthoria ectaneoides* (Nyl.) Zahlbr. (SK23707, nrITS voucher LD-M52); host thallus heavily damaged by lichenicolous fungus *Telogalla olivieri* s. l.)

Fig. 4b. *Xanthoria ectaneoides* (Nyl.) Zahlbr. (SK23707, nrITS voucher LD-M52)

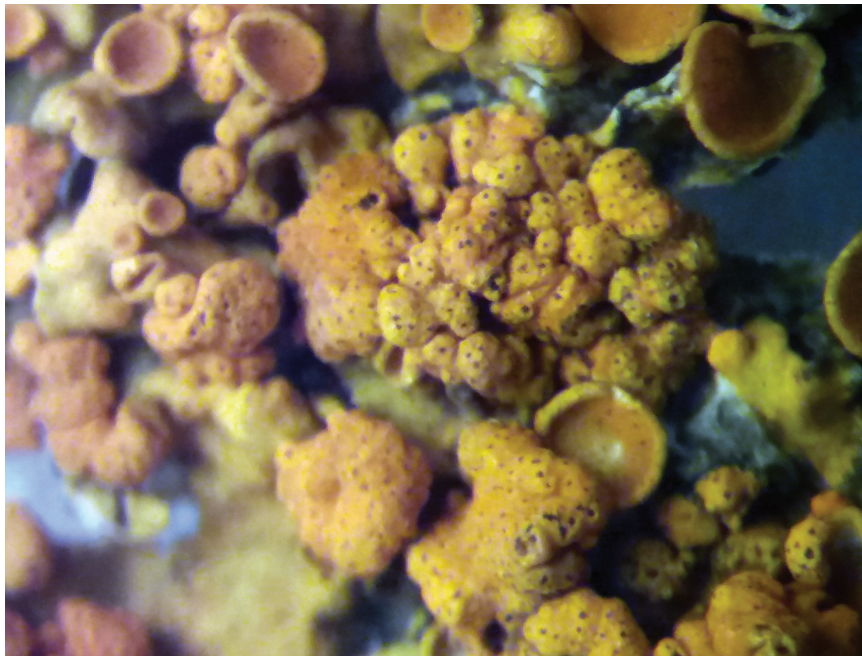

Fig. 4c. *Xanthoria ectaneoides* (Nyl.) Zahlbr. (SK23707, nrITS voucher LD-M52)

species appears as richly fertile in this study, with a variable development of the thallus.

Nylander's species *Physcia ectaneoides* (= *Xanthoria ectaneoides* (Nyl.) Zahlbr.) was accepted as a questionable taxon with numerous, very long and strap-shaped lobes, with a bulky appearance in the Mediterranean and Atlantic Europe, considered as a synonym of *X. aureola* (Gaya *et al.* 2012, 2015, Lindblom and Ekman 2005). This approach is not followed in this study since the two species are not the closest relatives but belong to different subclades.

The unfortunate confusion of measurements of conidia and apothecia in Nylander's original description have caused incorrect conclusions. At any case, *X. ectaneoides* wears the longest ascospores and widest septa of the genus. It is often supplied with sublobulae in the centre, which are lacking in closely related *X. coomae*. *Xanthoria ectaneoides* was earlier recognised by a bulky thallus due to numerous strap-like, overlapping lobes and by *X. parietina* type ascospores (see also *Xanthoria* sp. 2). However, true *X. ectaneoides* has no strap-like lobules forming a bulky thallus, but much shorter and narrower lobes, named '*secondary sublobules*', a term introduced here.

The systematic position of *X. ectaneoides* and the new species *X. pylporlykii* constitute a sister branch to *X. coomae* (see Fig. 1).

The position of *Xanthoria ectaneoides* is confirmed by five specimens, which are morphologically and anatomically identical with Nylander's type specimen of *Physcia ectaneoides*, however, the specimen is too old for a molecular study.

Most specimens determined as *Xanthoria ectaneoides* sensu German lichenologists based upon of numerous thalline sublobules on rocky surface, most likely belong to true *Xanthoria ectaneoides*. Richly fertile specimens of *Xanthoria ectaneoides* are common both in Scandinavia and Germany and can easily be confirmed by their nrITS-sequences (Table 1).

Specimens of *Xanthoria ectaneoides* examined: Nexø SK23703 (voucher LD-M61 for nrITS), SK23711 (voucher LD-M69 for nrITS), SK23707 (voucher LD-M52 for nrITS), SK23793, SK23795, SK23799, SK23800, SK23717, SK23719, SK23712, SK23793, SK23800. – Stehag SK23908, SK23890G, SK23890K, SK23905C, SK23901. SK23917B, SK23917, SK23971, SK23970, SK23968, SK23966, SK23964, SK23487, SK23488, SK23969, SK23963, SK23900. – Tårnby SK23932, SK23927D, SK23927C, SK23927B, SK23922E, SK23922C, SK23922B, SK23922, SK23917C, SK23917D, SK23917C, SK23939, SK23933F, SK23933E, SK23933D, SK23933C, SK23933B, SK23917, SK23930, SK23952. – Tarnby 918, 932, 931, 929, 928, 926, 924, 923, 922, 921, 920, 919, 933, 934, 945, 946, 947, 949, 950, 951, 954, 955, 956, 957, 958, 959, 962. – Marstal, concrete wall at sea coast, 27v23 SK23847; Sandby / Fanefjord 524, 527; S Malmo 528; Norra Vrams 446, 447, 491; Nyker 450, 451; Everöd 555 (nrITS voucher LD-M70), 568; Igelösa 774; Skanor kirka 360. – Germany: Rostock SK23A12, SK23A11, SK23A09, SK23A01, SK23A04; Cammin, 1x23 SK23A29; Russow SK23995, SK23994, SK23988, SK23987, SK23986, SK23985, SK23990; Alt Barlow SK23974, SK23975, SK23976, SK23978, SK23983, SK23977. – Farum SK23502 (nrITS voucher LD-M68), 506, 515, 572, 497, 535, 575, 571; Nylor 466, 467; S of Helsingborg 468, 469; SE Trelleborg 471, 484, 485; Igelösa 775; Tofta 779.

The *Xanthoria coomae* subclade is in need of an extended revision. *Xanthoria coomae* is commonly confused with the morphologically similar *Xanthoria parietina* (Fedorenko *et al.* 2009), whereas *X. ectaneoides* may include additional taxa.

Xanthoria sp. 2

The name *Xanthoria ectaneoides* was hitherto used along the coastal zone of Atlantic Europe for specimens with a narrow, strap-like, often semierect or semi-ascending lobes forming a rather bulky thallus, not considering the appearance of the ascospores. Such material is not conspecific with *Xanthoria ectaneoides* and therefore called '*Xanthoria* sp. 2' in the phylogenetic tree (Fig. 1). This material, positioned in the *Xanthoria calcicola* subclade, will be revised in a future study since it cannot belong in *Xanthoria ectaneoides* s. str. (Fig. 1).

Fig. 5. The type specimen of *Xanthoria aureola* (Ach.) Erichsen (lectotype, H-NYL 32723)

Xanthoria aureola (Ach.) Erichsen

Basionym: *Parmelia aureola* Ach. – Lichenogr. Univ.: 437 (1810).

Type: Sweden, 'Suecia' [Bohuslan province (= 'Bahusia'), on seashore rocks] (H-ACH 1300 – lectotype, designated by Lindblom and Ekman 2005).

The description of *Xanthoria aureola* needs to be revised since it includes descriptions of both *Xanthoria aureola* s. str. (apothecia almost always absent, poorly developed ascospores etc.) and *Xanthoria* sp. 2 (thalline lobes strap-shaped 0.3–1.3 mm wide, to slightly ascending, often irregularly overlapping in thallus centre, without vegetative diaspore etc.).

The small, sterile and old type specimen of *Xanthoria aureola* is unusable for both spore- and DNA-studies (Fig. 5). It has rather wide and heavy thalline, closely attached to substrate, sometimes with lobe margins bent downwards, supplied with minute isidia, i.e. the type of isidia unique for the *X. calcicola* group. Thus, *X. aureola*, having minute isidia, is also morphologically different from both the non-isidiate and richly sublobulate true *X. ectaneoides* and the non-isidiate, strap-like lobulate species with a bulky thallus, here called *Xanthoria* sp. 2.

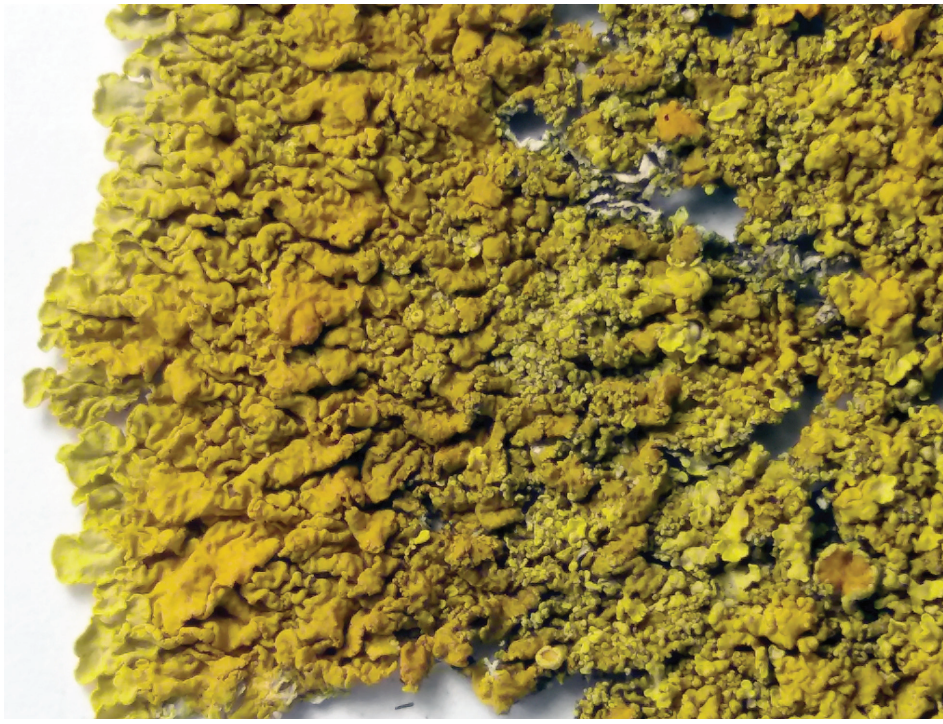

Fig. 6a. *Xanthoria* aff. *aureola* 1 (voucher LD-M84) from Gørløse locality, Bornholm, Denmark

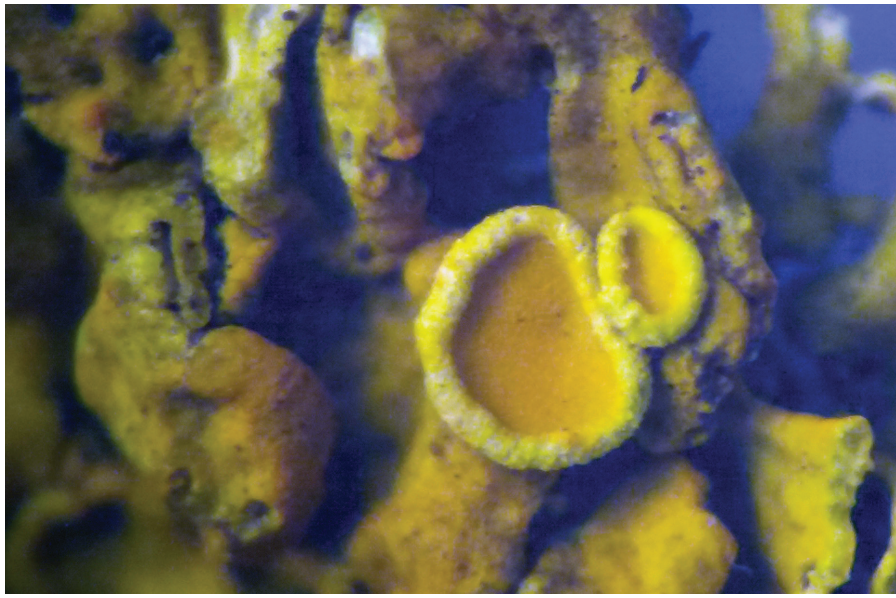

Fig. 6b. *Xanthoria* aff. *aureola* 1 (voucher LD-M84) from Gørløse locality, Bornholm, Denmark

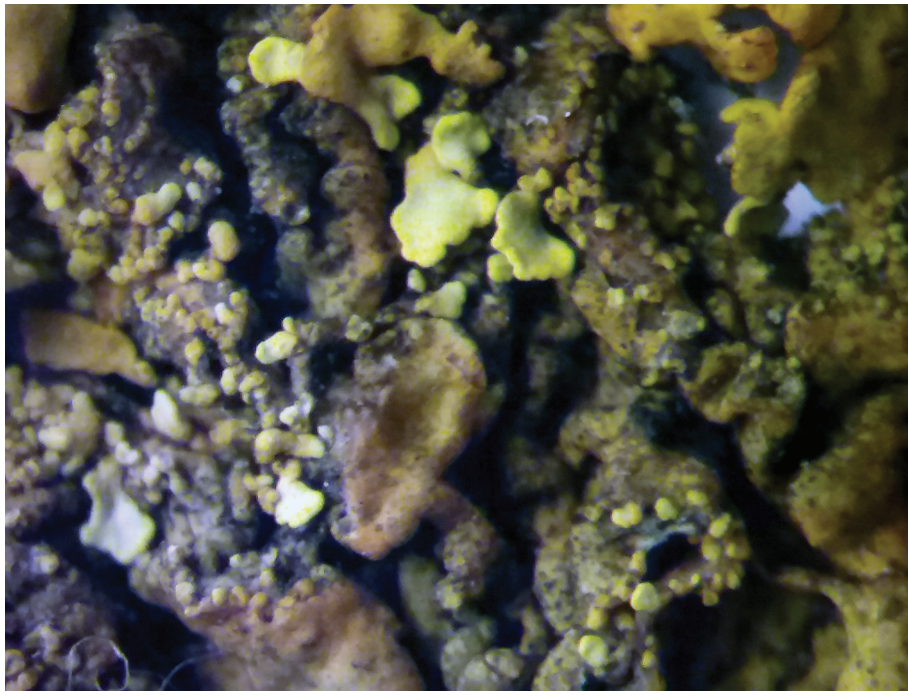

Fig. 6c. *Xanthoria* aff. *aureola* 1 (voucher LD-M84) from Gørløse locality, Bornholm, Denmark

Molecular data for *X. aureola* were published by Lindblom and Gaya with colleagues (Gaya *et al.* 2012, 2015, Lindblom and Ekman 2005, Llewyllen *et al.* 2023). They also revealed the position in the *Xanthoria calcicola* subclade for *X. aureola* (Gaya *et al.* 2012, 2015, Lindblom and Ekman 2005). Lindblom and Ekman (2005) proposed to synonymise *X. ectaneoides* (*Xanthoria* sp. 2) with the Acharian species *Xanthoria aureola.* However, *X. aureola* is neither conspecific with *Xanthoria* sp. 2 nor, and even less, with *X. ectaneoides* s. str. (Fig. 1).

Furthermore, *Xanthoria ectaneoides* sequences in the GenBank from Sicily, Italy and Tunisia probably belong to *Xanthoria mediterranea* (but not to *X. tendraensis*) or close, maybe undescribed relatives to these two taxa (see Khodosovtsev *et al.* 2023).

Xanthoria aff. *aureola* 1

A first attempt to select fertile collections for DNA-vouchers of *Xanthoria aureola* ~~were~~ made in this study. The richly fertile specimen with minute isidia from Gørløse in Zealand, Denmark (voucher LD-M84) was included in our phylogenetic analysis as *X. aff. aureola* 1 (Fig. 1).

Xanthoria pylyporlykii S. Y. Kondr., Kärnefelt et A. Thell, *spec. nova* (Fig. 7)

MycoBank No.: XXXXXXX

Similar to Xanthoria ectaneoides, *according to nrITS-sequences in sister position to* X. ectaneoides, *but differs in having wider thalline lobes, usually well-developed overlapping and irregularly orientated in the centre of the thallus, shorter ascospores and narrower ascospore septum.*

Type: Denmark, Søborg ruins SK23721 – holotype (voucher LD-M59 for nrITS) isotype as set for the Plantae Graecenses exsiccate is prepared; SK23721B – isotype; SK23723, SK23723C, SK23722B sub *Xanthoria pylyporlykii* with *Telogalla olivieri*, SK23723D sub *Xanthoria pylyporlykii* with *Athelia* killing the centre and infected by *Xanthoria* sp. 1 (particularly on killed portions) (KW-L); SK23723E sub *Xanthoria pylyporlykii* infected by *Xanthoria* sp. 1, SK23723F sub *Xanthoria pylyporlykii* infected by *Xanthoria* sp. 1, SK23723G sub *Xanthoria pylyporlykii* infected by *Xanthoria* sp. 1, SK23723H sub *Xanthoria pylyporlykii* infected by *Xanthoria* sp. 1 (KW-L) – isotypes).

Thallus foliose, usually well developed and regularly rounded, of rather wide range from small to 3–5(–9) cm across, more or less rounded to widely oval / ellipsoid or forming much larger irregular aggregations; often with damaged, blackening or completely killed and collapsed central portions, forming film-like centre sometimes with distinct wrinkles or indistinct or cov-

ered with numerous apothecia, while lobes with reticulate upper surface are more or less developed only in the peripheral zone (without apothecia) to 5–7(–10) mm wide; sometimes brighter, yellow in the peripheral zone with the thicker and darker, greenish centre (distinctly greenish especially in wet conditions and very similar to *Xanthoria calcicola*) are rather different and contrasting, especially when the secondary, flat and horizontally orientated lobes are present in the centre; sometimes forming incomplete circles ('arcs') 1.5–2 cm wide and to 10 cm long, or wide circles (to 10–20 cm diam., where the central portion is completely collapsed) where narrow peripheral zone and numerous apothecia from the inner side of such circles are developed, similar to *Kudratoviella anularis* (Kondratyuk *et al.* 2023)

Thalline lobes thin, paper-like, 5–7(–10) long and 1–1.5(–2) mm wide in the narrowest portions while to 3–5(–7) mm wide in the widened terminal portions; sometimes secondary lobules are overlapping and irregularly orientated in the centre and well developed, somewhat 'triangle' to 1–1.5(–3) mm across forming variegated colouration owing bright yellow tips and well contrasting to darker greyish- or greenish-whitish towards the centre, somewhat raised above the thalline centre, horizontally orientated.

Thallus in section to (75–)100–140(–180) μm thick, upper cortical layer 5–7(–12) μm thick, sometimes irregularly developed, paraplectenchymatous, lumina to 5–7(–12) μm diam./across; algal zone to (25–)30–50(–70) μm thick,

Fig. 7. Xanthoria pylyporlykii S. Y. Kondr., Kärnefelt et A. Thell (holotype)

medullar layer to 50–70 μm thick, the lower cortical layer to 10–15(–20) μm thick, paraplectenchymatous with vertically slightly elongated cells.

Apothecia 1–3(–5) mm diam., in section to 0.2–0.3 mm thick from not very numerous, more or less distant, to rather numerous and crowded, usually distinct due to dark orange discs, contrasting the yellow thallus, more or less raised, lecanorine, initially with a permanent thalline margin, developing to zeorine or biatorine, discs more or less plane to slightly concave (reminding of *Xanthoria calcicola*), undulating at overmature, thalline margin distinctly raised above the disc giving the apothecia a concave impression, often becoming crenulate, or only in form of portions to distinctly zeorine, crowded at overmature; in zeorine or biatorine apothecia: true exciple rather thin, permanent, concolorous with the disc or slightly lighter, rather distinct; in section, thalline exciple to 125 μm thick with cortical layer to 25–30 μm thick on underside; true exciple to 120–150 μm thick in the uppermost lateral portion and to 20–25 μm thick in the lower basal and basal portions; hymenium to 55–60 μm thick; ascospores sometimes with somewhat attenuated ends, more or less widened at the septa, (10–)11–15(–15.5) × 6–7.5(–8) μm, septa (5–)7–10 μm wide.

Conidiomata to 250–270 μm diam., hyaline, situated between the upper and the lower cortex of thallus; conidia very small ellipsoid, (1–)1.5–2.5(–3) μm.

Ecology: on rocky walls, tile roof of rock walls, on brick fragments of old ruins (Søborg, Hammershus castle ruins, etc.), often growing side by side with *Xanthoria ectaneoides* and growing together or overgrowing *Physcia adscendens*, *Phaeophyscia orbicularis*, etc. *Xanthoria pylyporlykii* is also confirmed from bark of *Acer platanoides* in coastal zone, so far only from a few localities in Æroskobing (SK23814, SK23815), Marstal (SK 23816, SK 23817) and Tranderup (SK23818), all within Ærø Island.

Xanthoria pylyporlykii is host for several lichenicolous fungi, e.g. *Telogalla olivieri*, *Bryostigma parietinaria*, *Pyrenochaeta xanthoriae*, *Athelia arachnoidea*, *Xanthoriicola epiphysciae* of which the first three are very common. The entire collection of *Xanthoria pylyporlykii*, 27 specimens at the locality Svendborg Landevej, southern Funen, and 23 specimens in Søby on Ærø were damaged by *Telogalla olivieri*, compared with only 30% in Fjenneslev, western Zealand. The number of infected specimens was much lower at other localities.

A rather high number of specimens of *Xanthoria ectaneoides* and *X. pylyporlykii* damaged by *Telogalla olivieri* was recently collected in Svaneke and Rønne on Bornholm.

Distribution: *Xanthoria pylyporlykii* is confirmed by more than 215 specimens from 44 localities around the western part of the Baltic Sea, i.e. in Skåne, southernmost Sweden, southern Denmark and northern Germany (Table 1), however it is probably distributed also in other Atlantic parts of Europe.

Etymology: It is named after Pylyp Stepanovych Orlyk (11[21] October 1672–26 May 1742), author of the famous 'Constitution of Pylyp Orlyk', Hetman of Ukraine in-exile, secretary and close associate of Hetman Ivan Mazepa as well as his successor. Pylyp Orlyk lived in Kristianstad with his family after an official invitation from the Swedish king Karl XII. Latin version of 'Constitution of Pylyp Orlyk' with his signature is still kept in Sweden.

Taxonomic notes: After morphological characters *Xanthoria pylyporlykii* combines characters of *Xanthoria ectaneoides* in having *Xanthoria coomae* type of thallus and secondary lobules in the centre and *Xanthoria calcicola* in having greenish (especially in wet condition) centre, which is different from peripheral zone.

Xanthoria pylyporlykii is similar to *Xanthoria ectaneoides*, its closest relative according to the phylogeny based on nrITS-sequences, however the new species usually differs in having a well-developed and regularly rounded thallus with a wider peripheral zone, (5–7(–10) mm wide vs. 1–3 mm wide in *X. ectaneoides*, in the lack of secondary sublobules (*vs*. especially numerous in the centre of *X. ectaneoides*; in having wider thalline lobes, usually well-developed, overlapping and irregularly orientated in the centre of the thallus, as well as and in having shorter ascospores with shorter septa.

Xanthoria pylyporlykii usually reminds of *Xanthoria calcicola* in having a dark greenish thallus centre wearing secondary lobes, but differs by much larger, flat and horizontally orientated secondary lobes with a smooth surface, as well as in having much wider ascospore septum.

Xanthoria pylyporlykii and *Xanthoria coomae* both have horizontally orientated, overlapping secondary lobes in the centre (Everöd, SK23498, SK23544, SK23545, SK23546), lobes developed only in the peripheral zone, however, *X. pylyporlykii* has a thinner thallus centre with a smooth surface (not wrinkled as in *Xanthoria coomae*). Furthermore, *X. pylyporlykii* differs by smaller, both narrower and shorter, and horizontally orientated thalline lobes in the narrower peripheral zone and in having shorter ascospores.

Two sequences in the GenBank, MT644879 and KJ027710, submitted as *Xanthoria parietina* probably represent *Xanthoria pylyporlykii*.

Selected specimens of *Xanthoria pylyporlykii*: Denmark, Søborg roof SK23723J, SK23723I sub *Xanthoria pylyporlykii* growing together with *Xanthoria ectaneoides*, SK23723K, SK23723L sub *Xanthoria pylyporlykii*, SK23723M, SK23723N, SK23723O, SK23723P, SK23723R. Paratype specimens of *Xanthoria pylyporlykii* from Søborg will be distributed as set of the Plantae Graecenses exsiccate. – Sweden, Skåne, Kristianstad municipality, Everöd, tile roof of rocky wall around church and cemetery, 4 March 2023 Coll.: S. Kondratyuk SK 23498 (KW-L) sub *Telogalla olivieri* s.l. on rather damaged thalli of *Xanthoria pylyporlykii* with *Xanthoriicola epiphysciae*; SK 23499 (LD) (nrITS voucher LD-M77) sub *Xanthoria pylyporlykii* partly infected by *Xanthoria* sp. 1c***; SK 23498; SK23540 (voucher LD-M73 for nrITS), Everöd SK23569 (voucher LD-M75 for nrITS); Everöd SK23546 sub

Xanthoria pylyporlykii with *Athelia arachnoidea*, SK23548 sub *Xanthoria pylyporlykii* with *Telogalla olivieri*, SK23554 sub *Xanthoria pylyporlykii*, growing side by side with *Xanthoria ectaneoides*, SK23555 sub *Xanthoria ectaneoides*, growing side by side with *Xanthoria pylyporlykii*, SK23556 sub *Xanthoria pylyporlykii*, growing side by side with *Xanthoria ectaneoides*. – Helsinge, roof, 26.03.2023 SK23751 (nrITS voucher LD-M79); SK23741B LD-M79 voucher 742; SK23741 [7 of 8 z 742] ~~zriz~~ 741 sub *Xanthoria pylyporlykii* and *Xanthoria* sp. 1***, SK23741H [3 of 8], SK23741C [6 of 8], SK23741D [5 of 8] sub *Xanthoria pylyporlykii* with *Athelia* in places of the centre, SK23741E [4 of 8], SK23741G [1 of 8] sub *Pyrenochaeta xanthoriae* on *Xanthoria pylyporlykii*, SK23741 'B' [8 of 8] zriz 742 = M79 sub *Xanthoria pylyporlykii* growing together with *X. ectaneoides*; Helsinge SK23747 (voucher for nrITS sequence LD-M55) sub *Xanthoria pylyporlykii* with *Pyrenochaeta xanthoriae*, and with small addition of *Xanthoria ectaneoides*. – Denmark, Søborg ruins SK23720 zriz 720; SK23720B smaller of 720; SK23720C *Xanthoria pylyporlykii* growing together with *Xanthoria* cf. *ectaneoides*; roof, SK23722 sub *Xanthoria pylyporlykii* partly infected by *Xanthoria* sp. 1c. – Nexo, 28.10.2022 on separate rocks near rocky wall SK23716 (voucher LD-M53 for nrITS), SK23716B. – Helsinge 26.03.2023, tile roof of rocky wall Coll.: S. Kondratyuk SK23748 (voucher LD-M72 for nrITS) sub *Pyrenochaeta xanthoriae* on *Xanthoria pylyporlykii* [centre killed by lichenicolous fungus]; SK23749 (voucher LD-M54 for nrITS), SK23747 (voucher LD-M55 for nrITS) sub *Xanthoria pylyporlykii* with *Pyrenochaeta xanthoriae*; 26iii2023 roof of eastern wall SK23751 (voucher LD-M71 for nrITS) sub *Xanthoria pylyporlykii* growing together with *Xanthoria ectaneoides* and partly infected by *Xanthoria* sp. 1. – ***; SK23584 sub *Xanthoria pylyporlykii* partly infected by *Xanthoria* sp. 1. – ***, SK23585 sub *Xanthoria pylyporlykii* partly infected by *Xanthoria* sp. 1. – *** and *Telogalla olivieri*, growing side by side with *Physcia adscendens* and *Phaeophyscia orbicularis*; SK23741G sub *Xanthoria pylyporlykii* with *Pyrenochaeta xanthoriae*. – Denmark, Søborg ruins SK23763 sub *Xanthoria pylyporlykii* with *Telogalla olivieri*, SK23764 sub *Telogalla olivieri* on *Xanthoria pylyporlykii*, SK23766B sub sub *Xanthoria pylyporlykii* with *Telogalla olivieri*, growing together with *Xanthoria calcicola*; SK23768C sub *Xanthoria pylyporlykii* with *Telogalla olivieri* and *Xanthoria* sp. 1. – ***, SK23768E sub *Xanthoria pylyporlykii* with *Telogalla olivieri*; SK23595 sub *Xanthoria pylyporlykii* with *Telogalla olivieri*, sets for the Plantae Graecenses exsiccate (one as *Telogalla olivieri* on *Xanthoria pylyporlykii*) with specimens of this collections (as SK23595B, SK23595C, SK23595D, etc) are prepared; SK23701 (voucher LD-M81 for nrITS) sub *Xanthoria pylyporlykii* partly infected by *Xanthoria* sp. 1***; SK23701B [2 of 5] sub *Xanthoria pylyporlykii* partly infected by *Xanthoria* sp. 1***; SK23701C [4 of 5] sub *Xanthoria pylyporlykii* partly infected by *Xanthoria* sp. 1***, SK23701D [3 of 5] sub *Xanthoria pylyporlykii* partly infected by *Xanthoria* sp. 1***, SK23701E [5 of 5] sub *Xanthoria pylyporlykii* partly infected by *Xanthoria* sp. 1***. – Stehag SK23878 sub *Xanthoria pylyporlykii* with *Telogalla olivieri*, SK23885B sub *Xanthoria pylyporlykii* killed by *Bryostigma parietinaria*, SK23885E sub *Xanthoria pylyporlykii* damaged by *Bryostigma parietinaria*, and by *Phoma* sp., SK23893C sub *Xanthoria pylyporlykii* with *Telogalla olivieri*, SK23893D sub *Xanthoria pylyporlykii* with *Bryostigma parietinaria*, SK23890L sub *Xanthoria pylyporlykii* with *Telogalla olivieri*, *Bryostigma parietinaria*, [and other lichens], SK23890H sub *Xanthoria pylyporlykii* with *Bryostigma parietinaria*, [and other lichens], SK23897C sub *Xanthoria pylyporlykii* with *Telogalla olivieri*, SK23897D sub *Xanthoria pylyporlykii* with *Telogalla olivieri*. – Farum SK23495 sub *Xanthoria pylyporlykii* killed by *Telogalla olivieri*, SK23498 sub *Xanthoria pylyporlykii* with *Telogalla olivieri*, SK23509 sub *Xanthoria pylyporlykii* with *Telogalla olivieri*, SK23511 sub *Xanthoria pylyporlykii* with *Telogalla olivieri*, SK23512 sub *Xanthoria pylyporlykii* with *Telogalla olivieri*, growing side by side with *Xanthoria ectaneoides*; SK23534 sub *Xanthoria pylyporlykii*, growing side by side with *Xanthoria ectaneoides*; SK23535 sub *Xanthoria ectaneoides*, growing side by side with *Xanthoria pylyporlykii*; SK23536 sub *Xanthoria pylyporlykii*, growing

side by side with *Xanthoria ectaneoides*; Farum 576 (nrITS voucher LD-M49). – Norra Vrams SK23492 sub *Xanthoria pylyporlykii* with *Telogalla olivieri*, SK23493 sub *Xanthoria pylyporlykii* with *Telogalla olivieri*. – Tofta SK23788D sub *Xanthoria pylyporlykii* with *Athelia archnoidea*. – Gørløse SK 23329 (voucher LD-M56 for nrITS). – Malmo 12.07.2022 SK22049 (and section 49, and section 587) (voucher LD-M78 for nrITS), SK22049B, SK22049C. – Coast 9.06.2022, wood SK22046 (voucher LD-M66 for nrITS).

***Initial thalli of *Xanthoria* sp. 1 are especially numerous on damaged and decaying portions of thalli of *Xanthoria pylyporlykii*. Status of *Xanthoria* sp. 1 is under revision including molecular phylogenetic study and will be discussed elsewhere. Within the first observations of the lichenicolous fungi associated with saxicolous specimens of *Xanthoria calcicola* s. lat. in southernmost Scandinavia (see Kondratyuk *et al.* 2023), it was found as lichenicolous lichen species with the field name '*Xanthoria* aff. *calcicola*'. It was recorded from a number of localities and originally was considered among lichenicolous fungi. However, after finding richly fertile specimens growing on rock surface as well as on bark of trees it was excluded from list of true lichenicolous fungi.

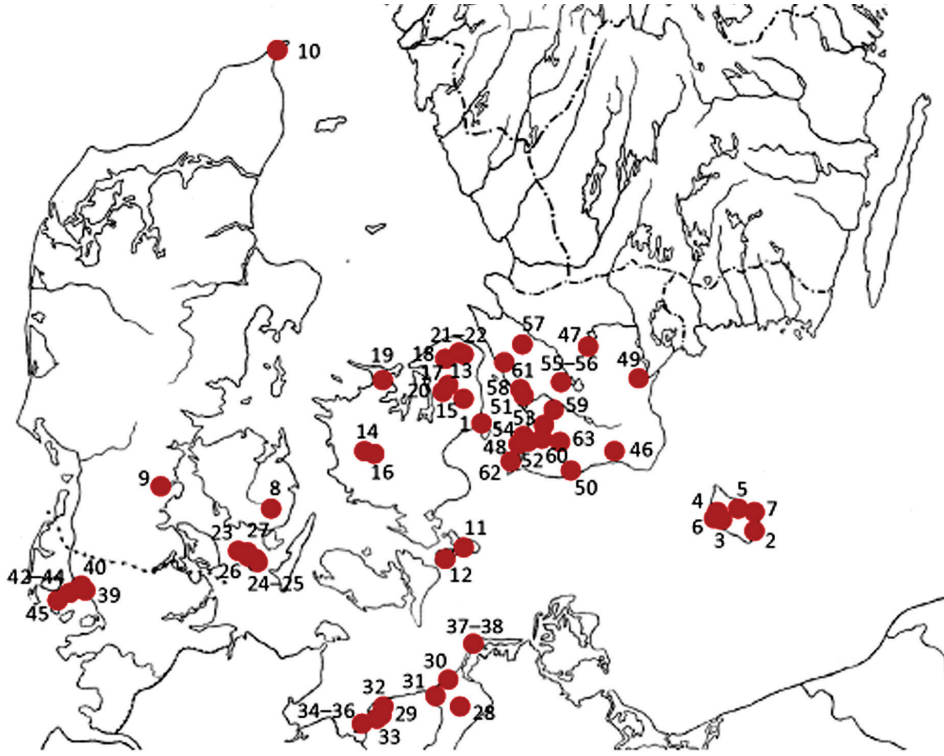

Fig. 8. Distribution of *Xanthoria ectaneoides* complex in the southwestern part of the Baltic Sea area: 1 = *Xanthoria ectaneoides*, 2 = *Xanthoria pylyporlykii*

Xanthoria pylyporlykii from the *Xanthoria coomae* subclade known so far from Sweden, Denmark and Germany which is similar to *X. ectaneoides* but differs in having a more developed thallus and in having small and rather richly dissected into narrower positions thalline lobes as well as in having ascospores with narrower ascospore septum is a rather common epilithic lichen in the investigated region growing side by side with *Xanthoria calcicola* and other taxa. It is characterised by rather variegated to very large size thallus similar to the *Xanthoria coomae* type of thallus, with a more or less film like centre, and thalline lobes, developed only in the peripheral zone, with narrower and shorter thalline lobes than those characteristic for *Xanthoria parietina*, usually with numerous to very crowded apothecia as well as minute secondary thalline lobules in the centre of the thallus.

Molecular data from *Xanthoria ectaneoides*, *X. ibizaensis* and *X. steineri* are for the first time included in a phylogeny of the genus. *Xanthoria ectaneoides* and *X. pylyporlykii* were both revealed to belong to the *Xanthoria coomae* subclade of the genus.

Additional specimen of *Xanthoria steineri* and position of this species in the phylogenetic tree of the genus *Xanthoria* based on nrITS sequences retrieved from the whole genome data of *X. steineri* are illustrated.

*

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NEW AND NOTEWORTHY LICHEN-FORMING AND LICHENICOLOUS FUNGI 14. *XANTHORIA PEDERSENII* AND *X. WENNERGRENII* – TWO NEW SPECIES FROM THE *XANTHORIA CALCICOLA* SUBCLADE (XANTHORIOIDEAE, TELOSCHISTACEAE) PROVED BY INTEGRATIVE APPROACH

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Two new for science species differing by complex of morphological, anatomical and molecular characters, i.e.: *Xanthoria pedersenii* and *X. wennergrenii* from the *Xanthoria calcicola* subclade of the phylogenetic tree of the genus *Xanthoria* (Xanthorioideae, Teloschistaceae) are described, illustrated and compared with closely related taxa.

Key words: ascospore septum type, ascospore type, Denmark, Germany, integrative approach, lichen-forming fungi, new species, Sweden, *Xanthoria*

INTRODUCTION

The name *Xanthoria calcicola* was proposed by the Ukrainian lichenologist A. Oxner at species level in 1937 for the infraspecific taxon *X. parietina* f. *congranulata* Cromb. described in the 19th century on the basis of two diagnostic characters, i.e. rugose centre of thallus and concave apothecia. Oxner has emphasised the importance of presence of isidia for this species.

Since the 1930s *Xanthoria calcicola* Oxner has been accepted as a rather common and widely distributed species in the Mediterranean and Atlantic Europe (Kondratyuk 2004, Nimis 1993, 2016, Nimis and Martellos 2023, Oxner 1937, Smith *et al.* 2009, Wirth *et al.* 2013). However, correlation of morphological and anatomical characters of taxa within *X. calcicola* complex was not hitherto analysed especially.

The aim of this paper is to present legal descriptions of two new for science species of the *Xanthoria calcicola* complex from the western part of Baltic Sea basin proved by integrative approach based on morphological, anatomical

and molecular data sets. This integrative approach in taxonomy of the genus *Xanthoria* (Fr.) Th. Fr. (i.e. correlation of morphological and anatomical characters (especially details of ascospores and ascospore septum) was elaborated by Austrian lichenologist J. Poelt with colleagues in the premolecular era in the 1990s (Giralt *et al.* 1993, Kondratyuk and Poelt 1997, Poelt and Petutschnig 1992*a*, *b*, etc.). A number of taxa described in the premolecular period with this approach has got confirmation in molecular phylogeny data set (Arup *et al.* 2013, Kondratyuk *et al.* 2013, 2017, 2020). Working hypothesis of this study was to check if correlation of three main groups of morphological (thallus, lobes, isidia, vegetative propagules), anatomical (including especially measurements of ascospores and width of ascospore septum), and molecular data on the same taxa of the *Xanthoria* are hitherto available.

Within our study of *Xanthoria calcicola* complex of the southwestern part of the Baltic Sea basin (see Kondratyuk *et al.* 2023, 2024) several species found to be included in this complex. Status of *Xanthoria ectaneoides* (Nyl.) Zahlbr. was thought to be synonymous to *Xanthoria aureola* (Ach.) Erichsen being positioned in the *Xanthoria calcicola* subclade. However, based on our revision by comparison of morphological and anatomical characters correlated with molecular phylogenetic data set, the *Xanthoria ectaneoides* complex was found to be positioned in the sister position to the *Xanthoria coomae* branch (excluded from the *Xanthoria calcicola* subclade) (Kondratyuk *et al.* 2024).

This communication includes the next portion of results, which connected to the taxa of the *Xanthoria calcicola* subclade of phylogenetic tree of the genus *Xanthoria*. Two taxa, i.e. *X. pedersenii* and *X. wennergrenii* are described below, as well as data on some other taxa, which are under revision with molecular data are mentioned, too.

Xanthoria is thought to be a well-known genus, but its taxonomy has been neglected for a long period, although seventeen lichen groups earlier including in the *Xanthoria* s. l. had been segregated in other genera since premolecular and molecular era (Kondratyuk *et al.* 2022*b*). Before the molecular era, this genus included *ca* 50 species (Kärnefelt 1989), but today only 13 species remain in genus *Xanthoria* in the strict sense (Kondratyuk *et al.* 2022*b*). Only a few papers thought to be confirming wide species concept of *Xanthoria parietina*, *X. aureola* were published with application of molecular data (Lindblom and Ekman 2005, Tsurykau *et al.* 2020). However, those results were based on less carefully identified voucher specimens, while molecular data themselves do not confirm wide species concept.

MATERIAL AND METHODS

Lichen-forming fungi of the *Xanthoria calcicola* complex occurring on various saxicolous substrates, i.e., rocks, bricks, and tiles, as well as on bark of trees

Table 1
List of localities of investigated epilithic communities of *Xanthoria calcicola* s. l. and portion of specimens infected by lichenicolous fungi ('–' means no infected thalli)

Locality	Date	Position	Number of specimens		
			totally collected	with *X. pedersenii*	with *X. wernergrenii*
DENMARK					
Bornholm, Nexø, on rock wall	28.10.2022	55.0629° N, 15.1250° E	56	1	–
Østerlars par., the church, on rock wall	29.10.2022	55.1648° N, 14.9656° E	2	2	–
Hammershus castle ruins, on brick walls at the highest point of ruins	27.10.2023	55.2713° N, 14.7554° E	57	–	7
Nyker par., the church, on rock wall	29.10.2022	55.1396° N, 14.7595° E	11	2	2
Fyn, Svendborg Landevej, on concrete	28.05.2023	55.1860° N, 10.7330° E	44	–	1
Møn, Borre par., the church yard, on tiles on the cemetery wall	22.09.2022	54.9959° N, 12.4432° E	6	1	–
Fanefjord par., the church, on tiles on the cemetery wall	11.10.2022	54.9013° N, 12.1511° E	23	5	–
Fjenneslev par., the church, on tiles on the cemetery wall	26.05.2023	55.4336° N, 11.6875° E	22	3	–
Gørløse par., the church, on tiles on the cemetery wall	3.12.2022	55.8853° N, 12.1991° E	22	10	–
Helsinge. the church yard, on tiles on the cemetery wall	26.03.2023	56.0208° N, 12.1969° E	170	2	–
Højby parish, the church, on tiles on the cemetery wall	25.06.2023	55.9128° N, 11.5996° E	15	2	1
Søborg par., the church, on tile roof	16.04.2023*	55.7352° N, 12.5120°S	70	6	–
Søborg par., the castle ruins, on modern brick inclusions	16.04.2023	55.0877° N, 12.3055°S	45	1	–
Ærø, Søby par., the church, on tiles on the cemetery wall	26.05.2023	54.9386° N, 10.2568° E	55	2	3
Marstal, the church, on tiles on the cemetery wall	26.05.2023	54.8550° N, 10.5170° E	59	4	6
Ærøskøbing, the church, on tiles on the cemetery wall	26.05.2023	54.8879° N, 10.4122° E	71	1	2
GERMANY					
Mecklenburg-Vorpommern, Rostock district, Cammin	2.10.2023	53.967° N, 12.333° E		–	1
Rostock district, Alt Bukow, the church, on tiles on the cemetery wall	2.10.2023*	53.9963° N, 11.6077° E		3	2

Table 1 (continued)

Locality	Date	Position	Number of specimens		
			totally collected	with *X. pedersenii*	with *X. wernergrenii*
Nordwestmecklenburg district, Blowatz-Dreveskirchen	2.10.2023*	53.9939° N, 11.5385° E		–	1
SWEDEN					
Skåne, Bromma par., the church, on rocky wall	28.09.2022	55.4707° N, 13.8001° E	34	2	1
Dalby par., the church, on rocky wall	date	55.6646° N, 13.3461° E		–	1
Malmö, Västra Hamnen, on granitic rocks	16.08.2022	55.6133° N, 12.9813° E	14	2	–
Norra Vram par., the church, on tiles on the cemetery wall	12.11.2022	56.0870° N, 12.9734° E	15	1	–
Ramlösa (S of Helsinborg), on roadside rocks near parking area	12.08.2022*	55.8056° N, 12.7333° E		1	–
Skanör par., the church, on vertical surfaces of thumbs at the cemetery	23.08.2022*	55.4195° N, 12.8497° E	1	1	–
Total					

in coastal zone were collected at 28 localities in southern Scandinavia (province Skåne of Sweden and southern Denmark (see also list of localities and maps in Kondratyuk *et al.* 2023, 2024) and Mecklenburg-Western Pomerania, Germany. However, for our taxonomic revision only about 10 plots were especially important owing to large number of substrate available for *Xanthorias*, as to time available for large collections and possibility to revisit some of them (i.e. Bromma, Everöd, Gørlose, Søborg, Marstal, Stehag, Norra Vrams, Helsinge, Farum, etc.).

Table 2
Sequences generated within this study.

Species, voucher number in the phylogenetic tree	Isolate	Country	nrITS
Xanthoria pedersenii	LD-M58	Denmark	LD-M58
Xanthoria pedersenii	LD-M60	Denmark	LD-M60
Xanthoria pedersenii	LD-M63	Denmark	LD-M63
Xanthoria pedersenii	LD-M67	Denmark	LD-M67
Xanthoria pedersenii	LD-M72	Denmark	LD-M72
Xanthoria wennergrenii	LD-M65	Denmark	LD-M65
Xanthoria wennergrenii	LD-M83	Denmark	LD-M83
Xanthoria wennergrenii	LD-M86	Denmark	LD-M86

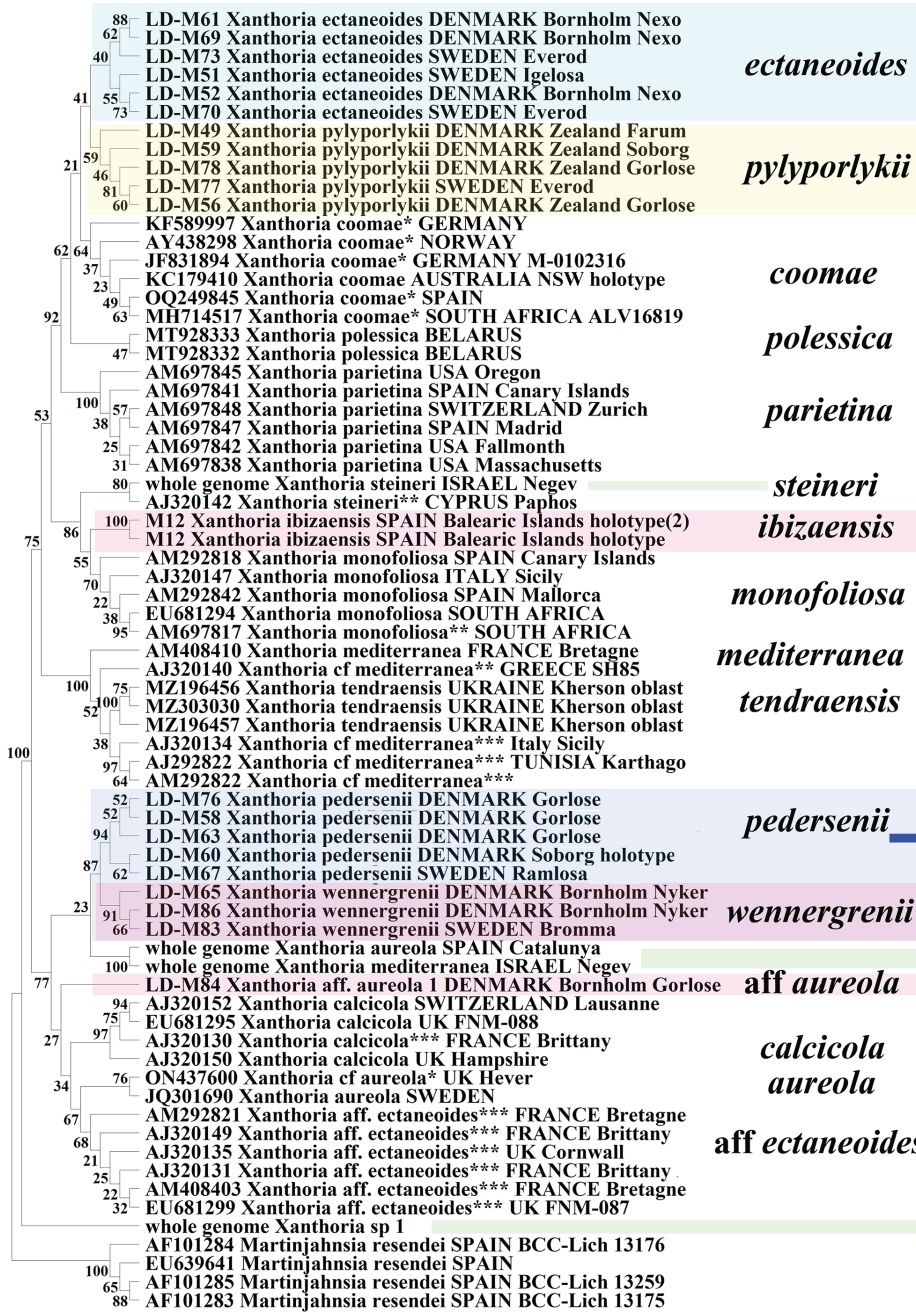


Fig. 1. Consensus MP tree after nrITS of the members of the genus *Xanthoria* for which hitherto molecular data available (* = data are submitted to GenBank under *Xanthoria parietina*, ** = data provided under *Xanthoria* sp., *** = data are provided under *Xanthoria ectaneoides*)

Specimens identified within this study are prepared for submission/deposition to C, GB, KW-L, LD and other herbaria. Furthermore, several sets including type specimens are prepared to be distributed within the Plantae Graecenses exsiccate.

A revision of morphological, anatomical, and molecular characters was done on the basis of own collections as well as of the type specimens of *Xanthoria aureola*, *X. calcicola*, *X. ectaneoides*, and some other taxa.

The specimens were sprayed with water preferably from ten minutes to half an hour before they were removed from the substrate. Mature apothecia were cut by hand. Fifteen sections of each apothecium were mounted in the same water droplet to contain a sufficient amount of ascospores, at least 50 in bright-field microscope, for statistic measurements. Ascospores were exclusively measured outside of asci and sections. At least 50 measurements of adult ascospores were performed and included in the further statistical analysis.

The specimens were studied and determined microscopically at the unit of Molecular Cell Biology, Department of Biology, Lund University. Voucher samples for extracting DNA were also prepared here.

RESULTS AND DISCUSSION

Molecular data on Xanthorias

To illustrate position of newly described taxa *Xanthoria pedersenii* and *X. wennergrenii* only a selection of the sequences available in the GenBank for members of the genus *Xanthoria* were included (see also Kondratyuk *et al.* 2024).

Sequences for the newly described *Xanthoria pedersenii* and *X. wennergrenii* produced within this study for the first time are shown in Table 2.

As it is shown from molecular phylogeny of the genus *Xanthoria* after nrITS sequences (Fig. 1) *Xanthoria pedersenii* and *X. wennergrenii* are positioned within the *Xanthoria calcicola* subclade of the phylogenetic tree of the genus *Xanthoria* (Xanthorioideae, Teloschistaceae).

New taxa

Xanthoria pedersenii S. Y. Kondr., I. Kärnefelt et A. Thell, *spec. nova*
(Fig. 2)

MycoBank No.: MB XXXXXXX

Similar to Xanthoria calcicola, *but differs in having thin, paper-like thalline lobes, sometimes long and irregularly orientated with more or less reticulate surface at tips and with wrinkled surface in the centre, in having smaller knob-like warts, which may resemble coarse isidia, which never fall off, in having much narrower as-*

cospores and much wider range of variation of ascospore septum, as well as in having slightly shorter, but wider conidia.

Type: Søborg, roof, 16.04.2023, coll.: S. Y. Kondratyuk SK23599 sub *Xanthoria pedersenii* growing together with *X. ectaneoides* – holotype, SK23599A, SK23599B; SK23599C sub *Xanthoria pedersenii* with *Xanthoria* sp. 1; SK23599D sub *Xanthoria pedersenii* with *Xanthoria* sp. 1; SK23599E, etc. till SK23599G sub *Xanthoria pedersenii* with *Xanthoria* sp. 1; SK23599K sub *Xanthoria pedersenii* with *Xanthoria* sp. 1; SK23599L sub *Xanthoria pedersenii* with *Xanthoria* sp. 1; SK23599N sub *Xanthoria pedersenii* with *X. pylyporlykii*; SK23599O sub *Xanthoria pedersenii* with *Xanthoria* sp. 1; and SK23599P sub *Xanthoria pedersenii* with *Xanthoria* sp. 1 – isotypes, this collection prepared to be distributed in the Plantae Graecenses exsiccate set).

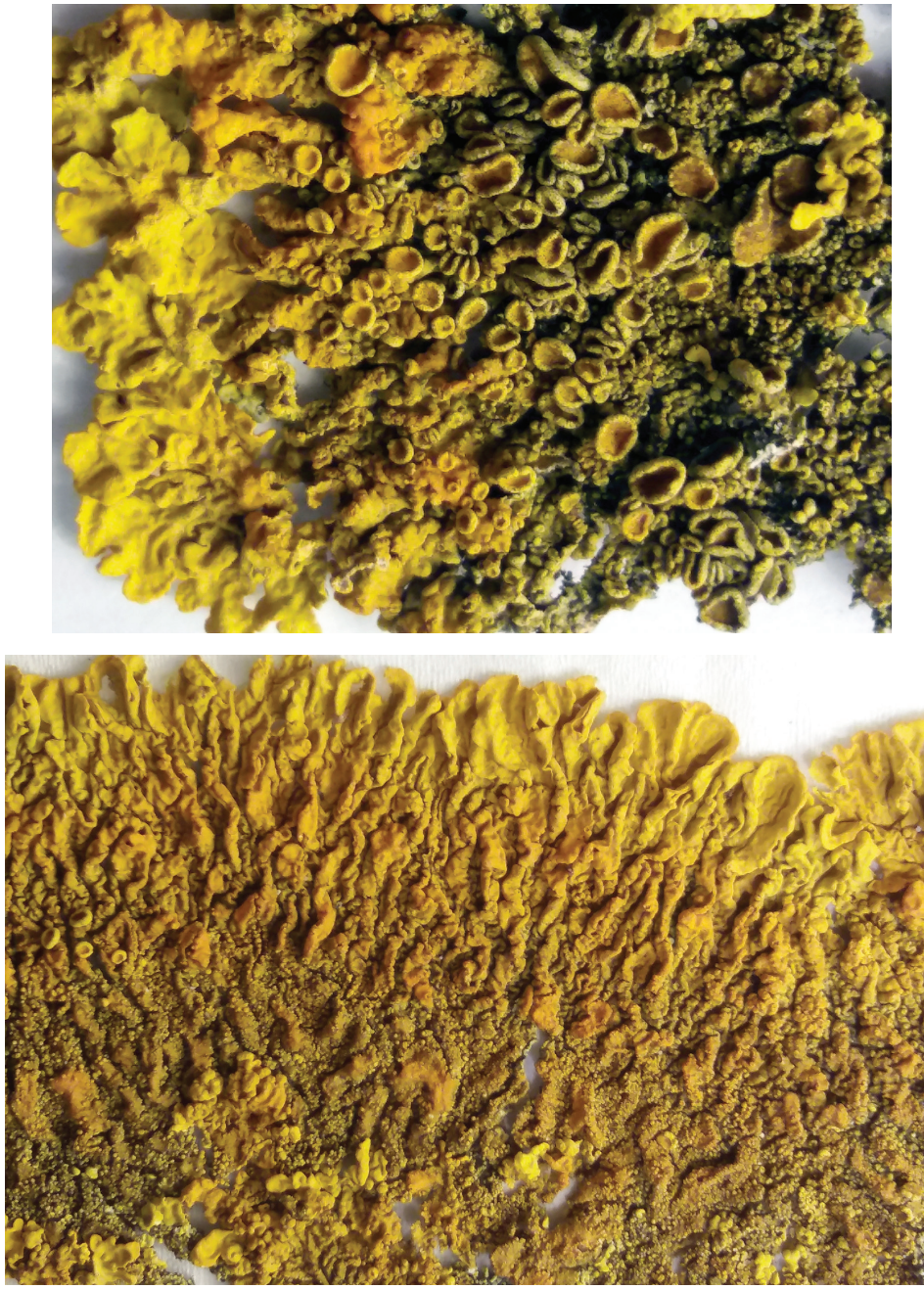
Thallus from 15–20 mm across to 3–7(–11) cm in diam./across while often forming larger aggregations; film-like in the centre, while dissections and lobes well developed in peripheral zone, *ca* (3–)5–7(–10) mm wide, sometimes badly presented or only in places well developed, but usually seen as incomplete circles, where only a half of thallus in semicircles (semi-acres), i.e. '*anularis*' morph (see also Kondratyuk *et al.* 2024), to 1.5–2 cm wide observed; thalline lobes (4–)5–7(–10) mm long, only rarely to 10–15(–18) mm long (SK22016, SK23599)*, and from 1–1.5(–2) mm wide in the narrowest portions, and widened towards the tips to (2–)4–5(–8) mm wide in peripheral zone, but dissected into smaller portions with total width to 5–7(–12) mm wide, very indistinct (especially towards the centre), very irregularly orientated; terminal portions of lobes to 2–3 mm wide, sometimes dissected into the smaller 0.5(–1) mm wide , and a. 1 mm long (well seen from underside) with more or less smooth surface (without or with rare wrinkles, i.e. thallus of *Xanthoria pylyporlykii* type, see Kondratyuk *et al.* 2024), light yellowish or whitish yellow, or greenish yellow, or greyish yellow, while centre wrinkled or uneven, dull orange or dirty greenish orange to dark brick or brownish orange or dark green-grey;

Secondary lobules to 0.3–1(–3) mm wide and to 1–3(–5) mm long to somewhat 'triangle' overgrowing terminal portions to 1–1.5(–3) mm across densely overgrowing overlapping and irregularly orientated with bright yellow tips well contrasting to dull orange, yellow or dull brownish yellow central portion of thallus, making variegated colouration of thallus centre are observed in places. Upper surface of lobes very wrinkled as radially as with cross wrinkles, very irregular especially in the centre. Lower surface white, with scarce white hapters of *Xanthoria parietina* type (sensu Kondratyuk and Poelt 1997) to 175 μm wide and 50–60 μm long.

* Material from *Fraxinus* bark, Ramlösa locality (voucher LD M67 SK22027) differs in having ~~so what~~ better developed/distinct thalline lobes towards the centre, and in having better seen cross wrinkles of these portions of thalline lobes. Thalline lobes of epiphytic specimens were from 3 mm wide in the narrower portions and to 4–10(–12) mm wide in terminal portions and to 10–15 mm long.

I would suggest to renumber the figures

Fig. 2. Xanthoria pedersenii, – a = general view (holotype); b = general view (isotype, 559E); c = enlarged peripheral zone with lobes (isotypes, 599A, 599F); d = enlarged portion of thallus with isidia (isotype, 559A, 599A)



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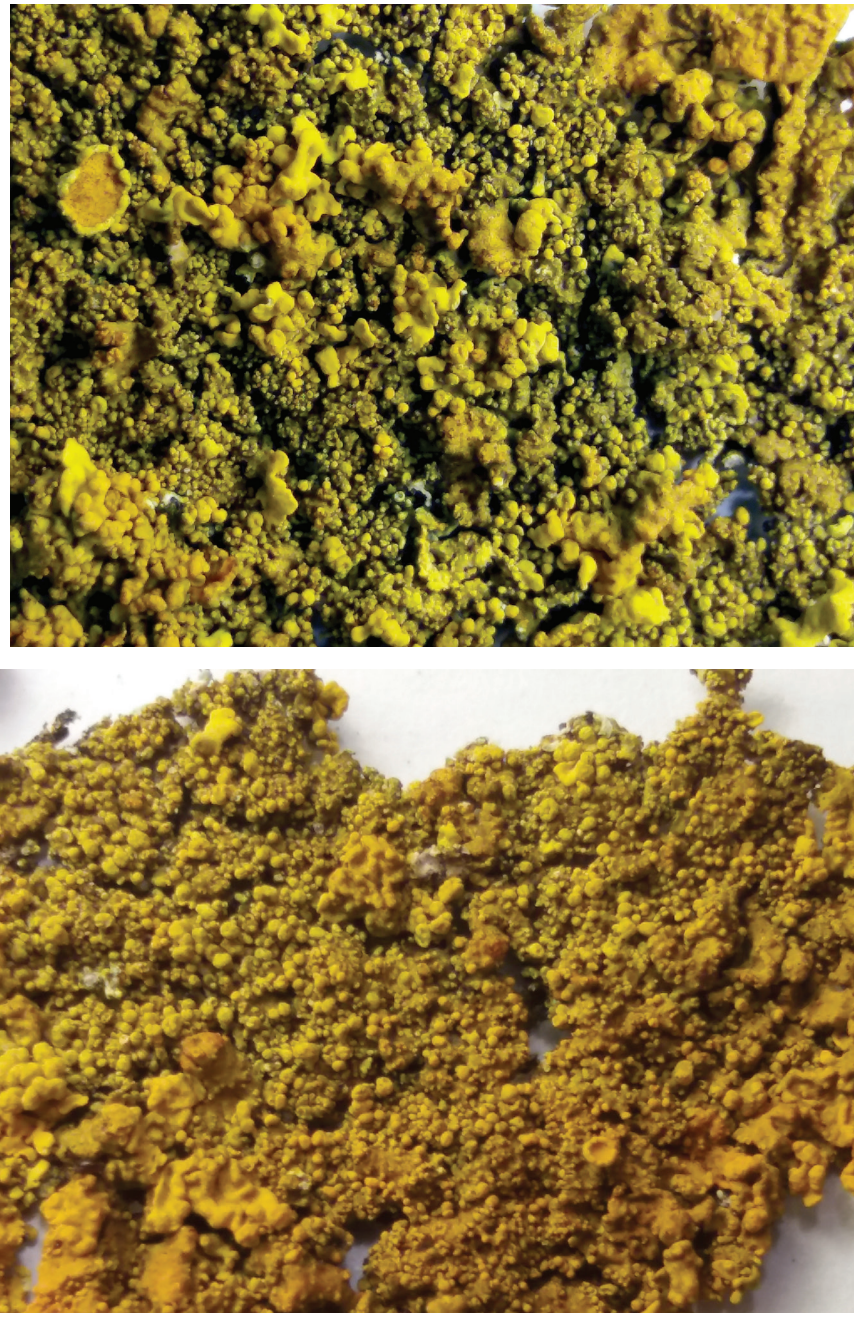

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Isidia present usually in places in the centre of thallus sometimes in several distant places of the same thallus, more often along thalline lobe edges (at the most uplifted portions of wrinkles); initial isidia very small, to 0.05–0.1 mm in diam./across, regularly rounded, more or less subconvex, usually furuncle-like (boil-bearing), i.e.: seem to be dissolving in very minute sorediate particles (similar to *Rusavskia sorediata* type) and disappearing, often aggregated in groups to 0.5(–1.5) mm across, seldom somewhat granular; rarely numerous and somewhat larger, to 0.1–0.15(–0.3)[–0.5] mm across, regularly rounded to almost spherical or pustule-like, more or less convex, somewhat lighter of darker thallus centre, rarely with conidiomata, while sometimes conidiomata as single as groups present among them too; rather rarely lobule-like isidia to 0.18–0.25 mm wide and 0.2–0.4 μm long observed.

Thallus in section to (100–)125–200(–225) μm thick, upper cortical layer 12–20(–25) μm thick, algal zone to (20–)30–50(–70) μm with algal cells 12.5–22 μm across; medulla to (50–)70–100(–125) μm thick if cavities not developed, or with cavities and hollows to 40 μm thick or with conglutinated hyphae groups to 15–20 μm thick; lower cortex (12–)15–20 μm thick, cell lumina 5–6(–7) μm across in both cortical layers, paraplectenchymatous.

Apothecia usually rare and distant, seldom very numerous in the centre, from very small to 0.5–1 mm in diam. and distant, often indistinct and easily overlooked to usually very large 2–4(–5) mm in diam. [to 0.25–0.45(–0.5) mm thick in section] and numerous especially in the centre, very uplifted above level of thallus to 1–1.5 mm high, and not attenuated at the basis (stipa to 0.7–0.8 mm in diam.), somewhat cup or 'wine glass' type, at first with thick uplifted thalline margin whitish yellow or greenish yellow, lighter and well contrasting to dull brownish orange centre of thallus sometimes with isidia-like granules; disc deeply concave, dull orange or brownish orange at first, soon becoming more or less plane, plate-like, to very undulating, with more or less crenulate to deeply crenulate or cracked margin to emarginate at overmature; especially numerous in the centre while usually remain distant, rarely covering the whole surface of the centre when numerous, massive, thalline margin thick, highly uplifted above apothecium disc level, disc becoming more or less plane and darker at overmature; in section thalline margin to 100–150(–200) μm thick, sometimes with hollow, cortical layer to 50 μm thick on underside, cell rounded thick walled 10–12.5 μm in diam./across; true exciple to 70–80 μm thick in the uppermost lateral and to 25–35 μm wide/thick in lower lateral and basal portions; hymenium to 50–60 μm high; subhymenium very thin, to 15–20 μm thick. Ascospores (7–)8–13(–15) × (4.5–)5.5–7.5(–8) μm and ascospore septum (3–)4–9(–11) μm wide.

Conidiomata to 200–250 μm in diam., hyaline, spherical, immersed in the thalline uplifted and constricted at basis formations, often in groups up to 3

to grape-like formations; conidia widely ellipsoid, somewhat attenuated from both ends or only one end, 2–3.5 × 1.5–2 μm.

Ecology: On siliceous and calcium-containing rocks and rarely on bark of trees (*Fraxinus* sp.) in coastal conditions, it should be emphasised that *Xanthoria pedersenii* in Søby, Aerø Island, Denmark often was overgrowing thalli of *Xanthoria pylyporlykii* heavily damaged or killed by the lichenicolous fungus *Telogalla olivieri*. It is the first record of *Xanthoria pedersenii* from *Fraxinus* bark, Ramlösa (voucher LD M67, SK22027), while special set of the former *Xanthoria calcicola* s. l. from the bark is prepared for repeated revision with the usage of molecular markers.

Etymology: It is named after Christiern Pedersen (*ca* 1480–16 January 1554), a Danish canon, humanist scholar, writer, printer and publisher, who is best known for his translation of the Bible into Danish, known as 'Christian III's Bible', a landmark in Danish literature and still in use today. His role in Denmark can be compared with role of humanist and theologian Erasmus Rotterdam in the Netherlands.

Distribution: This taxon found hitherto to be confirmed from 16 localities, where specimens were identified after microscopical and anatomical characters. The highest number of specimens was registered in Gørløse (10 specimens), Søborg (6 specimens), and Sandby (5 specimens), the other 12 localities with one-two specimens (see also Table 2 and Fig. 4).

Taxonomic notes: *Xanthoria pedersenii* is similar to *Xanthoria calcicola* in having wrinkled central portion with scarce isidia or in having hemispherical isidia-like formations with conidiomata, but differs in having thin, paper-like thalline lobes, which well-developed only in narrow peripheral zone, in having smaller (shorter and narrower) lobes, sometimes long and irregularly orientated with more or less reticulate surface at tips and with wrinkled surface in the centre, in having smaller knob-like warts which may resemble coarse isidia, which never fall off (to 0.2–0.3 mm in diam. *vs*. 0.3–0.6 mm wide warts), in having much narrower ascospores ((7–)8–13(–15) × (4.5–)5.5–7.5(–8) μm *vs*. 10–15 × 7–9 μm) and much wider range of variation of ascospore septum ((3–)4–9(–11) μm *vs*. 5–6.5 μm wide) as well as in having slightly shorter, but wider conidia (2–3.5 × 1.5–2 μm *vs*. 2–4 × *ca* 1 μm)*.

Xanthoria pedersenii is similar to *Xanthoria pylyporlykii* after thin thalline lobes and often narrow overlapping and irregularly orientated lobes in the centre, but differs in having rather thick, not uplifted, with downwards bent margins, in having greenish, and wrinkled surface especially in the centre,

* We were not able to identify 'yellowish crystals 2–5(–6) mm wide, 0.15–0.4 mm thick', in our collections from the southwestern part of the Baltic Sea basin region mentioned from Mediterranean collections of *Xanthoria calcicola* (Nimis and Martellos 2023), which considered sometimes as diagnostic character of *Xanthoria calcicola* (Lindblom and Ekman 2005).

thallus usually contrasting to more yellowish terminal portions, and in very uplifted and only slightly attenuated at the basis, distant apothecia as well as in having minute isidia of furuncle type often degrading, dissolving especially along ridges of lobe wrinkles and in having shorter ascospores and narrower of ascospore septum.

Xanthoria pedersenii is similar to *Rusavskia* species in having separate/distinct long and narrow thalline lobes and furuncle-like isidia dissolving in smaller portions, but differs in having plane thalline lobes, or only slightly seem to be semi-convex lobes owing to bent downwards marginal portions, in the lack of true soredia, and in having much smaller isidia-like formations and in having minute and in small amount of dissolving fragment soon disappearing (and forming 'sorediate' thallus afterwards, as well as in having longer ascospores and wide ascospore septum.

It should be noted that if *Xanthoria pedersenii* includes one more taxon elected in this paper as '*Xanthoria* sp. 1' material (see below/above) its distribution will be much wider within region studied and its importance in succession exchanges of lichen cover on rocky surfaces of southwestern part of the Baltic Sea basin will be better seen too.

Other specimens examined: Gørlose, 3.12.2022, coll. S. Kondratyuk SK22011 (voucher LD-M63 of nrITS sequence) sub *Xanthoria pedersenii* growing side by side with *X. pylyporlykii*; SK22016 (voucher LD-M76 of nrITS sequence) sub *Xanthoria pylyporlykii* growing side by side with *X. pedersenii*; Gorlose, SK22017 (voucher LD-M58 of nrITS sequence) sub *Xanthoria pedersenii* with *Telogalla olivieri*; SK22018; SK22005-3; SK22007, SK22008, SK22009. – Ramlösa, 12.08.2022, coll. S. Kondratyuk (voucher LD-M67 for nrITS sequence) on bark of *Fraxinus*; Bornholm, Osterlars kirka, 29.10.2022, coll. S. Kondratyuk section 280 (specimen differs in having shorter ascospores and narrower septum); sections 281, 282. – Fanefjord, 11.10.2022, coll. S. Kondratyuk section 167 (voucher LD-M85 for nrITS sequence) sub *Xanthoria pedersenii* with *Telogalla olivieri*; section 168 (differs in having wider ascospores). – Alt Barlow 2.10.2023, coll. S. Kondratyuk section 972 lobulo-isidiate; section 981 (long and narrow separate lobes well distinct with wrinkled surface in the centre); section 982 SK23981C sub *Xanthoria pedersenii* partly infected by *Xanthoria* sp. 1; Marstal 27.05.2023, coll. S. Kondratyuk roadside rock, section 833; Marstal 27.05.23 concrete wall along coast, section 844 sub *Xanthoria pedersenii* with *Telogalla olivieri,* section 845 (differs by very narrow ascospores), section 846 (differs by very narrow ascospores); Aeroskovling 28.05.2023, coll. S. Kondratyuk granite, coast, section 843 sub *Xanthoria pedersenii* with *Telogalla olivieri*; Hojby 25.06.2023, coll.: AT and NT, SK23872 (differs in having very narrow ascospores); SK23873 ascospores to 8 μm wide; Aerø: Søby church 26.05.2023, coll. S. Kondratyuk SK23825, SK23826 (differs by very narrow ascospores); Helsinge 26.03.2023, coll. S. Kondratyuk sect 579 (voucher LD-M82) SK23578; Nyker kyrka, 29.10.2022, coll. S. Kondratyuk on bark of *Acer pseusoplatanoides* SK22273, SK22275; Borre church, 22.09.2022, coll. S. Kondratyuk section 159; Skanor kirka 23.08.2022, coll. S. Kondratyuk section 361; Malmö, Västra Hamnen, coll. S. Kondratyuk SK23442, SK23443.

It should be mentioned that in field conditions in wet situation central portion of thallus of *X. calcicola* becoming greenish or darker in contrast to light or bright yellow, while in dry conditions thallus is usually dark reddish orange where only bright yellow thalline margin of apothecia are very distinct.

Some similarities to member of *Xanthoria calcicola* group also show several taxa of the *Xanthoria ectaneoides* group. They have also much darker greenish thallus in the centre while peripheral zone is usually brighter/lighter yellowish (especially in field conditions). In laboratory (herbarium) conditions colour of thallus depends on the conditions of drying and preserving specimens after taking from field conditions.

Collections from Marstal (concrete wall along coast SK23845, SK23846), Søby (SK23825, SK23826), and Hojby (SK23872) differing by much narrower ascospores is waiting for confirmation of its position in this branch with molecular data and included here with some hesitation.

Status of specimens from bark of *Acer pseudoplatanus* from coastal conditions of Bornholm Island (Denmark), as well as from artificial material from inland conditions (Lund, Sweden) is still waiting for additional checking their position after molecular data.

One more taxon, i.e. *Xanthoria* '*pseudocalcicola*' ad int. (not shown in the tree) differing in the lack of true isidia and in the lack of true exciple in basal and lower lateral portion as well as in having paraplectenchymatous true exciple only in the upper lateral portion (*vs*. pseudo-prosoplectenchymatous and well developed in basal and both upper and lower lateral portions) may belong to another genus or will be placed in separate branch of the genus *Xanthoria*.

Position of another taxon similar to *Xanthoria pedersenii* material but differing in parasitic style of life, i.e.: growing on damaged or decaying central portions of thalli of other lichens, especially of *Xanthoria pylyporlykii*, and *X. ectaneoides* (especially damaged by *Telogalla olivieri*, *Bryostigma parietinaria*, *Pyrenochaeta xanthoriae* or *Athelia arachnoidea*, too) as well as in having minute thalli (to 5 mm in diam./across) wrinkled in the centre, rarely seen with apothecia will be also checked after molecular data in the nearest future. Unfortunately, only one voucher (LD M61 section 703 from Nexø) of this taxon was hitherto included in the phylogenetic analysis, and it was positioned within the *Xanthoria ectaneoides* branch (see Kondratyuk *et al.* 2024). However, such parasitic material may be in future to be shown to be paraphyletic, as far data on ascospores of different specimens of such parasitic thalli are not the same. Part of this material named here as *Xanthoria* sp. 1.

On another side, it should be emphasised that this taxon is usually presented by very small thalline portions, and it is highly risky to take them for

DNA analysis (i.e. high risk of contamination by host tissue), while fertile collections were hitherto collected only in a few separate localities (i.e. Nexø, Bjernede, Fjenneslev, etc.) and single fertile specimens were rather small. However, we will ~~have~~ plan to check position of parasitic collections after molecular data.

Among these three fertile collections two (Bjernede and Fjenneslev) after measurements of ascospores are closer to *X. pedersenii*, while Nexø specimen as after ascospores as after molecular data is positioned in *Xanthoria ectaneoides* branch.

Among taxa of the *Xanthoria calcicola* subbranch there is one more still undescribed taxon showing somewhat similarity to species of the genus *Rusavskia* owing to radially orientated wrinkles of thalline lobes making impression that thalline lobes are very long, while they are indistinct in fact in the centre. Convex isidia-like formations with conidiomata are somewhat not always well developed. Two collections of this taxon from Denmark (Fanefjord 11.10.2022, SK22167 voucher LD M85), and Sweden, Bromma (SK23588 = voucher LD M62) form~~ing~~ separate branch with the highest level of support in out position to *X. pedersonii* and *X. wennergrenii*.

We are looking for additional specimens of this branch for making final decision about its status.

Xanthoria wennergrenii S. Y. Kondr., I. Kärnefelt et A. Thell, *spec. nova* (Fig. 3)

MycoBank No.: MB XXXXXXX

Similar to Xanthoria calcicola *and positioned after nrITS in sister position to the latter species, but differs in having smaller thalline lobes well developed in the peripheral zone of thallus only, pustule-like isidia and forming more or less bulky centre of thallus as well as longer ascospores and wider ascospores septum.*

Type: Denmark, Bornholm, Hammershus castle ruins, 55.2713° N, 14.7554° E, on brick walls at the highest point of ruins, 27.10.2023, coll.: S. Y. Kondratyuk SK23A55 – holotype, set with 6 isotypes will be distributed in the Plantae Graecenses exsiccate.

Thallus foliose, from very small to 1–2.5 cm across, sometimes to very large, to 5(–8) cm in diam./across, irregular at first than more or less regularly rounded, with more or less well developed peripheral zone contrasting to thick or bulky 'isidiate' central portion including extremely uplifted lobes, pustule or isidia like formations, extremely fragile, and sometimes completely transformed/eroded to isidiate-phyllidiate mass in the centre or unfortunately very damaged/eaten. If thalli well developed and large size peripheral zone

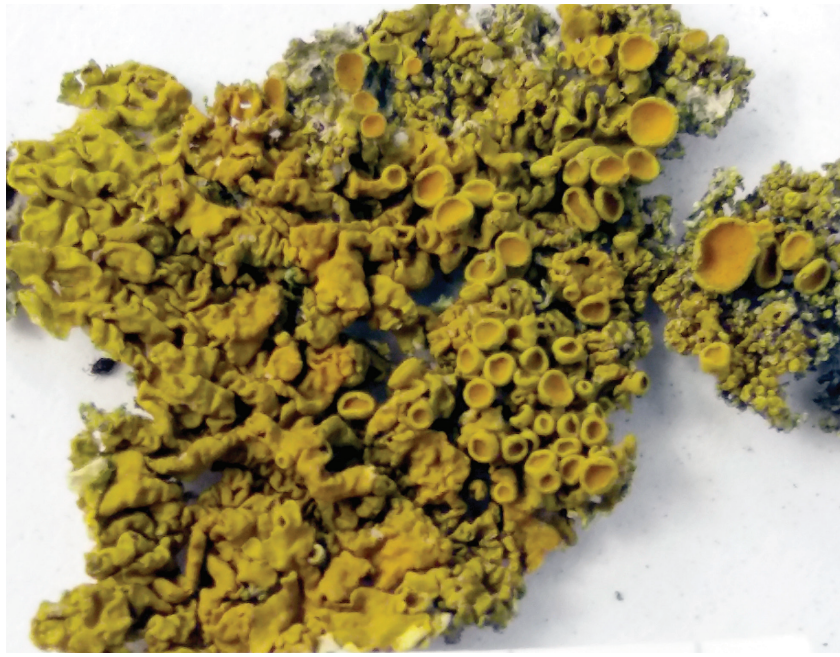

Fig. 3. Xanthoria wennergrenii – a = general view (holotype); b and c = enlarged portion of thallus with isidia (holotype); d = enlarged portion of thallus with isidia damaged by moss mites (SK22278)

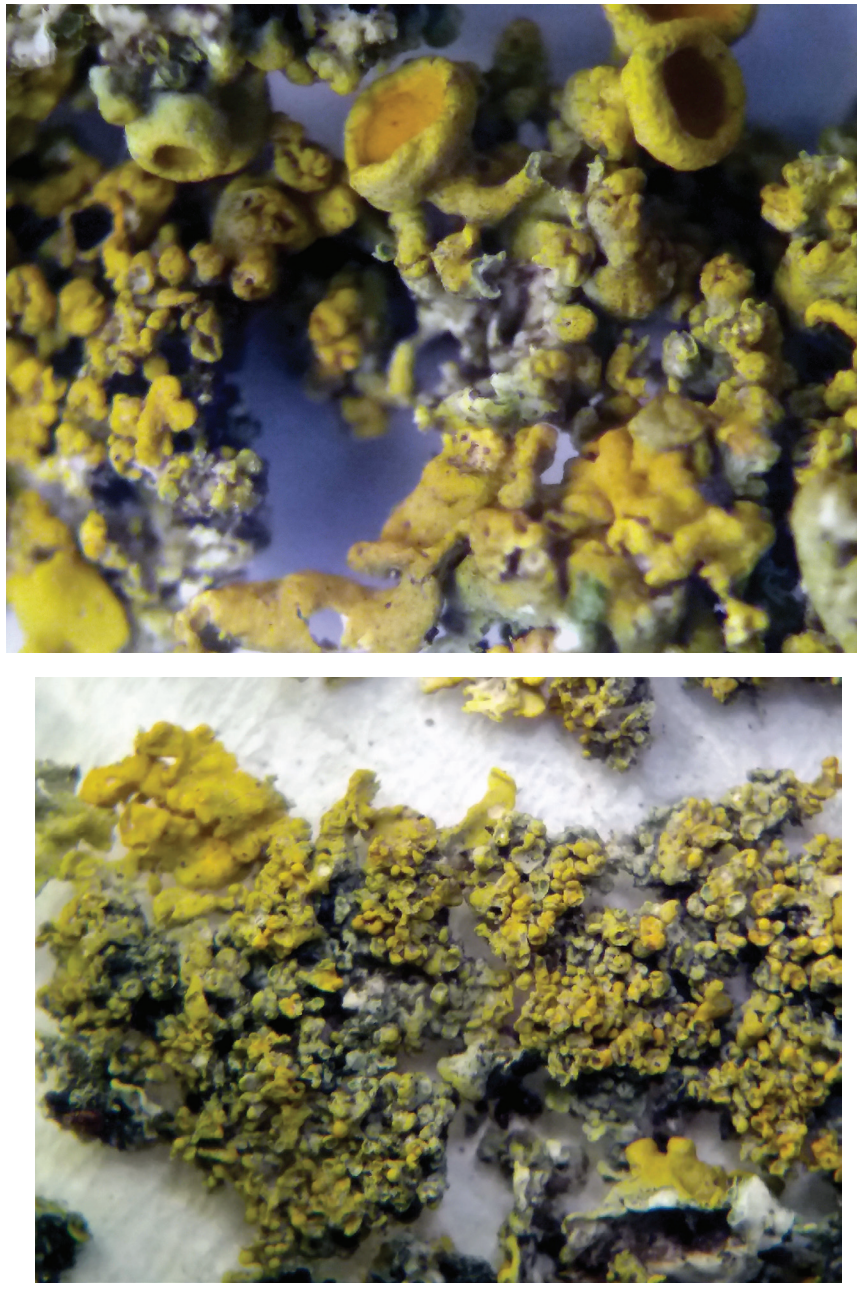

Fig. 3. Xanthoria wennergrenii – a = general view (holotype); b and c = enlarged portion of thallus with isidia (holotype); d = enlarged portion of thallus with isidia damaged by moss mites (SK22278)

(without isidia and apothecia) rather wide to (5–)15 mm wide bright yellow well contrasting to dull greenish or dull greenish-greyish yellow centre, and long well developed thalline lobes present; if thalli small (*ca* 1 cm across) lobes and central portion can be very small, but they are very different and centre well contrasting to plane lobes in peripheral portion.

Thalline lobes (4–)8–10(–15) mm long and 1(–2) mm wide in the narrowest place to (1–)3–5(–7) mm wide at the tips, somewhat widened and sometimes dissected into several portions towards the tips, in latter case total width of lobe with dissected portions to 5–7(–12) mm wide, rather thin, upper surface with more or less smooth surface or with scarce irregular wrinkles, soon becoming irregularly wrinkled very waved especially toward centre, with more or less well-developed cross wrinkles, seem to be rather thick owing to bent downwards edges of lobes or more or less uplifted marginal zones of lobes forming radiating ridges/wrinkles; in the centre upper surface at first becoming warted with warts more or less hemispherical and rather low at first to 0.2–0.3 mm in diam./across mostly more or less regularly rounded, rather densely packed in the centre soon becoming irregularly widened or uplifted and even more crowded, at this stages partly eaten pustules are also observed, like irregularly gaped pustule to 0.5(–1) mm across with hollow inside, very indistinct and isidiate or eroded/eaten by insects (and seem to be sorediate), with very indistinct secondary lobules erect or somewhat ascending, overlapping and forming indistinct ramified, coral-like or phyllidiate surface, where tips of lobes or isidia/pustule-like formations of wide range from 0.05–0.15 mm in diam. with gradual transition to more or less hemispherical or cylindrical, finger-like formations to *ca* 0.1–0.2 mm in diam./wide and to 0.5 mm long or thalline portions horizontally and irregularly orientated making mixture with ascending secondary lobes 0.2–0.3 mm wide to 0.5(–1) mm long/high make impression of totally irregularly orientated pustule-like* isidiate mass, and where portions of latter lobules as far they are very crumble/fragile and often heavily damaged/eaten by insects seem to be isidiate or lobulate in the centre. Centre sometimes completely covered somewhat flattened (pressed from the top), rather wide warts containing conidiomata or young apothecia to 0.3–0.5(–0.7) mm in diam./across, rarely such spherical formations contain several conidiomata reaching to 1(–1.2) mm in diam. across; such subspherical formations often produce regenerating secondary sublobules to (0.5–)1 mm across.

In comparison with much wider 0.3–0.5(–0.7) mm wide and to 1–1.5 mm long/high and more numerous and large is size pustule-like formations of more or less ascending or terete portions of like in the centre forming rather thick (to 1–1.5 mm thick) very crumble/fragile centre.

* Eaten or broken portions make impression that pustules are hollow inside.

Apothecia from rather small *ca* 0.2–0.4 mm in diam. and hardly distinct among isidiate/lobulate/phyllidiate/pustulate mass in the centre, to much larger to 1–3(–4.5) mm in diam./across very often 'pererodzhenykh' or eaten (see below moss mites), from rather rare to very numerous in places very uplifted above thallus or pustulate-isidiate mass of the centre from urceolate or concave to very waved or more or less plane disc; disc brown dull orange.

Ascospores (12–)13–17(–19) × 5–7(–8) μm (i.e. ascospores of *Xanthoria ectaneoides* type) and ascospore septum (5–)6–10(–12) μm wide (i.e. ascospore septum of *Xanthoria coomae* type) (see Kondratyuk *et al.* 2024).

Ecology: On tile roof as well as on vertical rocky walls near church, where growing side by side with *X. ectaneoides*, which is also overgrowing as this species as other *Xanthoria* species.

Distribution: So far, this species is confirmed from Bornholm Island of Denmark, where it was collected in Nyker and Hammerhus castle ruins. Additional specimens were also collected in 9 localities of Ærо, Fyn and Mon islands of Denmark, as well as dryland of Denmark, Skåne, Sweden and Rostok, Germany (Table 2, Fig. 4).

Etymology: It is named after Axel Wenner Gren (5 June 1881 – 24 November 1961), a Swedish entrepreneur, founders of several foundations including Foundation owing to support from which senior author have had possibility to carry out taxonomic revision of *Xanthoria calcicola* group in Southern Scandinavia.

It is interesting to emphasise that pretreatment of material of *Xanthoria wennergrenii* in freezing camera (or with minus temperature) is very important for its future preservation in herbarium, while Wenner Gren was creator of Electrolux company, which ~~produces~~ such type of ~~technics~~ from Werner Gren type till now.

Taxonomic notes: *Xanthoria wennergrenii* is similar to *Xanthoria calcicola* in having distinct contrast between peripheral and isidiate centre and positioned after nrITS in sister position to the latter species, but differs in having very thin and much smaller thalline lobes well developed in the peripheral zone of thallus only, in having very irregularly developed peripheral zone with more or less developed lobes, in having rather thick and very crumble central isidiate portion, in having pustule-like isidia and forming more or less bulky centre of thallus as well as in having longer ascospores (i.e. ascospores of *Xanthoria ectaneoides* type) and in having wider ascospores septum (i.e.: ascospore septum of *Xanthoria coomae* type), as well as in forming separate branch in phylogenetic tree of the genus *Xanthoria*.

X. wennergrenii is often growing side by side with *X. ectaneoides*. When these two species are represented only by very small thalli (usually to 1 cm across) with a few apothecia, with a few lobes, often irregularly developed/present and more or less uplifted, to pustulate or heavily eroded surface in very small portions their identification can be problematic.

However, *X. wennergrenii* differs from *X. ectaneoides* in the lack of well-developed secondary sublobules, as well as in having shorter ascospores and ascospore septum.

Problems with material of this taxon ~~when~~ it is presented by very small thallus, which are very fragile and only portions are collected. Originally it was planned to select collection from Nyker, Bornholm, Denmark, but after finding much richer collection from Hammershus castle ruins, type collection of this species selected from the latter locality. It should be mentioned that all collections of this taxon were seen only as rather small thalli, and Hammershus collection is somewhat different in having larger peripheral zone in much larger thalli, as well as in presence of cross wrinkles on upper surface of thalline lobes. The further collecting and checking position of such collection after nrITS phylogeny is in progress, and hypothesis if this species is more or less homogenic will be additionally tested.

Xanthoria wennergrenii is similar to *Xanthoria calcicola* and positioned after nrITS in sister position to the latter species, but differs in having smaller thalline lobes well developed in the peripheral zone of thallus only, in having pustule-like isidia and forming more or less bulky centre of thallus, as well as in having wider ascospores septum (i.e.: ascospore septum of *Xanthoria coomae* type).

Xanthoria wennergrenii is similar to *Xanthoria pylyporlykii* after having ascospore septum of *Xanthoria coomae* type, but differs in having pustule-like isidia and forming more or less bulky centre of thallus, and in having longer ascospores (i.e. ascospores of *Xanthoria ectaneoides* type), as well as in positioning after nrITS phylogeny in the *Xanthoria calcicola* subclade (while *X. pylyporlykii* is positioned in sister position to *Xanthoria coomae* of the *X. coomae* subclade).

After *Xanthoria ectaneoides* type of ascospores and *Xanthoria coomae* type of ascospore septum *Xanthoria wennergrenii* would be expected to be member of the *Xanthoria coomae* subbranch the same as *Xanthoria ectaneoides* and *X. pylyporlykii*. However, it is positioned within the *Xanthoria calcicola* subbranch.

It should be mentioned that thalli of *Xanthoria wennergrenii* are very sensitive to attacks of moss mites. If the latter are especially abundant, they can completely destroy lichen thalli, or especially their central portion. Heavily damaged thalli of this species were observed in several localities of Bornholm in 2022. Still unbroken thalli could be collected only in Nyker locality, which were morphologically, and anatomically investigated later. Finally, these two vouchers from this collection have been included in molecular phylogenetic study.

For preserving lichens of this taxon for the future depositing in herbarium collection it is especially important to have pretreatment in freezing camera just after arriving from field. The bulky central portion of thallus is probably very convenient for hosting of moss mites and other insects. And it

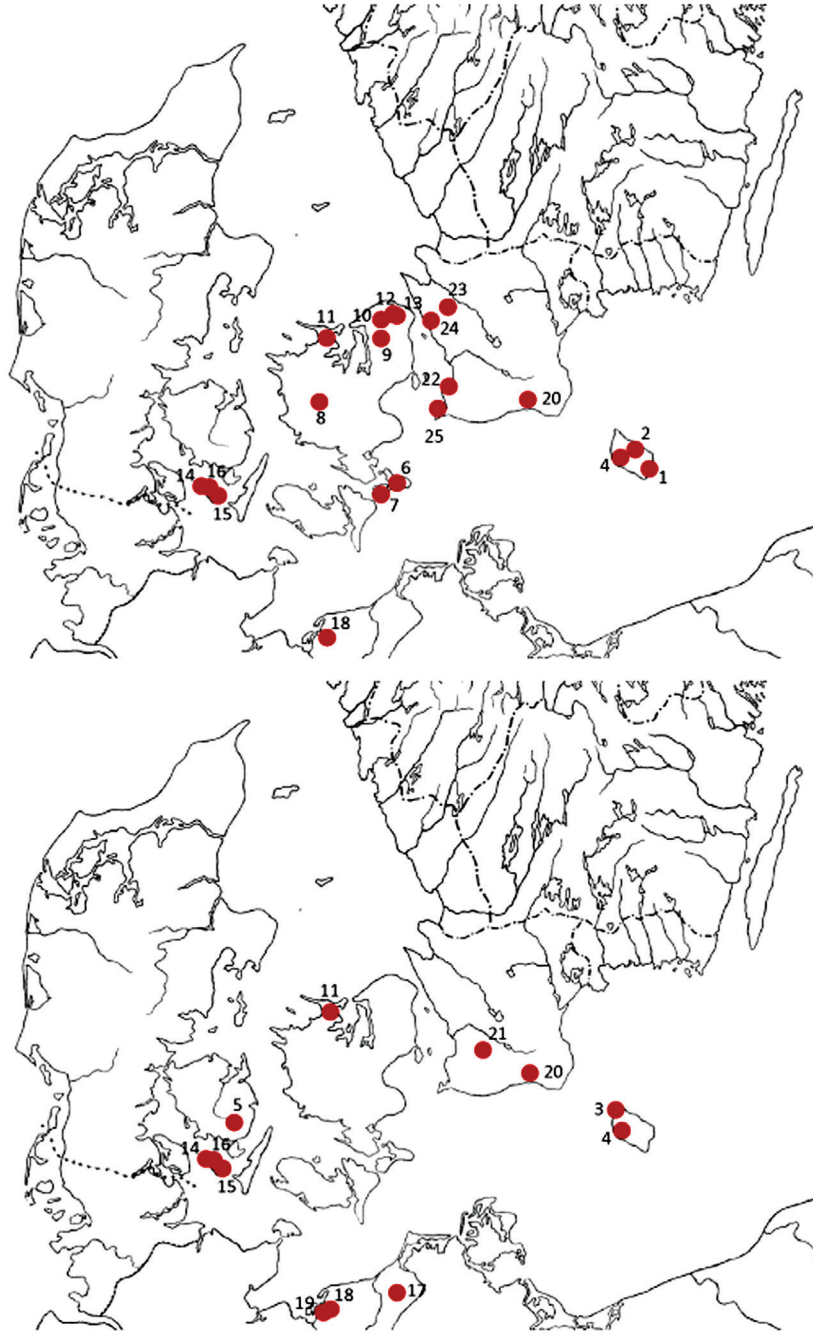

Fig. 4. Distribution of *Xanthoria calcicola* complex in western part of the Baltic Sea basin: 1 = *Xanthoria pedersenii*, 2 = *Xanthoria wennergrenii*

is almost impossible to collect lichen specimens of this species without moss mites. Thus, to protect lichen specimens for future preserving in collection step with freezing camera is especially important.

Other specimens examined: Sweden: Bromma, 28.09.2022, coll. S. Kondratyuk SK22149K (voucher M83 for nrITS sequence); SK22149G sub *Xanthoria wennergrenii* growing together with *X. pylyporlykii*; SK22149E sub *Xanthoria wennergrenii* growing together with *X. pylyporlykii*; SK22278 sub *Xanthoria wennergrenii* growing side by side with *X. pylyporlykii*, and *Physcia adscendens*. – Nyker 29.10.2022, coll. S. Kondratyuk SK22278 (voucher M65 for nrITS sequence), sub *Xanthoria wennergrenii* growing side by side with *X. ectaneoides* and *Physcia adscendens*; Nyker SK22278D (voucher M86 for nrITS sequence); SK22278C. – Dalby, s.d., coll. S. Kondratyuk SK24B21. – Denmark: Hojby 25.06.2023, coll.: N. and A. Thell SK23871*, SK23874; Søby 26.05.2023, coll. S. Kondratyuk SK23826**, *; SK23806, SK23850, SK23851; Aeroskovbing 26.05.2023, coll. S. Kondratyuk SK23840, SK23841; Marstal, 26.05.2023, coll. S. Kondratyuk SK23835, SK23836, SK23847, SK23848, SK23849. – Germany: Cammin, 1.10.2023, coll.: S. Kondratyuk SK 23A28*; Alt Barlow, 26.05.2023, coll. S. Kondratyuk SK23979, SK23980; Blowach 2.10.2023, coll. S. Kondratyuk SK24A19; Svandeborg Landevej, 28.05.2023, coll. S. Kondratyuk SK23814.

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* Specimens differ in having somewhat shorter ascospores (close to *Xanthoria parietina* type of ascospores, see Kondratyuk *et al.* 2024) and their position will be repeatedly checked in the future investigations.

** Specimen from Søby differs also in having much narrower ascospores and shorter ascospore septum. Its position within this taxon will be checked especially with the usage of nrITS phylogeny in future.

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TWO NEW NATURAL ACID NORWAY SPRUCE COMMUNITIES IN THE HIGH MOUNTAINS OF THE WESTERN CARPATHIANS

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Two new associations of natural acid Norway spruce communities (the order Piceetalia abietis Pawłowski ex Pawłowski et al. 1928 nom. corr.) from Slovakia are introduced: *Solidagini virgaureae-Piceetum abietis* and *Parido quadrifoliae-Piceetum abietis*. Their original relevés and floristical characteristics are published as well as their differentiation against the most common acid Norway spruce community in the Central Europe – the association *Lophozio-Piceetum abietis* Volk in Braun-Blanquet et al. 1939 (syn.: *Vaccinio myrtilli-Piceetum* Šoltés 1976, *Calamagrostio villosae-Piceetum* auct. non Schlüter 1966). The distribution of hitherto known phytocoenological relevés of the two considered communities *Solidagini virgaureae-Piceetum* and *Parido quadrifoliae-Piceetum* and the field experience indicate that their distribution within the Western Carpathians is bound to high mountain ranges (hochgebirge mountain ranges).

Key words: forest community, hochgebirge mountain range, phytocoenology, *Picea abies*, Piceetalia abietis, Slovakia, syntaxonomy, Tatra Mountains

INTRODUCTION

Natural acid Norway spruce communities are generally thought to be species-poor monotone and little variable phytocoenoses throughout large areas without the considerable habitat and phytocoenotic changes. This commonly accepted view was also adopted in the earlier first version of syntaxonomic revision of the class Vaccinio-Piceetea Br.-Bl. et al. 1939 in Slovakia (Kučera 2012) and recently by Kliment *et al.* (2022). These surveys more or less followed the older concept of Šomšák (in Mucina *et al.* 1985) introduced for acid Norway spruce communities (order *Piceetalia abietis* Pawłowski ex Pawłowski *et al.* 1928 nom. corr.).

Therefore only three associations of non-wetland natural acid *Picea abies* communities were accepted for Slovakia by Kučera (2012) following that traditional concept: *Vaccinio myrtilli-Piceetum* Šoltés 1976, *Athyrio alpestris-Piceetum*

Hartmann ex Hartmann et Jahn 1967 and **Chrysanthemo rotundifolii-Piceetum* Krajina 1933 nom. invers. Moreover, Kliment *et al.* (2022) recognised only two such acid Norway spruce associations.*

However, the recent more detailed and specialised study revealed important phytocoenological and regional differences within Slovakian acid *Picea abies* forest communities which correspond to geobotanical patterns found in other Central European lands (Moravia, Silesia, Czechia, Austria, Germany) (see below). The aim of this contribution is to present two new natural acid Norway spruce communities distinguished during works on the second version of syntaxonomic revision of natural Norway spruce communities from Slovakia (cf. Kučera 2023). As ~~the~~ most of the here presented relevés come from unpublished theses, all the considered relevés have to be published to ensure a formal valid publication of the considered syntaxa names.

MATERIAL AND METHODS

The field research was performed by Ľ. Vidličková, E. Elchison and K. Rajcová in the years 1985–1986. Their original relevés were stored by P. Kučera in Turboveg for Windows database software (Hennekens c1998–2020) (cf. Hennekens and Schaminée 2001) and submitted to Slovak Vegetation Database (Šibík 2012, *https://www.givd.info/ID/EU-SK-001*). Within the works on the recent syntaxonomic revision of the Vaccinio-Piceetea Br.-Bl. in Braun-Blanquet et al. 1939 (see Kučera 2022, 2023), they were retrieved from the SVD and partially revised/corrected.

Selection of data for the mentioned syntaxonomic synthesis and dataset adjustments were made using the regulations explained in detail by Kučera (2023), i.e. limitations concerning date of the dataset, plot sizes, elimination of relevé samples representing successional stages or missing ground layer species, species merging, etc. The consequent statistical analyses were performed using the programs JUICE (Tichý c1998–2020) (cf. Tichý 2002) and SYN-TAX 2000 (Podani 2001*a*). For the mentioned study, the conclusive ordinal hierarchical clustering was executed to evaluate also quantitative information provided by ordinal Braun-Blanquet's scale and the Podani's discordance coefficient was used as it takes into account also the presence vs. absence relation (Podani 2001*b*).

* The name is traditionally inverted according the Art. 10b of the ICPN (Theurillat *et al.* 2021). Nevertheless, the later reconsiderations supported the view that the traditionally distinguished association *Chrysanthemo rotundifolii-Piceetum* represents a community of the class Mulgedio-Aconitetea Hadač et Klika in Klika 1944 and it should be labelled following the original Krajina's proposition as *Piceo-Chrysanthemetum rotundifolii* Krajina 1933 (see Krajina 1933, Kučera 2012, 2024).

As the result, and following the proposal of Kučera (2019) on delimitation of the orders Piceetalia abietis and Sphagno palustris-Piceetalia, seven groups of natural acid Norway spruce phytocoenoses are recognised within the alliance Piceion abietis Pawłowski ex Pawłowski et al. 1928 nom. corr. in Slovakia (see Kučera 2023): [#1]*Lophozio-Piceetum*, [#2]*Athyrio distentifolii-Piceetum*, [#3]*Solidagini virgaureae-Piceetum*, [#4]*Parido quadrifoliae-Piceetum*, [#5]*Lycopodio annotini-Sorbetum*, [#6]*Listero cordatae-Piceetum*, [#7]*Sphagno capillifolii-Piceetum*. However, due to the large extent of that study on the syntaxonomic revision of acid Norway spruce communities, the formal valid publication of both the names and the original diagnoses of the syntaxa *Solidagini virgaureae-Piceetum* and *Parido quadrifoliae-Piceetum* have to be excluded into this paper.

The differential attributes of the here presented syntaxa (fidelity and frequency values) and the resulting tables were elaborated within JUICE; the concept of fidelity was used (Chytrý *et al.* 2002). Fidelity calculations (φ-values) are based on the presence/absence data, however, in contrast to my previous works, without standardisation of the relevé group sizes. Considering the here evaluated number of classified units and the amount of relevés as well as the species diversity of Piceetalia abietis communities in relation to their habitat diversity, use of this approach mostly reduced φ-values for species represented in more than one association and especially excluded low frequent species (e.g. *Veratrum album* subsp. *lobelianum*, *Polytrichum commune*) from being counted among differential species (φ-value ≥ 0.25). Performing the Fisher's exact test, zero fidelity was given to species with significance $P > 0.05$ in a particular cluster (Tichý and Chytrý 2006).

The formal sequence of species groups in Table 1 is adjusted according to the template of differential tables of the Vegetation of the Czech Republic, Vol. 4 (Chytrý *et al.* 2013), i.e. trees, shrubs, differential and other species of the field and ground layers separately; constancy and fidelity values equal and higher than "50" are highlighted in boldface type. Statistically determined diagnostic species are ranked according to the fidelity values. The conventional levels of statistical significance (0.05, 0.01 and 0.001, Fisher's exact test) for species are indicated with asterisks (*, **, ***). Due to rather low total number of available natural Norway spruce relevés, species with the lowest diagnostic value (φ-value ≥ 0.25 at Fisher's exact test 0.05-limit) were also retained as "diagnostic species" for individual syntaxa, bearing in mind their availability for the future comparisons and the potential use during field research.

Statistical fidelity values calculated for the individual species of *Dryopteris carthusiana* agg. are not respected because the relevant included taxa are usually not sufficiently recognised in the field: for example *D. expansa* was recognised in the Norway spruce habitats documented by phytocoenological relevés only recently, cf. relevés of Kučera (2012) within the unit *Vaccinio*

myrtilli-Piceetum Šoltés 1976 (correct name *Lophozio-Piceetum* Volk in Braun-Blanquet et al. 1939) from the Veľká Fatra Mts; however, *D. expansa* and its hybrids grow also in the Tatra Mountains (P. Kučera, not.) although they were not recognised within the that region. Nonetheless, the respective three basic species were retained in the table for the future comparisons.

Species taxa names follow the checklists of Marhold *et al.* (1998), Kubinská and Janovicová (1998) and Pišút *et al.* (1998), with exception of the *Soldanella marmarossiensis* agg. (*S. hungarica* auct. slov., Valachovič *et al.* 2019). Syntaxonomic nomenclature is regulated according to the 4th edition of the Code (Theurillat *et al.* 2021).

RESULTS AND DISCUSSION

Each of the above-mentioned seven communities of the natural acid Norway spruce woodlands of Slovakia has specific pattern of floristical and ecological features. The most easily recognisable attributes of three recently recognised Piceion abietis communities for Slovakia (Kučera 2019, 2023) are (1) more or less scree character of habitat, either with dominance of *Sorbus aucuparia* in the tree layer (#5*Lycopodio annotini-Sorbetum*) or with Western Carpathian relict occurrence pattern of *Listera cordata* as well as *Linnaea borealis* on boulder scree sites (#6*Listero cordatae-Piceetum*), or, (2) specific type of humid soil-regime indicated by marginal presence of *Eriophorum vaginatum* and various *Sphagnum* species (flat, partially water-logged nutrient-poor habitat, #7*Sphagno capillifolii-Piceetum*) [floristically and ecologically different from #2*Athyrio distentifolii-Piceetum* also inhabiting humid, however, nutrient-richer habitat and indicated by dominance of *Athyrium distentifolium*].

In addition to the two traditionally recognised associations #1*Lophozio-Piceetum abietis* Volk in Braun-Blanquet et al. 1939 nom. corr. (see below; syn.: *Vaccinio myrtilli-Piceetum* Šoltés 1976, *Calamagrostio villosae-Piceetum* auct. non Schlüter 1966, see Kučera 2023) and #2*Athyrio distentifolii-Piceetum abietis* and the above-mentioned three communities (#5–#7), the performed statistical analysis of the communities of the order Piceetalia abietis in Slovakia by Kučera (2023) differentiated also two groups of phytocoenoses (i.e. #3 and #4) consisting of relevés, which would be included in the *Lophozio-Piceetum* in the traditional classification schemes.

The association *Lophozio-Piceetum abietis* represents the most common natural acid Norway spruce community of the Western Carpathians and its phytocoenoses are characteristic by usually low number of the field layer species. The most frequent species are *Vaccinium myrtillus*, *Calamagrostis villosa*, *Oxalis acetosella*, *Avenella flexuosa*, *Homogyne alpina*, *Dryopteris dilatata*, *Luzula sylvatica* subsp. *sylvatica*, *Vaccinium vitis-idaea*, sometimes accompanied by

Dryopteris expansa, *D. carthusiana*, *Prenanthes purpurea*, *Rubus idaeus*, *Lycopodium annotinum*, *Huperzia selago*, etc. However, the field layer often consists from only 3–5 species and, as a whole, the *Lophozio-Piceetum* phytocoenoses do not have regular positive diagnostic species differentiating the community within the alliance Piceion abietis. The respective forest community is widely distributed in Slovakia, from the Oravské Beskydy Mts and the Veterné hole Mts in the northwestern part of the country to the Stolické vrchy Mts in the southeastern part. Floristically similar stands occur also in the Tatra Mountains.

On the contrary, the here described two new natural acid *Picea abies* communities [#3]*Solidagini virgaureae-Piceetum* and [#4]*Parido quadrifoliae-Piceetum* are clearly distinguished by the presence of species which are usually absent or at most low frequent in the stands of the association *Lophozio-Piceetum*. Typical examples of such species are *Calamagrostis arundinacea*, *Solidago virgaurea*, *Milium effusum*, *Paris quadrifolia*, *Stellaria nemorum* or *Athyrium filix-femina* (see Table 1) – the last-mentioned genus is usually represented by the species *A. distentifolium* in the stands of the natural acid *Picea abies* communities within the lower mountain ranges of the Western Carpathians.

These two groups of phytocoenoses have also a distinct character of distribution, different from the *Lophozio-Piceetum*: they were phytocoenologically recorded only in the Západné Tatry Mts up to the present and at the same time they are not confirmed from the mountain ranges with the above-mentioned lower absolute elevation such as the Oravské Beskydy Mts, the Veľká Fatra Mts, the Veporské vrchy Mts, the Stolické vrchy Mts or from the large parts of the Nízke Tatry Mts (see Kučera 2023, figs 2, 3). Due to the both floristical and chorological attributes the mentioned groups are evaluated as separate associations of the order Piceetalia abietis under the names *Solidagini virgaureae-Piceetum* and *Parido quadrifoliae-Piceetum* (Kučera 2023); however, due to the large extent of that study, formal valid publication of these names ~~are presented~~ in ~~this~~ separate paper.

Relevé records of these two communities are bounded mostly to the region of the Tichá dolina Valley and Kôprová dolina Valley; however, this is mostly result of the spatially limited field documentation of the natural Norway spruce communities within the Slovakian Tatra Mts region up to the present. In addition, the natural distribution of Norway spruce forests was there strongly negatively influenced by historical land management (deforestation and high-mountain grazing).

The original relevés of the two new associations *Solidagini virgaureae-Piceetum* and *Parido quadrifoliae-Piceetum* are presented in Table 1. For an easier floristical differentiation from the association *Lophozio-Piceetum* the synoptic column representing the latter syntaxon is added in the table as well (extract-

ed from Kučera 2023). On the contrary, the association **Athyrio distentifolii-Piceetum abietis* is not included in this comparison due to its specific floristical and ecological character generally expressed by the dominance of *Athyrium distentifolium* (or codominance with *Adenostyles alliariae*) in the respective woodlands, corresponding to the special habitat conditions of this plant community (see Jirásek 1996): as such phytocoenoses the latter association are considerably distinct and hard to be confused in the field with the here presented two new communities or any other syntaxon of the alliance Piceion abietis.*

In addition, to avoid any confusion, a separate column representing the original diagnosis of the association *Calamagrostio villosae-Piceetum* Schlüter 1966 is also included in Table 1. Although considered to represent a true natural acid Norway spruce in the last decade (Chytrý *et al.* 2013, followed by Kliment *et al.* 2022), this community in fact represents one of the types of substitutionary, non-natural *Picea abies* phytocoenoses developed in middle montane altitudes of Central European mountain ranges (see Schlüter 1966, 1969) (for more detail see Kučera 2012, 2023).

For a complete comparison and differentiation of the communities of the order Piceetalia abietis in Slovakia see Kučera (2023).

Solidagini virgaureae-Piceetum abietis P. Kučera, *ass. nova, hoc loco*

Nomenclatural type: Table 1, rel. 12, holotypus, *hoc loco*.

Original diagnosis: Table 1, rels. 1–21.

The canopy of stands of this association is dominated by *Picea abies* with admixed *Sorbus aucuparia* (subsp. *glabrata*). *Larix decidua* was probably a natural component of the canopy; however, the currently known relevés did not record this species, most probably due to historical land management.

In the understorey shrub species *Lonicera nigra*, *Ribes petraeum* and *Sambucus racemosa* are rarely present.

The field layer is distinguished by the set of constant species *Adenostyles alliariae*, *Solidago virgaurea*, *Senecio nemorensis* agg., *Luzula luzuloides*; *Adenostyles* frequently with high cover-abundance values (even above 50–75%). Less frequent significant species are *Milium effusum*, *Athyrium filix-femina*, *Calamagrostis arundinacea*, *Cicerbita alpina*, *Hieracium murorum*: their presence in the non-carbonate rocks in the elevations above 1,500–1,550 m a.s.l. distinctly

* It should be only reminded that the separate subassociation *Athyrio distentifolii-Piceetum athyrietosum filicis-feminae* Hartmann et Jahn 1967 (Chytrý *et al.* 2013, Hartmann and Jahn 1967, Jirásek 1996, 2002) represents anthropogenically degraded stands of former upper montane mixed *Fagus* forests and as such the respective phytocoenoses syntaxonomically belong to the class Carpino-Fagetea Jakucs ex Passarge 1968 and not to the *Athyrio distentifolii-Piceetum* (Kučera 2012).

contribute to a clear differentiation from the phytocoenoses of the association *Lophozio-Piceetum abietis* Volk in Braun-Blanquet et al. 1939 nom. corr.

The other species typical for natural Norway spruce woodland are represented as well: *Vaccinium myrtillus*, *Oxalis acetosella*, *Homogyne alpina*, *Luzula sylvatica* subsp. *sylvatica*, *Dryopteris dilatata* [*D. expansa* was not recognised in the time of the field studies], *Gentiana asclepiadea*, here and there also *Calamagrostis villosa*; *V. myrtillus*, *O. acetosella* and *C. villosa* could reach cover-abundances over 25–50%.

The ground layer is poor in species: the most frequent species are *Dicranum scoparium*, *Polytrichum formosum* and *Plagiothecium curvifolium*.

The phytocenological field documentation of this association was originally presented in the master theses of Moravčíková (1987, within the tables 2, 4, 6) and Rajcová (1987, within the tables I.1, I.5) from the Krížna dolina Valley and Kôprová dolina Valley from the Západné Tatry Mts (see Kučera 2023, fig. 3). As further thorough research of the other valleys of the Západné Tatry Mts and the High Tatras was not continued in that time, these two manuscripts contain the only available documented relevés of the community known to the present.

The phytocoenoses of the association *Solidagini virgaureae-Piceetum* were documented up to the elevation 1,600 m a.s.l. The stands are bound to considerably steep slopes on granitoid rocks – mainly with inclination (30–)40–50° (Moravčíková 1987) – and oriented usually towards southeast to southwest.

Due to the considerably different ecological habitat conditions of this community in comparison to the association *Athyrio distentifolii-Piceetum abietis* Hartmann ex Hartmann et Jahn 1967, these two units represent the ecological counterparts to some extent as the latter community is ecologically bound to special wet habitats (cf. Jirásek 1996, 2002). Significant floristical distinction between these two communities comprise for example differences in constancy (incl. absence) of species *Athyrium distentifolium*, *Stellaria nemorum* vs. *Adenostyles alliariae*, *Solidago virgaurea*, *Hieracium murorum*, *Avenella flexuosa* (cf. Chytrý *et al.* 2013, Jirásek 1996).

Parido quadrifoliae-Piceetum abietis P. Kučera, *ass. nova, hoc loco*

Nomenclatural type: Table 1, rel. 27, holotypus hoc loco.

Original diagnosis: Table 1, rels. 22–32.

The canopy of the stands of this second newly recognised natural acid Norway spruce association of the Western Carpathians is dominated by *Picea abies*, admixed are both *Larix decidua* and *Sorbus aucuparia* (subsp. *glabrata*).

In the understorey *Pinus mugo* and *Lonicera nigra* were documented in some stands.

The field layer of the typical stands is characterised by conjoint presence of species *Stellaria nemorum*, *Paris quadrifolia*, *Melampyrum sylvaticum*, *Athyrium filix-femina*, *Senecio nemorensis* agg., *Calamagrostis arundinacea*, *Luzula luzuloides* and *Vaccinium vitis-idaea*. Here and there are growing *Blechnum spicant*, *Epilobium montanum*, rarely also *Galeobdolon montanum*, *Chaerophyllum hirsutum* and *Luzula pilosa*.

The dominant field layer species is usually *Vaccinium myrtillus*, accompanied by common species of Western Carpathian species of montane woodland: *Homogyne alpina*, *Luzula sylvatica* subsp. *sylvatica*, *Oxalis acetosella*, *Calamagrosis villosa*. Rarely *Dryopteris carthusiana* or *D. dilatata* become dominants of the field layer [*D. expansa* was not recognised in the time of the field study].

Differential species of the ground layer are *Plagiochila porelloides* and *Sphagnum girgensohnii*, together with less frequent *Plagiomnium undulatum*. Common forest species *Dicranum scoparium* and *Polytrichum formosum* belong to constant species, less frequently were found also *Hylocomium splendens* or *Plagiomium affine*.

The field documentation of this association was originally presented in the master thesis of Naďová (1987, within the tables 1, 3) from the Tichá dolina Valley of the Západné Tatry Mts. Additional relevé of Horák (1971) from the region of the valleys Račková dolina and Jamnícka dolina was also assigned to this association in the current statistical analysis (Kučera 2023, cf. fig. 3). Similarly, as in the case of the association *Solidagini virgaureae-Piceetum*, the small amount of the known phytocoenological relevés is caused by insufficient field documentation of Norway spruce forests of the Tatra Mountains.

Up to the present, the *Parido quadrifoliae-Piceetum* phytocoenoses were documented mostly on steep slopes (usually 25–38°) oriented towards southwest to west (occasionally in northwest and northeast slope orientation). The highest elevated stand was noted at the elevation 1,530 m a.s.l. though the community is probably distributed even in higher elevated sites. The association is developed only over non-carbonate rocks, mostly granitoids.

A part of the relevés assigned to this association lack the species *Melampyrum sylvaticum*, *Paris quadrifolia* or *Stellaria nemorum*; however, the presence of *Athyrium filix-femina* and *Calamagrostis arundinacea* and absence of differential species of the association *Solidagini virgaureae-Piceetum* indicate that these relevés most probably represent a variation of the association *Parido quadrifoliae-Piceetum* (see Kučera 2023).

The somewhat richer composition of the field layer species might remind the association *Luzulo sylvaticae-Piceetum* Wraber 1963 originally described by Wraber (1963). However, contrary to the usual syntaxonomic evaluations, the original diagnosis of the association *Luzulo sylvaticae-Piceetum* Wraber 1963 for the most part does not represent a natural Norway spruce plant commu-

nity. The respective phytocoenological relevés document mostly secondary, strongly human influenced stands with artificially reduced appearance of *Abies alba* and *Fagus sylvatica*, originated in the habitats of the former natural mixed *Fagus sylvatica* forests (see Wraber 1963, Kučera 2023; cf. also re-evaluation of the natural distribution of Norway spruce communities in the Western Carpathians by Kučera 2012, 2022, 2023).

Concluding remarks to the differentiation of the associations Solidagini virgaureae-Piceetum and Parido quadrifoliae-Piceetum

It might by argued that the here presented two new communities *Solidagini virgaureae-Piceetum* and *Parido quadrifoliae-Piceetum* do not represent a separate syntaxa in the level of association; instead, they would be variants or subassociations of one of the earlier described syntaxa, e.g. *Lophozio-Piceetum* as one of the most widespread community through the Central European mountain ranges (incorrectly labelled as *Calamagrostio villosae-Piceetum* auct., e.g. Chytrý *et al.* 2013, Exner 2007, Kliment *et al.* 2022, Seibert 1992).

However, two crucial features should be reminded:

A) Natural acid *Picea abies* communities of the supramontane vegetation zone (order Piceetalia abietis Pawłowski ex Pawłowski et al. 1928 nom. corr.) are considerably species-poorer than their calcareous counterparts of the order Cortuso-Piceetalia P. Kučera 2022 (= Athyrio-Piceetalia auct. non Hadač 1962) therefore the different Piceetalia abietis habitats and their communities corresponding to separate associations cannot differentiate by long lists of differential species as in the case of individual Cortuso-Piceetalia associations (cf. Kliment *et al.* 2022, Kučera 2012, 2022).

Thus, also a lower number of exclusive differential species do indicate an ecologically different and floristically individual community within the order Piceetalia abietis. In the case of the Western Carpathians, this specific attribute is highlighted by generally lower number of available indigenous species in comparison with the Alps or the Southern Carpathians (absence of species of genus *Erica*, *Rhododendron*).

Further, it should be reminded that there exist also selected communities which lack exclusive diagnostic species and as such they are negatively differentiated (cf. Kučera 2022, 2023, see also Chytrý *et al.* 2013, p. 374–376, Exner 2007 + Willner *et al.* 2007, tab. 3, cf. also Kliment *et al.* 2022, tab. 16) and they are widely recognised in national surveys. The example of the synthesis of the communities of the class Juncetea trifidi Hadač in Klika 1944 in Slovakia (Dúbravcová and Jarolímek 2007, ut Caricetea curvulae Br.-Bl. 1948) shows that in the case of species-poor acid plant communities merely a quantitative species differentiation of associations is accepted in syntaxonomic classifications.

B) Phytocoenological data from the Western Carpathian mountain ranges of Slovakia as well as from the geographically close lands of Poland, Czech Republic, Austria and Germany (the hochgebirge mountain range of the Alps with glacially formed landscape excluded) indicate that natural acid Norway spruce communities corresponding to *Solidagini virgaureae-Piceetum* and *Parido quadrifoliae-Piceetum*, i.e. with species as *Calamagrostis arundinacea*, *Athyrium filix-femina*, *Luzula luzuloides*, *Senecio nemorensis* agg., *Solidago virgaurea*, *Paris quadrifolia*, *Stellaria nemorum* and at the same time with absence of *Athyrium distentifolium*, are absent either in mittelgebirge mountain ranges of Slovakia (e.g. the Oravské Beskydy Mts, the Veľká Fatra Mts, the Stolické vrchy Mts, etc., Kučera 2023) or in Poland (Kasprowicz 1996, Matuszkiewicz 1977, Matuszkiewicz and Matuszkiewicz 1960, cf. Matuszkiewicz 2002) as well as in the Czech Republic (Jirásek 1996, Neuhäuslová and Eltsova 2003, Sofron 1981, cf. Chytrý *et al.* 2013), Austria (Ewald *et al.* 2011, Petermann *et al.* 1979, cf. Exner 2007) or Germany (Seibert 1992); anthropogenic secondary *Picea abies* have to be excluded (cf. Kučera 2012). It should be strongly emphasised that *Picea* phytocoenoses with the occurrence above-mentioned species *Calamagrostis arundinacea*, etc. from the non-supramontane altitudes of these Central European mountain ranges (cf. Kučera 2022) represent substitutionary *Picea* forests replacing the original Carpino-Fagetea communities.

This geobotanical (chorological) pattern confirms that the considered two communities *Solidagini virgaureae-Piceetum* and *Parido quadrifoliae-Piceetum* do not represent some kind of variability of the associations *Lophozio-Piceetum abietis* or *Athyrio distentifolii-Piceetum abietis*, which could develop anywhere where those two latter units occur. The development of the phytocoenoses of *Solidagini virgaureae-Piceetum* and *Parido quadrifoliae-Piceetum* are in the Central Europe limited to ~~most~~ highest mountain ranges, i.e. in Slovakia mostly slopes of the Západné Tatry Mts and the High Tatras (in the carbonate region of the Belianske Tatry Mts are developed communities of the order Cortuso-Piceetalia); their occurrence is also possible in the slopes to the north of the main ridge of the Nízke Tatry Mts.

This difference in development of Central European Norway spruce forest communities correspond to a certain difference between the Central European hochgebirge mountain ranges and the Central European mittelgebirge mountain ranges (cf. Troll 1973; some areas of the Krkonoše Mts have a transitional position). The first group is distinct by their different geomorphological development during the Pleistocene and Holocene ruled by series of Quaternary glaciations. Their high peaks (reaching high above the alpine forest line) have long steep slopes and their soils are "younger", presumably with higher nutrient supply in comparison to the mountains and their slopes of the mittelgebirge mountain ranges with lower absolute elevation, e.g. those

of the Veľká Fatra Mts, the Oravské Beskydy-Beskid Żywiecki Mts or the Bayerischer Wald-Šumava Mts (for the most part).

*

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DATA TO THE MALAYSIAN LIVERWORT FLORA, IV: A NEW SPECIES OF *MASTIGOPELMA* MITT. (LEPIDOZIACEAE) FROM CAMERON HIGHLANDS, PENINSULAR MALAYSIA

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The new species, *Mastigopelma latiffiana* G. E. Lee, E. Pesiu et X. L. He, discovered in Peninsular Malaysia, is described and illustrated as a new addition to the genus *Mastigopelma* Mitt., of the family Lepidoziaceae. The new species is corticolous, growing on a tree branch in a moist montane forest in Cameron Highlands, Pahang, at 1,900 m alt. It is characterised by the leafy shoots arising from stolons, branches, when present, all ventral-intercalary, and terminal branching lacking; asymmetrical leaves with almost straight ventral margins, rounded to truncate leaf apices, entire-margined leaves, thickened cell walls with nodular trigones, and glistening-homogeneous oil bodies (2–4 per cell); and retuse to blunt teethed underleaf apices. This discovery has increased the total number of known species of *Mastigopelma* Mitt. to five. An identification key to all the species of the genus is presented.

Key words: Lepidoziaceae, liverworts, Malaysia, new species

INTRODUCTION

Malaysia has a rich liverwort flora, with a diversity of nearly 800 liverwort species have been known (Lee *et al*. 2022*a*), encompassing more than 10% of the world's liverwort species diversity. Recent research efforts have further contributed to expanding our knowledge of the liverwort flora of Malaysia, including the discovery of new species from Genting Highlands, Pahang, such as *Lejeunea malaysiana* G. E. Lee et Pócs (Lee *et al.* 2022*b*), floristical studies in Sabah (Pócs *et al.* 2020) and Terengganu (Pesiu *et al.* 2021, Sarimi *et al.* 2021), as well as the recent liverwort checklist and guidebook publications (Lee *et al.* 2022*a*; Lee and Gradstein 2021). Here, we describe another new liverwort species from Cameron Highlands, Pahang, in Peninsular Malaysia.

Cameron Highlands is located on a high plateau at the headwaters of the Bertam River, west of Pahang. It spans an elevation range of 300 to 2,060 me-

ters above sea level. The area experiences heavy rainfall with averages of 2,650 mm annually, and frequent mist from clouds has contributed to high moisture levels (Saw 2010, Wyatt-Smith 1963). The approximate size of the land area is 69,699 square kilometres. Topographically, the area consists of approximately 50% mountainous terrain, 30% undulating areas, 15% valleys, and 5% plains (Aik *et al.* 2021). In the upper hill dipterocarp forest and below (below 1,200 m), the soil types in the Cameron Highlands are categorised as sandy clay loam, limestone, and slate (Hamzah *et al.* 2014). On the other hand, in montane ericaceous forests and above (above 1,200 m), the predominant soil type is granite (Hamzah *et al.* 2014). The highlands are heavily forested and most well-known for the 'Mossy Forest', which occurs on the summit of the surrounding hills. The forest canopy is about 1.3 to 18 metres high, flattened and compact with a single layer of trees and ground floor (Saw 2010). The predominant tree families found in the area include Araliaceae, Araucariaceae, Clethraceae, Cunoniaceae, Ericaceae, Fagaceae, Lauraceae, Myrtaceae, Pentaphylacaceae, Podocarpaceae, Sapindaceae, Symplocaceae and Theaceae (Saw 2010).

The new species, belonging to the genus *Mastigopelma* Mitt., subfamily Bazzanioideae and family Lepidoziaceae, grows in a moist montane forest at an altitude of 1,900 m along the forest trail to the Puncak Mat summit. The summit forms a brief crest characterized by relatively steep edges. The surrounding forest is distinctly different from the neighbouring forest due to the much shorter stature of the trees. Within this area, most of the trees typically reach heights of three to five meters, hardly surpassing that limit. They exhibit twisted and gnarled features, covered with dense bryophytes (mostly leafy liverworts), lichens, orchids, ferns, and pitcher plants.

DESCRIPTION OF THE NEW SPECIES

Mastigopelma latiffiana G. E. Lee, E. Pesiu et X. L. He, *spec. nova*
(Figs 1–2)

Diagnosis: Affinity to *M. fragile* (Steph.) N. Kitag. in lacking conspicuous teeth on the leaf margin and having a papillose cuticle but differing from it by the large nodular trigones, retuse to blunt teeth underleaf apices, and entire-margined leaves without any tooth-like or projecting marginal cells.

Type: Malaysia, Pahang: Cameron Highlands, Taman Eko Rimba (TER) Mossy Forest, 12 km from Brinchang town. Mossy cloud forest. Along the forest trail to Puncak Mat at 1,900 m alt., on tree branch, 04° 31.168′ N, 101° 23.286′ E, 11.III.2023, *G. E. Lee, E. Pesiu, N.S. Atiqah 23003* (holotype: UMTP, isotype: UKMB).

Etymology: The species is named in honour of the renowned plant taxonomist, Emeritus Professor Abdul Latiff Mohamad, who has dedicated his life to studying Malaysian flora. He is one of the pioneers in plant taxonomy research in Malaysia and a strong advocate and conservation lobbyist, pas-

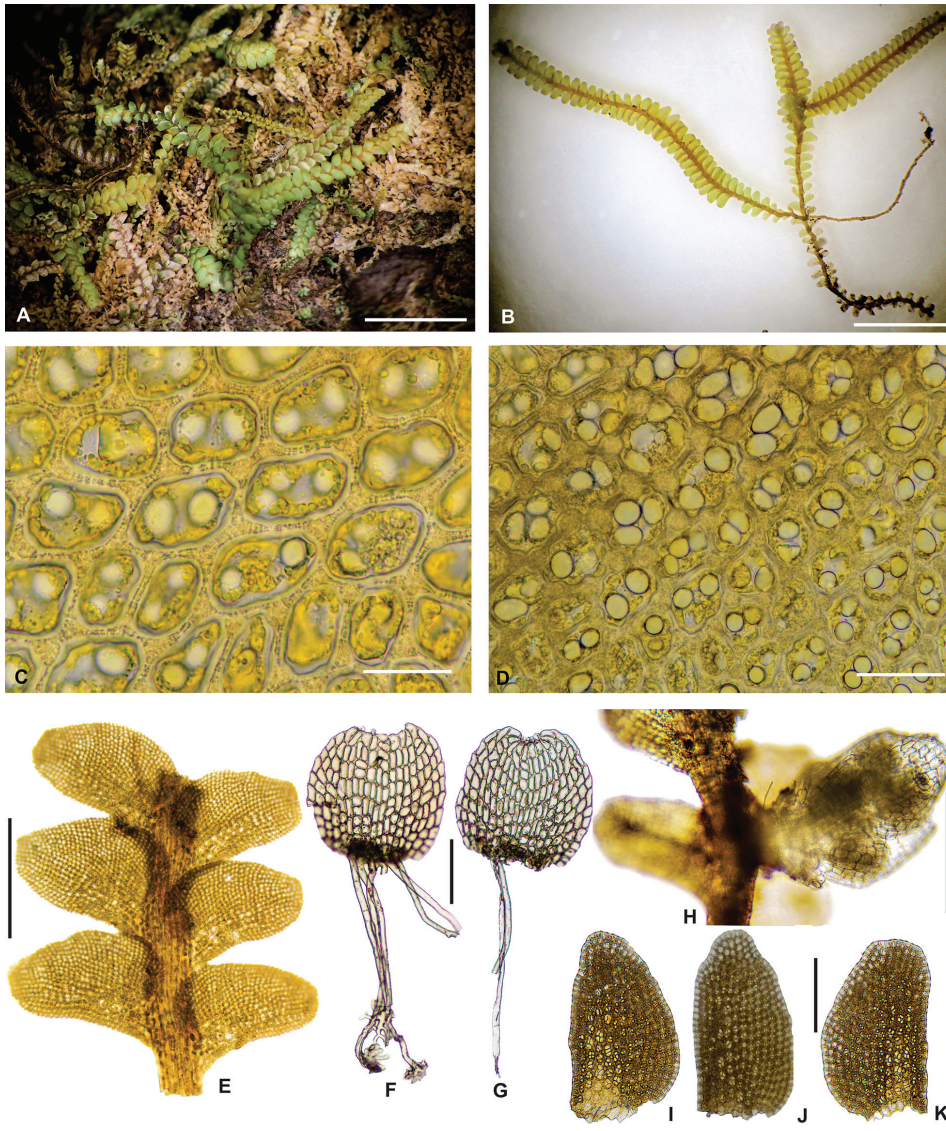

Fig. 1. Mastigopelma latiffiana G. E. Lee, E. Pesiu et X. L. He, sp. nov. – A = habit; B = plant in ventral view; C = median leaf cells; D = basal leaf cells and nodular trigones; E = part of the plant in dorsal view; F–G = underleaves; H = androecial shoot; I–K = leaves. All from the holotype. Scale bars: A–B, 3 mm; C–D, 50 μm; E, H, 0.4 mm; F–G, 100 μm; I–K, 200 μm

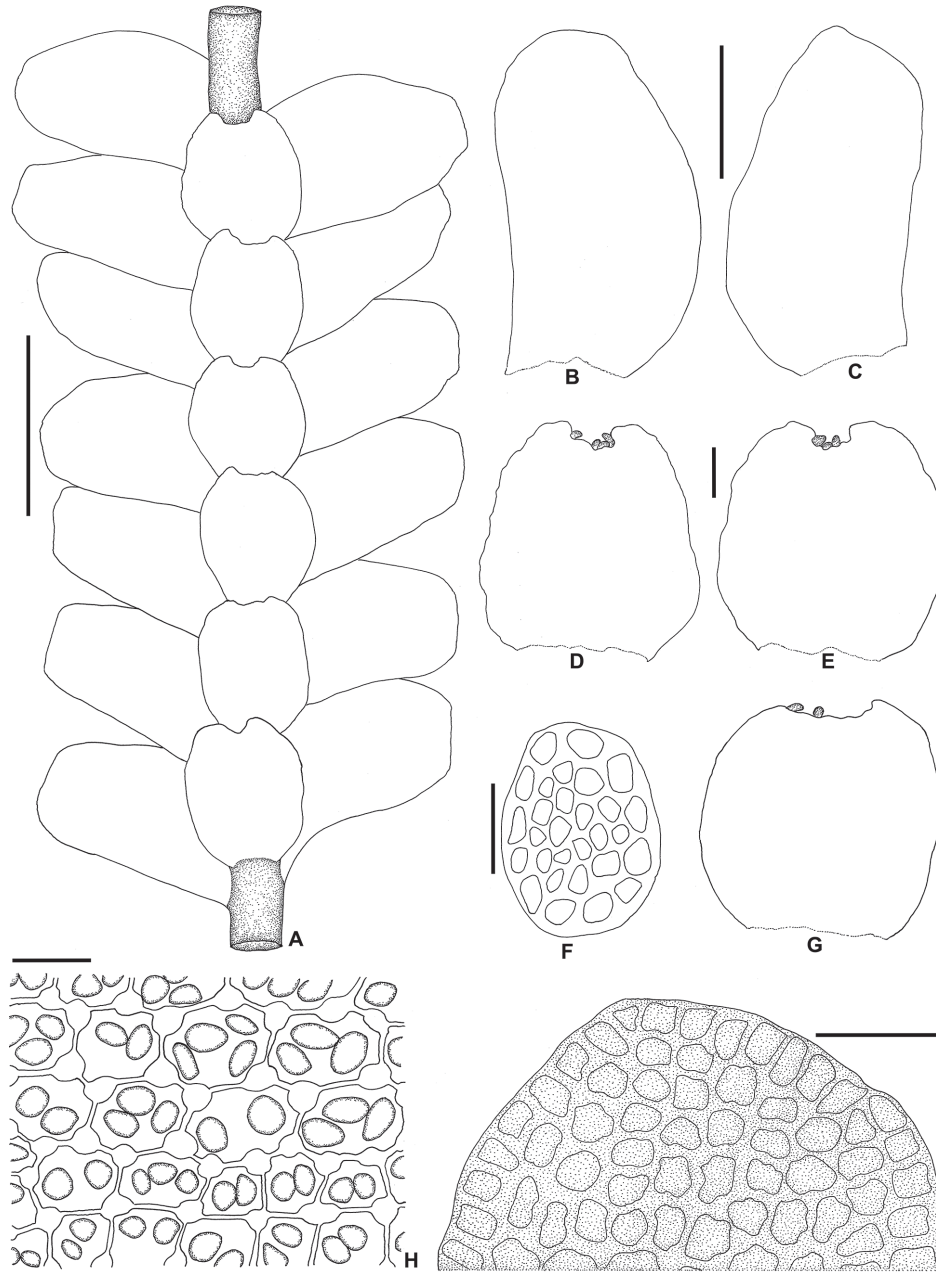

Fig. 2. Mastigopelma latiffiana G. E. Lee, E. Pesiu et X. L. He, sp. nov. – A = part of the plant in ventral view; B–C = leaves; D, E, G = underleaves (hyaline marginal cells shown in gray); F = cross-section of stem; H = basal leaf cells and nodular trigones; I = apical leaf cells. All from the holotype. Scale bars: A, 0.4 mm; B–C, 200 μm; D–I, 50 μm

sionately championing the conservation of Malaysia's biodiversity. Additionally, he has profound admiration for the tiny world of bryophytes. His unwavering support for his students to study bryophytes in Malaysia has inspired the first author, G. E. Lee, his former student, to work on Malaysian liverwort biodiversity.

Description: Plants dioicous (only androecium seen), rigid, light green to blue-green in appearance when fresh. Shoots creeping loosely on the substrate or other liverworts, with ventral flagella or unbranched. Leafy shoots or branches arising from short stolons, when present, always ventral-intercalary (*Bazzania*-type), emerging from the axils of underleaves, and terminal branching lacking. Rhizoids few, fascicled on ventral side of the stem and restricted to the base of underleaves. Stems 90–130 μm in diameter, about 5–6 cells high in cross-section, cells of uniform size throughout, with slightly thickened walls. Leaves approximate to overlapping, sometimes distant, spreading to widely spreading, slightly falcate, ovate, asymmetrical with almost straight ventral margin; leaves 400–550 μm long, 200–300 μm wide, usually widest at the lower or middle portion of the leaf; leaf apex flat, broadly rounded or sometimes truncate; leaf margin entire throughout; leaf cells round to rectangular, apical cells 9–13 μm long, 7–10 μm wide, median cells 10–18 μm long, 9–12 μm wide, basal cells 15–25(–30) μm long, 10–12 μm wide; cell walls thickened with well-developed trigones or nodular trigones, more distinct at the base, cuticle roughened by minute papillae; oil bodies 6–13 μm long, 3–6 μm wide, 2–4 per cell, mostly globose, glistening-homogeneous, smooth, rarely segmented with indistinct granules. Underleaves approximate to overlapping, to 2.0–2.5 times wider than the stem, ovate or subquadrate, 200–230 μm long, 210–230 μm wide; apex usually more or less retuse or with blunt teeth, hyaline marginal cells 2–4, restricted at the apex in the central notch, not observed in other parts of underleaves; margin entire throughout; base straight, perpendicular to the stem. Androecia present in the axils of underleaves; bracts in 2 pairs, concave, margin entire. Gynoecia not seen.

Distribution: Peninsular Malaysia; only known from the type collection in Cameron Highlands (Pahang).

Habitat: The type material was collected on a tree branch in a shaded area. It was found growing in a moist montane forest at an altitude of 1,900 m along the forest trail to the Puncak Mat summit. The forest floor is thickly covered by liverworts such as *Mastigophora diclados* (F. Weber) Nees, *Lepicolea rara* (Steph.) Grolle and various *Bazzania* spp.

DISCUSSION

The new species, *Mastigopelma latiffiana*, is characterised by: 1) leaves slightly falcate, asymmetrical with an almost straight ventral margin; 2) leaf

apices flat, rounded or sometimes truncate; 3) leaf margins entire throughout; 4) leaf cells with well-developed trigones, more prominent nodular trigones at the basal cells; 5) oil bodies 2–4 per cell, glistening-homogeneous, smooth; 6) underleaves ovate or subquadrate with 2–4 hyaline marginal cells at the apex; and 7) underleaf apices retuse or with blunt teeth. *Mastigopelma latiffiana* is morphologically closely related to *M. fragile* (Steph.) N. Kitag. among the five species of *Mastigopelma* Mitt., due to its non-dentate or serrate leaf margins and leaf apices lacking teeth. The discovery of *M. latiffiana* in Peninsular Malaysia represents an extension of the genus within the Malesian-Oceanic region, which includes Borneo, the Philippines, Papua New Guinea and Samoa. The new species in Peninsular Malaysia is the fifth species in the westernmost locality of the genus, extending its range into the Malesian region.

The genus *Mastigopelma* was initially described by Mitten (1873) for *M. simplex* Mitt. on the basis of its highly distinctive growth mode, i.e., the new leafy shoots arise from the basal stolons, solely ventral-intercalary branching. After a span of 77 years, another new species was described by Herzog (1950) from Borneo, namely *M. pulvinulatum* (De Not.) Grolle (= *M. bilobum* Herzog). Subsequently, Grolle (1970) described the third new species of the genus from Papua New Guinea as *M. subfissum* Grolle. At the same time, Kitagawa (1972) made a new combination of *Mastigobryum fragile* Steph. and assigned it to *Mastigopelma fragile* (Steph.) N. Kitag. Morphologically, the genus *Mastigopelma* Mitt. closely resembles *Bazzania* Gray and *Acromastigum* A. Evans, however, the former genus lacks pseudo-dichotomous branching and has the presence of stolons from which leafy shoots arise. Differences among all the species of *Mastigopelma* Mitt., including the new species, *M. latiffiana* are provided in the key below:

Key to the species of *Mastigopelma* Mitt.

1 Leaf margin entire or crenulate with more or less projecting marginal cells. Leaf apex rounded or truncated and without tooth 2

1 Leaf margin dentate to serrate. Leaf apex strongly toothed 3

2 Underleaf margin entire, apex with weakly blunt teeth or retuse. Basal leaf cells with large nodular thickenings trigones (Peninsular Malaysia) *M. latiffiana*

2 Underleaf margin crenulate, apex without blunt teeth. Basal leaf cells without large nodular thickenings trigones (Micronesia, the Philippines) *M. fragile*

3 Underleaf apex divided into (3–)4 lobes (Papua New Guinea) *M. subfissum*

3 Underleaf apex entire or retuse with 2–4 distant small toothed 4

4 Leaf apex distinctly divided into 2 lobes or short-bifid (Borneo) *M. pulvinulatum*

4 Leaf apex not divided into 2 lobes, usually with numerous conspicucous teeth (Samoa) *M. simplex*

CONCLUSIONS

The discovery of new species in Cameron Highlands, Pahang, is expected, as Pahang is recorded as the second largest state after Sabah regarding the number of species reported in Malaysia (Lee *et al.* 2022*a*). The state of Pahang in Peninsular Malaysia consists of several highlands with moist montane forests above 2,000 m alt., providing highly conducive habitats for bryophytes. Furthermore, the well-developed road infrastructure in Cameron Highlands makes the place easily accessible. Nevertheless, many mountains and forests in Malaysia remain largely unexplored from a hepaticological perspective, leaving significant potential for many more discoveries to be made.

*

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FISSIDENS POKHRENSIS NORK. – A NEW RECORD TO KERALA

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The Fissidentaceae is one of the moss families with the genus *Fissidens.* A species of *Fissidens viz*., *F. pokhrensis* has been collected from the Neeliyarkottam sacred grove of Kannur District, which is a new record to the moss flora of Kerala. Sacred groves remain neglected in the study of bryoflora.

Key words: bryoflora, Fissidentaceae, sacred grove

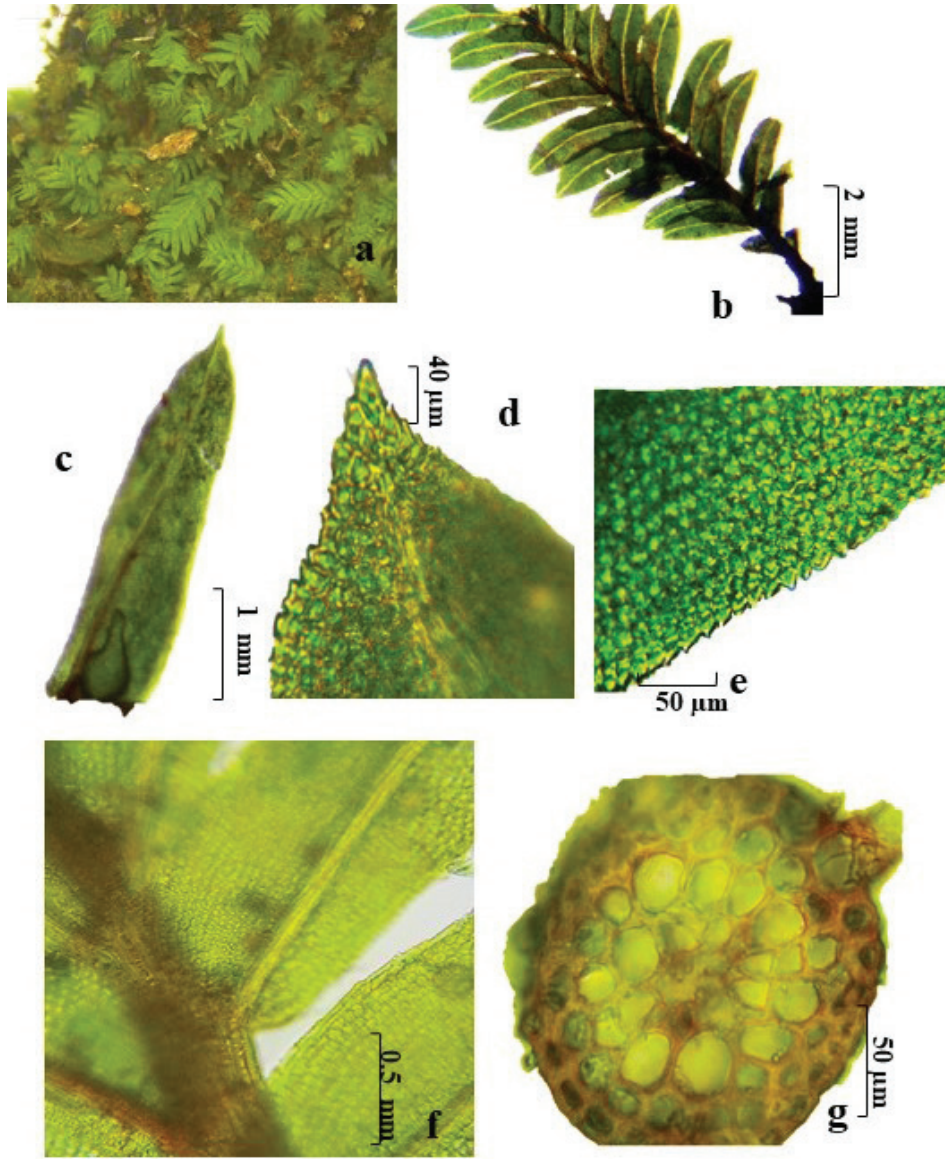
INTRODUCTION

Floristical diversity of vascular plants and ecological aspects of sacred groves are fairly well-studied especially in South India by Gadgil (1987), Chandran *et al.* (1998), and Godbole (1996). However, studies are scarce on the bryoflora of the sacred groves. There is only one report on the bryophytes of sacred groves in Kerala (Jyothilakshmi *et al*. 2016). The present study is focused on the diversity of bryophytes associated with the sacred groves of Kannur District. Sacred groves of different microclimatic conditions were selected for the study. Village forests, mainly traditional sacred grove called Neeliyarkottam, located between 11° 59′ 5.86″ N latitude and 75° 21′ 49.11″ E longitude situated in Morazha village in Taliparamba Taluk of Kannur District, is an evergreen patch of forest. A species of *Fissidens viz*., *F. pokhrensis* has been collected from this sacred grove. Incidentally, this species is a new record to the moss flora of Kerala. A description supported by a photographic plate is provided. Specimens are deposited at Sir Syed College, Taliparamba (SSCT).

TAXONOMIC NOTE

The Fissidentaceae is one of the acrocarpic and haplolepideous monogeneric moss families with the genus *Fissidens* Hedw. The term "*Fissidens*" was derived from the Latin words '*fissus*' meaning a cleft and '*dens*' meaning

tooth, referring to the families characteristic split peristome teeth. The genus is cosmopolitan in distribution. The significant characteristics of the genus *Fissidens* are the distichous arrangement of leaves and the presence of sheathing or vaginant laminae clasping stem.



Resolution is too low!

Fig. 1. Fissidens pokhrensis Nork.: a = plant habitat; b = single thallus separated; c = leaves and branching; d = leaf apex; e = leaf cells; f = central stipe (cross-section)

Fissidens pokhrensis Nork. ex S. S. Kumar (Fig. 1)

Misc. Bryol. Lichenol. 8: 120. f. 2 (1979); R. S. Chopra and S. S. Kumar, Moss. W. Himal.: 51, t. 50 (1981); J. Lal, Checklist Indian Moss.: 62 (2005); A. E. D. Daniels and P. Daniel, Bryofl. S.-most. W. Ghats, India: 48 (2013); A. E. D. Daniels *et al*., Bryofl. India Gandhi Natl. Pk.: 68 (2018); A. E. D. Daniels and K. C. Kariyappa, Bryofl. Agasthyamalai BR.: 85 (2019). – Type: India, Himachal-Pradesh, Simla, 2,300 m, Nov. 1966, *Norkett* 12192 (2) (holotype: BM).

Plants 2–3.2 mm tall; leaves 5–8 pairs, curled when dry, 1.3–2 × 0.6–1.01 mm, ovate-lingulate, serrulate at the margin, acute at apex; vaginant laminae half of leaves, unequal, open; costa ending below leaf apex; laminal cells quadrate-hexagonal, multipapillose on walls; apical cells 4–8 × 3–6 μm; median cells 6–10 × 4–8 μm; basal cells 8–12 × 6–10 μm; limbidium present only on vaginant lamina base of perichaetial leaves. Sporophyte not seen.

Habitat: Corticolous on a tree.

Distribution: Endemic to India. Western Himalaya and Western Ghats of Tamil Nadu and Kerala (Kannur Dist.) (Daniels and Daniel 2013, Gangulee 1969–1980).

Specimens examined: Western Ghats, Kerala, Kannur Dist., Taliparamba Taluk, Neeliyarkottam, *ca* 37 m, 25.6.2021, *P. Neethu et al*., 8521 (SSCT).

*

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NEW RECORDS FOR THE BRYOPHYTE FLORA OF VIETNAM, 5
Epihyllous liverworts of Tam Đảo Mountains, Vietnam

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In November 1998, guided by Prof. Trần Ninh we revisited the Tam Đảo mountain range, extensively researched by him before. Since it was converted into a National Park, with areas stretching to three provinces, Vĩnh Phúc, Thái Nguyên, and Tuyên Quang. The previous collections indicated that many more novelties can be expected from there. As a result, we collected 38 epiphyllous liverwort species. Among these 6 were new to the flora of Vietnam: *Cololejeunea fructu-marginata*, *C. papillosa*, *C. spathulifolia*, *Colura bisvoluta*, *Lejeunea dipterota* and *Microlejeunea sechuanensis*. One species is new to science: *Cololejeunea dinhensis*. Furthermore, *Cololejeunea rotundilobula* proved to be a new synonym of *Cololejeunea sigmoidea*.

Key words: *Cololejeunea*, endemism, Indochina, new species, new synonym

INTRODUCTION

In October 1963 I made a brief visit to the Tam Đảo Mountains, where I began collecting epiphylls. Only three records from that time were published (Pócs 1969). Even then, it was evident, that this humid area with relatively intact wet tropical forests harboured a very rich bryoflora. In the meantime, Trần Ninh from the Hanoi University of Science started to study its bryophytes, focusing especially on the mosses of the area, which is currently a national park, but he also collected liverworts. Trần Ninh published several papers, including the description of new species (Ninh 1980, 1981) and a comprehensive moss check list for the present Tam Đảo National Park (Ninh 1993). In 1999 two Hungarian botanists, Gabriella Kis and Erzsébet Fráter with her husband, Géza Kósa, guided by Vietnamese experts, visited shortly the mountains and collected 18 species of epiphylls at the foothills (Pócs 2023).

I revisited the area from 18 to 22 November 1998, with the guidance of Trần Ninh. We were accompanied by Dr Nguyễn Quốc Binh, a specialist of Zingiberaceae at the Botany Department of the Institute of Ecology and Biological Sciences (Vietnam) and by Géza Kósa, a dendrologist from the Institute of Ecology and Botany, Hungarian Academy of Sciences. We aimed to collect as many epiphyllous liverworts, as possible (20–30 host leaves per localities). In this paper I intend to publish the results after identifying them. The nomenclature fol-

lows Söderström *et al.* (2016). Voucher specimens are deposited in the herbarium of Hanoi University of Science (HNU) and in the herbarium of our university (EGR). The bryophyte specimens collected in Vietnam and incorporated into our herbarium until 2020 are enumerated in Lương *et al.* (2020). I changed in the title of the publication series from "liverworts and hornworts" to "bryophytes" following the renewed evolutionary concept (Bechteler *et al.* 2023), which suggests that bryophytes are monophyletic. Additionally, this change is practical as it allows for the publication of mosses in this series in the future.

MATERIAL AND METHODS

The epiphyllous collection

We collected epiphylls at the following places, as indicated by their locality numbers listed below. All identified epiphyllous liverworts are presented in Table 1.

No. 9897: Mossy elfin woodland with Melastomataceae, Ericaceae (*Vaccinium* sp.) and Theaceae shrubs and small (1–3 m) trees on the Đinh Rung Rinh summit at 1,335–1,345 m alt. 21° 28.76′ N, 105° 37.88′ E. Coll.: T. Pócs and Trấn Ninh, 18 Nov. 1998.

No. 9898: Montane rain forest NW from Tam Đảo town, NE slope of Mt Đinh Rung Rinh at 1,050–1,150 m alt. 21° 28.9′ N, 105° 38.2′ E. Coll.: T. Pócs and Trấn Ninh, 18 Nov. 1998.

No. 9899: Montane rain forest SE of Tam Đảo town, on the stony SW slopes of Mt Mỏ Quạ, at 910 m alt. 21° 26.9′ N, 105° 38.7′ E. Coll.: T. Pócs and Trấn Ninh, 19 Nov. 1998.

No. 98100: Microphyllous forest on the rocky summit ridge of Mt Mỏ Quạ, E from Tam Đảo town, at 980–1,045 m alt. 21° 26.5′ N, 105° 38.8′ E. Coll.: T. Pócs and Trấn Ninh, 19 Nov. 1998.

No. 98102: Locality is missing ??????

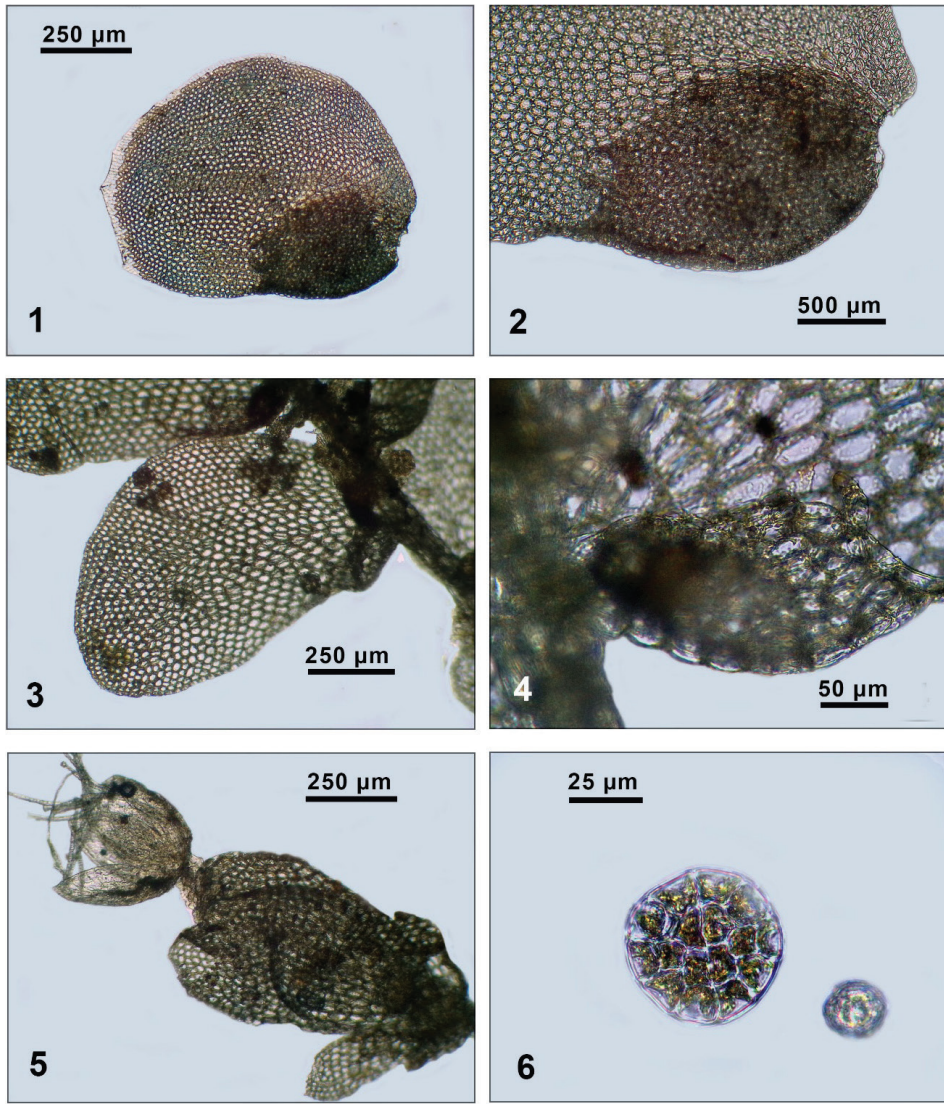
No. 98103: Mt Cái Keng N of Tam ảo town. Montane rain forest on the S slopes between 1,015 and 1,185 m, with many *Cylindrokelupha alternifoliolata* trees, rich in bryophytes along streamlet. 21° 27.6′ N, 105° 38.8′ E. Coll.: T. Pócs and Trấn Ninh, 21–22 Nov. 1998.

No. 98105: Montane rain forest in the valley E from Thác Bạc waterfalls, SW of Tam Đảo town, at 870–940 m alt. 21° 27.23′ N, 105° 39.05′ E. Coll.: T. Pócs, 22 Nov. 1998.

RESULTS

Species new to the bryoflora of Vietnam

The following list enumerates the species new to the bryoflora of Vietnam, including those reported only once before, as documented in the check-



Figs 1–2. Cololejeunea fructu-marginata Tixier, leaf and lobule, ventral view (from 98102). – *Figs 3–6. Cololejeunea spathulifolia* (Steph.) H. A. Mill., 3: leaf; 4: lobule, ventral view; 5: gynoecium with sporophyte; 6: gemma (from 98102)

Table 1

The occurrence of collected species in the different localities (Loc. 9897–98 and 9899–100 are united, as they are very close to each other). The numbers in the five columns are the traditional dominant values according to Braun-Blanquet (1964). In the last column frequency values are indicated

Species/Locality no.	9897–9898	9899–98100	98102	98103	98105	FR
Cheilolejeunea turgida (Mitt.) W. Ye et R. L. Zhu	+	–	–	–	–	I
Cheilolejeunea trapezia (Nees) R. M. Schust.	1	1	–	–	–	II
Cheilolejeunea xanthocarpa (Lehm. et Lindenb.) Malombe	+	1	–	–	–	II
Cololejeunea appressa (A. Evans) Benedix	1	+	–	+	–	III
Cololejeunea dinhensis sp. n.	2	3	–	2	–	III
Cololejeunea fructu-marginata Tixier	–	–	+	1	–	II
Cololejeunea haskarliana (Lehm. et Lindenb.) Schiffn.	–	–	2	–	–	I
Cololejeunea inflata Steph.	–	2	–	–	–	I
Cololejeuna lanciloba Steph.	–	–	–	–	2	I
Cololejeunea peraffinis (Schiffn.) Schiffn.	1	–	–	+	–	II
Cololejeunea papillosa (K. I. Goebel) Mizut.	1	–	–	–	–	I
Cololejeunea sigmoidea Ast et Tixier	1	–	2	2	–	III
Cololejeunea spathulifolia (Steph.) H. A. Mill.	–	–	+	–	–	I
Cololejeunea tenella Benedix	+	–	–	–	–	I
Cololejeunea trichomanis (Gottsche) Besch.	–	–	3	+	2	III
Colura bisvoluta Herzog et Ast	+	–	–	–	–	I
Diplasiolejeunea cobrensis Gottsche ex Steph.	+	–	–	–	–	I
Diplasiolejeunea rudolphiana Steph.	+	–	–	–	–	I
Drepanolejeunea commutata Grolle et R. L. Zhu	2	–	–	+	–	II
Drepanolejeunea erecta (Sateph.) Mizut.	2	2	+	1	+	V
Drepanolejeunea foliicola Horik.	1	–	–	–	–	I
Drepanolejeunea spicata (Steph.) Grolle	1	–	–	–	3	II
Drepanolejeunea tenera K. I. Goebel	1	–	–	–	–	I
Frullania alstonii Verd.	–	1	+	–	–	II
Lejeunea adpressa Nees	+	–	–	–	+	II
Lejeunea cf. *dipterota* (Eifrig) G. E. Lee	–	–	–	–	+	I
Lejeunea parva (Sw.) Nees	–	1	–	+	+	III

Table 1 (continued)

Species/Locality no.	9897–9898	9899–98100	98102	98103	98105	FR
Leptolejeunea maculata (Mitt.) Schiffn.	1	–	–	2	–	II
Leptolejeunea subacuta A. Evans	2	1	2	1	–	IV
Metzgeria consanguinea Schiffn.	1	–	–	–	–	I
Metzgeria furcata (L.) Corda	–	–	–	2	–	I
Microlejeunea punctiformis (Taylor) Steph.	–	1	+	1	–	III
Microlejeunea szechuanensis P. C. Chen	–	–	–	1	–	I
Myriocoleopsis minutissima (Sm.) R. L. Zhu et Pócs	+	–	–	–	–	I
Radula tjibodensis K. I. Goebel	+	–	–	–	–	I
Radula acuminata Steph.	–	–	2	1	–	II
Radula gedena Gottsche ex Steph.	1	–	–	+	–	II
Tuyamaella molischii (Schiffn.) S. Hatt.	–	1	–	–	–	I
Number of species	24	11	10	16	7	

lists of Bakalin and Sinh (2016) and Shu *et al.* (2017). Voucher specimens are deposited in HNU and their duplicates in EGR.

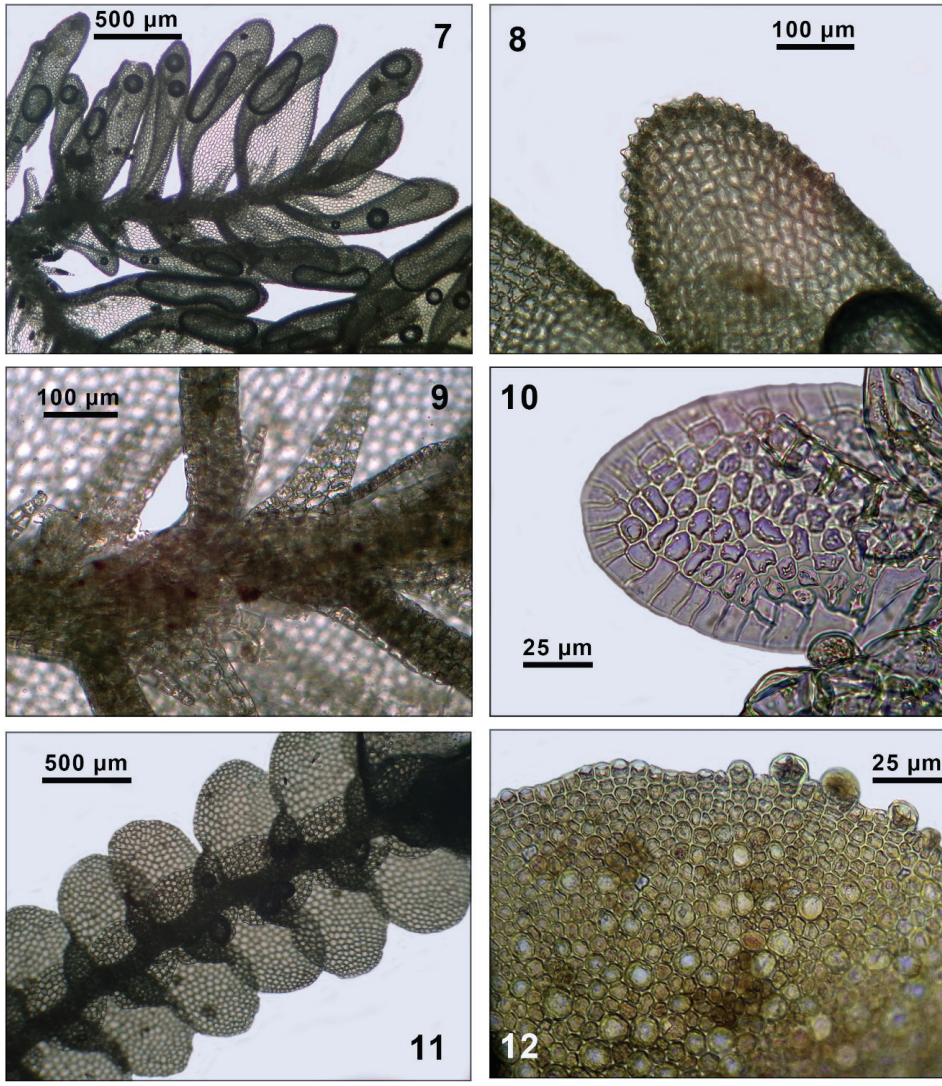
Cololejeunea fructu-marginata Tixier – Nos 98102, 98103 (EGR, HNU) (Figs 1–2) – A well-defined species of subgenus *Pedinolejeunea* Benedix ex Mizut. 1961. It has two unequal lobule teeth and lobe margin formed by two or more rows of hyaline cells at the apex. New to Vietnam, hitherto known from Thailand and Malaysia (Tixier 1985).

Cololejeunea papillosa (K. I. Goebel) Mizut. (Syn. *Aphanolejeunea papillosa* (K. I. Goebel) Herzog) – No. 9898 (HNU) – It is a tiny species of subgen. *Aphanolejeunea* characterised by many reduced, linear leaves formed by cells in two rows with smooth (not dentate) margin. A rare tropical American–Asian–Australasian disjunct, new to Vietnam (distributional data by Pócs and Piippo (1999), under the name of *Aphanolejeunea borneensis* (Herzog) Pócs).

Cololejeunea spathulifolia (Steph.) H. A. Mill. – No. 98102 (HNU, EGR only microslide) (Figs 3–6) – Species characteristic by its leaf shape and lobule with one tooth, the smooth leaf cells and the very short bracts of perianth. Gemmae 16 cells (new observation). Hitherto is known only from the Solomon Islands and Thailand, new to Vietnam. The reference of Chantanaorrapint and Pócs (2014) to Tixier (1985) of its presence in Réunion, Vietnam, New Caledonia and Hawaii was by mistake.

Colura bisvoluta Herzog et Ast – No. 9897 (HNU, EGR only microslide) (Figs 7–10) – In Tam Šảo only one well developed specimen occurred in our collection. It agrees in all properties with the Malaysian and Thai specimens

illustrated and described by Jovet-Ast (1954) and Sangrattanaprasert *et al.* (2018). But it differs from them by the inner valve cells, which have much larger, almost confluent trigones (Fig. 10). It was known from Sumatra, Thailand, Malaysia and Australia, new to Vietnam.



Figs 7–10. Colura bisvoluta Herzog et Ast, 7: habit, ventral view; 8: lobule apex; 9: underleaves, ventral view; 10: valve (all from 9897). – *Fig. 11. Lejeunea* cf. *dipterota* (Eifrig) G. E. Lee, habit, ventral view (from 98105). – *Fig. 12. Radula gedena* Gottsche ex Steph., lobe cells (from 98103)

Lejeunea cf. *dipterota* (Eifrig) G. E. Lee – No. 98105 (EGR, only microphoto) (Fig. 11) – The sterile specimen was very similar to the one described and illustrated by Eifrig (1936 under *Taxilejeunea dipterota*) and by Lee (2013), but without seeing a perianth I could not confirm its identity with certainty. The circular-reniform underleaves almost covering the lobules with fully incurved margin refer to this species. This case ~~it~~ would be new to Vietnam, known from Java and Malaysia (Sabah) before.

Microlejeunea szechuanensis P. C. Chen – No. 98103 (EGR) – It is a taxon of a bit uncertain position. It is differentiated from the widespread *Microlejeunea punctiformis* (Taylor) Steph. by its asymmetric, falcato-ovate leaf, broadening upwards and by the 3–5 cells long underleaf segments. Previously only known from Sichuan, SW China, new to Vietnam (Miller *et al.* 1967).

Species reported previously only from one locality in Vietnam

Cheilolejeunea turgida (Mitt.) W. Ye et R. L. Zhu – No. 9898 (only microslide) – This generally rare species is known in Vietnam only from Tam Đảo, which we could confirm. It was first reported by Zhu and Lai (2003), under the name of *Leucolejeunea turgida* (Mitt.) Verd. (Shu *et al.* 2017). It is distributed from Sri Lanka and the Himalayas to southern China and Thailand (Kitagawa 1968, Ye and Zhu 2010, Zhu and So 1999 with map;).

Radula gedena Gottsche ex Steph. – No. 9898 (HNU); No. 98103 (EGR) (Fig. 12) – Rare among the epiphyllous species of *Radula.* Easy to recognise by the uneven size of lobe cells, a good number of them being larger (12–16 μm) than the average (8–10 μm). In addition, numerous small discoid gemmae are all around the lobe margin. It was known before only from the Central Highland of Lâm Đồng Province in southern Vietnam (Pócs *et al.* 2013) and from the Hoang Lien Mountains in northern Vietnam (Bakalin *et al.* 2023). It is known to be scattered from Java to Japan and Thailand (Yamada 1979).

NEW SPECIES

Cololejeunea dinhensis Pócs, *spec. nova*
(Figs 13–18)

The new species belongs to Sectio Leonidentes *Benedix, Feddes Rep. Spec. Nov. Beiheft 134: 38, and is related to* Cololejeunea ensifera *Tixier (1968) and to* Cololejeunea ocelloides *(Horik.) Mizut. (1961, syn.:* Cololejeunea leonidens *Benedix 1953). It differs from both, with its first lobule tooth being much larger than the second tooth, which is narrow and, in most cases, much shorter, consisting of only a few cells.*

Type: Vietnam, Vinh-Phuc Prov., Tam-Đảo Mts, montane rain forest NW from Tam-Đảo town, on the NE slope of Mt Rung Rinh at 1,050–1,150 m elevation. 21° 28.9′ N, 105° 38.2′ E. Epiphyllous. Coll.: T. Pócs and Trấn Ninh, 18 November 1998. Holotype: 9898/AB (EGR); isotype (HNU); paratypes: 9898/A, 9899/V, 98103/BQ (EGR).

Etymology: The Vietnamese meaning of Đinh is a nail, peak or any pointed object (and rung rinh means 'moving' or 'vibrating'). The new species is named after the summit Đinh Rung Rinh and the long, pointed first lobule tooth.

Description: Relatively large species with 1.2–1.8 mm wide, pale green shoots form roundish colonies of 1.2–1.8 cm, appressed to the host leaf surface of shrubs or *Pandanus* sp. A 2–3 cell wide broad vitta can be seen even by a hand lens. The 60–80 μm thick stem irregularly branching and composed of 1 medullary and 6 cortical cells, of one is the ventral merophyte. Cortical cells are rectangular, about 40 × 20 μm, the ventral, merophyte cells irregular in shape and size. The leaves are ovate-oblong, asymmetric, reniform, slightly falcate, up to 720 × 560 μm size, leaf insertion angle to the stem 30°, ventral margin forms an angle of 140–150° with the keel. Lobe cells mostly square or rhomboid (8–18 × 6–12 μm), thin walled without trigones, each with a small papilla on both sides. Marginal cells just smaller (4–8 × 6–8 μm), hardly differentiated from the other lobe cells, rarely rectangular, perpendicular to the margin. The vitta is striking, honey yellow and shining under stereo microscope, always much exceeding the lobule in length, composed of two rows of 6–7 larger (up to 180 × 40 μm) cells diminishing toward the end. A third, smaller row accompanies them with sometimes scattered and obliquely located cells. The vitta cell walls have bulbous trigones and 2–3 intermediate thickenings. The lobule is of ⅓–¼ of lobe length and width, ovate and evenly inflate with a long, linear-lanceolate, first tooth consisting of 3–6 uniseriate cells and the usually shorter triangular second tooth, crossing each other. The second tooth usually small and consists only of 1–4 cell, sometimes obsolete and is always overgrown by the first tooth, crossing each other. An ellipsoidal, translucent hyaline papilla of 16 × 18 μm is between them, attached to the distal margin of lobule. The first tooth is at 2(–3) cells distance from the keel. Stylus bicellular, 40 × 15 μm.

I could not observe any gametangia on the investigated, more than 120 individuals. But on the other hand, the vegetative propagation is enhanced by the many discoid, endogenous gemmae of brownish colour, developing mostly on the ventral side of the lobes (generally 10–30, up to 50 per leaf). Normally the gemmae consist of 20–24 cells, round or very slightly reniform, 40–45 μm in diameter.

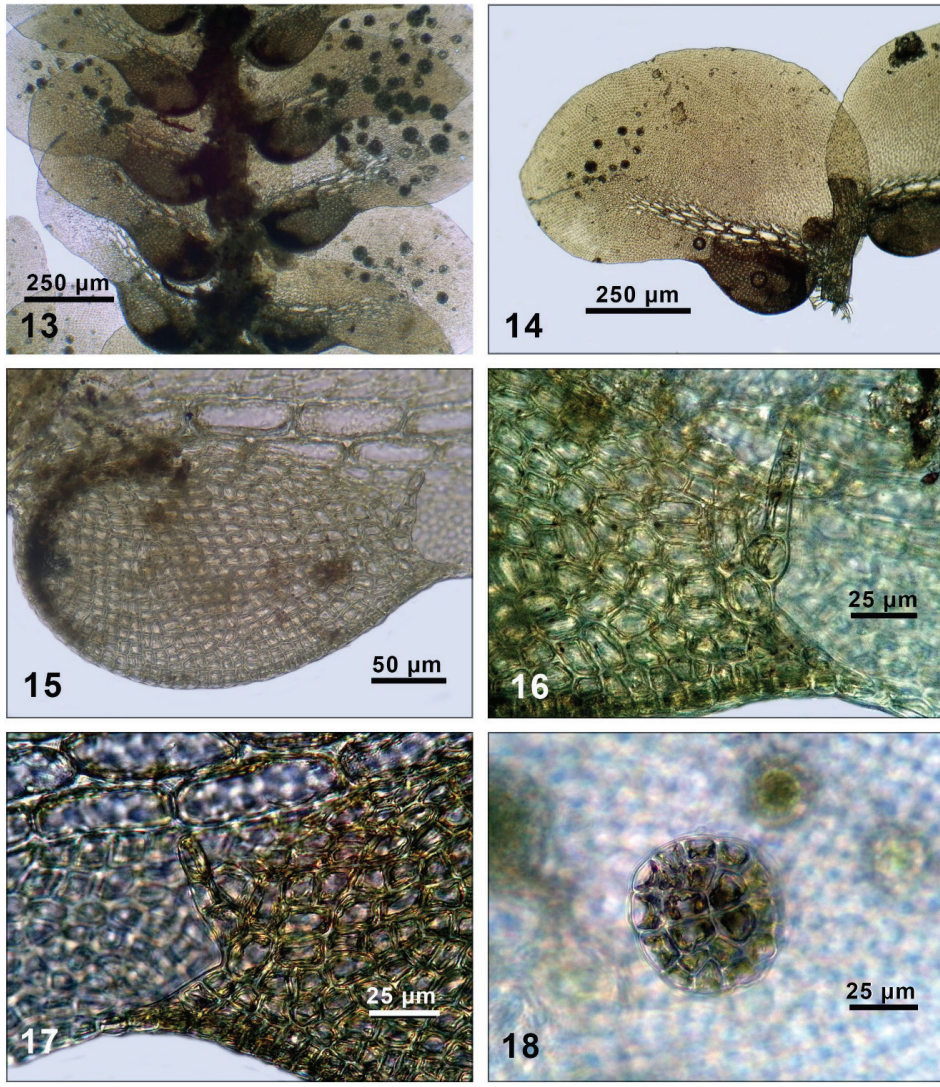
Several differences distinguish it from the related species of Leonidentes section. It differs from *C. bachmaensis* Tixier (1968), by the much longer vitta consisting of 2 or more rows of ocelli and in its small lobule; from *C. ensifera*

Table 2

Comparison of the morphological differences among *Cololejeunea dinhensis*, and related species of Sect. *Leonidentes*. (Species without true hyaline margin, with vitta much exceeding the length of lobule and with lobule teeth crossing each other)

Properties	*C. dinhensis* sp. n.	*C. bachmaensis*	*C. ensifera*	*C. crassipapillata*	*C. gresicola*	*C. ocelloides*
Leaf shape	subsymmetric ovate, slightly falcate, ventral margin forming an angle of 150–170° to the keel	rotundato-ovate, dorsal margin strongly arched, ventral margin almost straight	shortly obtuse-ovate, dorsal margin strongly arched, ventral margin almost straight	rotundato-ovate, asymmetric, ventral margin forming an angle of 160–170° with the keel	subsymmetric ovate with rounded apex, ventral margin almost straight	subsymmetric ovate, slightly falcate, ventral margin forming an angle of 160–170° with the keel
Angle of leaf insertion	30°	30°	30°	70°	70°	60–70°
Lobe margin cells	square, rarely rectangular and perpendicular to margin	rectangular or square	rectangular or square	rectangular, perpendicular to margin, rarely square	rectangular	rectangular, perpendicular to margin
Lobule/lobe length %	25–30	40–50	50–60	40–50	50–60	40–50
Lobule shape	broadly ovate. inflated	broadly ovate. inflated	elongate, inflated	ovate, inflated	ovate, inflated	ovate, inflated
Vitta	6–7 cell long, 2+1 rows	6–7 cells long,1 (+1) row	6–7 cells long, 2 rows	6 cell long, 2+1 rows	–11 cells long, 3 rows	4–10 cells long, 2–4 rows
Style cells	2	2	2–3	1	1	1
1st lobule tooth	long, linear-lanceolate, consisting of 3–6 uniseriate cells, much longer than the 2nd tooth	linear-lanceolate, formed by 2–3 cells, not exceeding the 2nd tooth	long, linear-lanceolate, consisting of 4–5 uniseriate cells	linear-lanceolate, 1–2 cells long, shorter than the 2nd tooth	linear-lanceolate, 2 cells long with thick walls, shorter than the 2nd tooth	linear-lanceolate, 2–4 cells long, much shorter than the 2nd tooth
2nd lobule tooth	small, narrow triangular to lanceolate or reduced to 0–few cells	narrow triangular, 2–3 cells broad at its base	triangular, as long as the 1st tooth, 3–4 cells broad at base	large, broad triangular, 3–5 cells broad at base	large, broad triangular, 3–4 cells broad at base	large, triangular, 2–4 cells broad at its base
Cells between 1st tooth and lobe margin	1–3	4	4	1–3	2	2–4

Tixier (Tixier 1968) by the smaller second tooth and the much shorter, ovate lobule and the vitta having a third row; from *C. crassipapillata* Tixier (Tixier 1968) by its bicellular stylus and much longer first and smaller second tooth; from *C. gresicola* Tixier (Tixier 1968) and from *C. ocelloides* (Horik.) Mizut. (Mizutani 1961) by its other leaf shape, smaller lobule and longer first lobule tooth. These differences are summarised in Table 2.



Figs 13–18. Cololejeunea dinhensis sp. n., 13: habit; 14: leaf; 15: lobule; 16–17: lobule teeth; 18: gemma (all ventral view, from the holotype, 9898/AB)

NEW SYNONYM

The format of this section follows Söderström *et al.* (2012).

Cololejeunea sigmoidea Ast et Tixier, Rev. Bryol. Lichenol. 31 (1/2): 273 (Jovet-Ast and Tixier 1962). – Type: Vietnam, Benom da Treu, forêt dense 1,800 m alt., leg. P. Tixier (holotype PC!).

= *Cololejeunea rotundilobula* (P. C. Wu et P. J. Lin) Piippo, J. Hattori Bot. Lab. 68: 134 (Piippo 1990). = *Pycnolejeunea rotundilobula* P. C. Wu et P. J. Lin, Acta Phytotax. Sin. 16: 69 (Wu and Lin 1978). – Type: China, Hainan, Jianfengling, Tianchilinchang, 1,150 m, on the leaves of *Symplocos viridissima* Brand, 6 Feb. 1962, P. C. Chen et al. 456 (holotype IBSC, isotype: HSNU), *syn. nova.*

Although I have not seen the type of *Pycnolejeunea rotundilobula*, have seen many large populations in the Tam Đảo material, which have shown all transitions from specimens without saccate lobule to populations, which have saccate lobules on the majority of leaves and reduced lobules only on the rest. Populations completely without saccate lobules are rare; in most cases one can find a few leaves with lobule sacks. Asthana and Srivastava (2003: 99, Plate 15) illustrated also this kind of specimen, as *C. sigmoidea*. Usually well-developed, larger specimens have more saccate lobules. The variation of saccate and reduced lobules is also known by other *Cololejeunea* species, such as *Leptolejeunea epiphylla* (Mitt.) Steph. The difference between *C. sigmoidea* and *C. rotundilobula* in leaf shape given by Wu and Lin (1978) and referred to them by Zhu and So (2001: 249, 254), after examination of many specimens, is not existing. Therefore, these properties are inadequate to separate the two species.

DISCUSSION

The Tam Đảo range has even within Vietnam high bryophyte diversity. Several species are endemic to this mountain or at least for the northernmost part of Vietnam. Trấn Ninh (1993) enumerates the formerly published *Campylopus eberhardtii* Par., *Leucoloma tonkinense* Broth. et Par., *Callicostella eberhardtiana* Broth. et Par. and *Heterophyllium microalare* (Broth. et Par.) Broth. He himself (Ninh 1981) described several new moss species, which seems to be restricted to this area (*Calymperopsis vietnamensis* Ninh, *Calyptrochaeta pocsii* Ninh and *Distichophyllum duongii* Ninh). Concerning liverworts, there are no old records of endemics from the Tam Đảo Mountains (Pócs 1965). More recently was described *Cololejeunea tamdaoensis* Tixier (Tixier 1968).

After the original delimitation of section *Leonidentes* (Benedix 1953), Tixier (1968) dealt in details with this species group. From the section only 2 more widespread species were known from the Indo-Malesian region (Tixier 1978), and he added 17 new taxa from the Southeast Asian tropics. It can be

questioned whether these new species are distinct or not. Taken in account their differentiating characters in leaf shape and insertion, lobe margin, vitta, lobule shape and dentition and their quite restricted distribution in certain mountainous areas of Vietnam, Cambodia, Thailand, and the Philippines, it seems that Indochina and the surrounding area is a real hot spot of their biodiversity and allopatric speciation. The recently described *Cololejeunea dinhensis* Pócs joins this group and well differs from *C. tamdaoensis* Tixier described from the same mountains.

Renner (2020) emphasises the importance of the investigation of cryptic bryophyte species, analysing in detail the results of widespread research in this field, both by refined morphological and molecular methods. Therefore, related species, distinguished even by little but important morphologic differences, should be kept apart until molecular methods clarify their evolutionary status. This is valid also for such species group as the Indochinese members of the *Leonidentes* section of *Cololejeunea.*

*

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SEA BARLEY (*HORDEUM MARINUM*) SEED GERMINATION ECOLOGY AND SEEDLING EMERGENCE

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Sea barely is weedy grass in agricultural landscapes and infrastructure habitats (roads, railroads, etc.) in Golestan province (the northern part of Iran). This study investigated the germination of sea barley in response to temperature, water potentials, salinity, pH levels, waterlogging, heat stress and also seedling emergence in response to burial depth. Results showed that sea barley seeds germinated over a wide range of temperatures from 5 to 35 °C, with the highest germination at 25 °C. Seed germination was rapidly reduced with increasing osmotic potential so that germination declined by 36% at –0.2 MPa. This was also the case for the salinity stress, and germination declined by 30% at 40 mM NaCl. Seed germination was the highest (> 65%) in 6 to 7 pHs and no germination was observed at alkali levels. Heat stress completely inhibited the germination of seeds at all tested temperatures and durations. Sea barley seed germination was higher than 50% after being waterlogged for 45 days, and some germination (12%) still occurred 60 days after waterlogging. The highest seedling growth occurred at 1–2 cm soil depth and was negligible at ≥5 cm soil depths. The results of this study indicate that deep tillage or flamethrower may be good options to mitigate the negative impacts of this weed.

Key words: burial depth, heat stress, management, osmotic potential, roadsides

INTRODUCTION

Sea barely (*Hordeum marinum* Huds., Poaceae) is a self-pollinating winter annual grass with original distribution in Europe, Western Asia, and North Africa. It is also introduced into Australia, America, South Africa, and Japan (https://powo.science.kew.org). This species has been mentioned as a common weedy grass in roadsides, railways, and barley fields of Iran (Sohrabi *et al.* 2017).

Sea barley is distinguished by the well-developed auricles that clasp the stem, and by the typically hairy, flat, narrow leaves about 3 to 8 mm wide. This plant can reach a height of over 100 cm, but 30 to 60 cm is much more common (Di Tomaso *et al*. 2013). Sea barely produces a bristly thick spike, 2.54 to 7.6 cm long, from April through June. The central axis of the spike

breaks apart at the nodes at maturity. Reproduction is only by seed (Cope and Gray 2009). This weed can be prevalent in continuously disturbed areas with humid winters and dry summers. It competes with crops for the limited moisture in spring and prevents the establishment of native perennial species where it is adventive (Di Tomaso *et al*. 2013).

Seed germination is a key demographic process in plants and the ability to predict seed germination could determine the competitiveness and enhance weed management in crops by facilitating the implementation of more effective weed control strategies and the optimization of weed control timing (Craven *et al*. 2019, Hassanpour-Bourkheili *et al*. 2020, Leblanc *et al*. 2004, Myers *et al*. 2004, Sohrabi *et al*. 2011, ~~2012,~~ 2014, 2023, Sumudunie and Jayasuriya 2019). Sea barley as a seaside species is likely to occur in wetland regions (Kotula *et al*. 2014, Maršálová *et al.* 2016). However, the impacts of saline conditions and waterlogging in seaside regions on seed germination of sea barley are not well understood. This issue deserves to be discussed, as salinity is considered to be a significant environmental threat in most agricultural production areas, especially in hot arid and semi-arid areas (Chen *et al.* 2019) such as Iran with serious climate change-related challenges (reduced rainfall, increased air temperature and soil evaporation). Waterlogging resistance of the sea ~~barely~~ has been reported (Kotula *et al*. 2014), but there is no information about this species in Iran. Sensitivity to acidic or alkali levels of soil is an essential factor to determine the distribution of weed species, each species has a different preference for soil pH (Gherekhloo *et al*. 2023, Sohrabi Kertabad *et al*. 2013). Fire impacts competition due to reduced germination, which is done through seed mortality or triggering seed germination by dormancy breaking (Zomer *et al.* 2022). Thus, fire is an important ecological management tool, and it is critical to understand how weeds like sea barley respond to high temperatures. Also, different tillage practices can be recommended to control weed seedlings based on their ability to emerge from certain burial depths (Sohrabi *et al*. 2016, Khodapanah *et al*. 2023).

Detailed knowledge about the range of environmental conditions favourable to sea barley seed germination and seedling emergence of this weed could help to predict its occurrence potential in new areas and thus be useful to develop effective long-term management methods. Therefore, the present paper studies the influence of environmental factors including temperature, osmotic potential, pH levels, waterlogging, heat stress, and burial depth on germination response of sea barley seeds.

MATERIAL AND METHODS

Seed source – Seeds of sea ~~barely~~ were collected from different barley production farms, roadsides, and railways (at least from more than 50 locations) at the maturity stage in June 2017 in the north of Iran (37° 04′ 12″ N, 54° 04′ 37″ E – 36° 85′ N, 54° 27′ E). The seeds were then pooled and stored at ambient temperature during the experiment period (18–21 °C).

General germination protocol – The experiment was carried out based on a completely randomized design with four replicates. To obtain the highest germination percentage, the seeds were exposed to cold stratification pretreatment for 14 days at 3 °C (Taheri 2016). The seeds (25 seeds per Petri dish) were placed in 9 cm Petri dishes (each serving as a replicate) on a Whatman no. 1 filter paper moistened with 5 ml of deionized water or other solutions (described below). Germination tests were carried out in the darkness in an incubator at the temperature with the highest germination percentage (see section "Response to temperature"). Petri dishes were checked 2–6 times a day for a 14-day period. Germination was defined as a visible radicle with a 2 mm length).

Response to temperature – The Petri dishes containing the seeds were exposed to 5, 10, 15, 20, 25, 30, 35 and 40 °C temperatures. Further experiments were carried out at the temperature that resulted in the highest germination (25 °C). Other conditions in this experiment were similar to those described in section "General germination protocol".

Drought stress – Solutions with osmotic potentials of 0, –0.2, –0.4, –0.6, –0.8, –1 and –1.2 MPa were prepared by dissolving polyethylene glycol (PEG-6000) based on Michel and Kaufman (1973). Other conditions in this experiment were similar to those described in section "General germination protocol".

Salt stress – The seeds were treated with seven levels of salt concentration (0, 40, 80, 120, 160, 200 and 240 mM), which were prepared by using NaCl. Twenty-four hours before the experiment, filter papers were placed in a prepared solution to simulate the usual salt condition. Other conditions in this experiment were similar to those described in section "General germination protocol".

pH solutions – Buffered pH solutions were prepared using potassium hydrogen phthalate in combination with 0.1 M HCl to obtain pH solution levels of 4, 5, and 6. A 25 mM sodium tetraborate decahydrate solution was used in combination with 0.1 M NaOH to prepare solutions with pH levels of 7, 8, or 9 (Gherekhloo *et al*. 2023). Seeds were placed on a moist paper containing 5 mL of the appropriate pH solution. Other conditions in this experiment were similar to those described in section "General germination protocol".

Heat stress – The effect of heat stress on seed germination (25 seeds with four replicates) was simulated in the laboratory at different temperatures (40,

80, 100,120, 140, and 240 °C) in a heated air oven for varying durations (0, 2.5, 5 and 7.5 min).

For heating and waterlogging stress, un-germinated seeds were tested with 1% TTC (2, 3, 5-triphenyltetrazolium chloride) at 25 °C to assess viability. After 12 h, seeds showing a pink to reddish colour were considered viable.

Seed burial depth – To determine the effect of burial depth on the emergence, ten seeds with four replicates were buried at 1, 3, 5, 7 and 9 cm depths in 18 cm diameter plastic pots filled with soil (50% clay, 25% sand and 25% peat). Pots were placed randomly inside a greenhouse under day/night temperature conditions (27/20 °C) in a light/dark (12/12 h) period. Pots were watered daily to maintain the soil at the field capacity. Seedlings were checked daily and were considered to have emerged when coleoptile was visible at the soil surface. Once a seedling reached this stage, it was removed from the pot.

Waterlogging – Plastic pots were filled with soil under the conditions described in section "Seed burial depth". Then, 25 seeds were placed in fabric bags, which were then sewn. The bags were buried in the soil at 1 cm depth. Each treatment consisted of three pots, and each pot contained three fabric bags. These pots were then placed inside larger pots with 30 cm depth and 25 cm diameter. The larger pots were filled with water so that the water level was a few centimetres above the soil (in smaller pots) (Atabaki *et al*. 2023). The bags remained in the soil for a period of 3, 7, 15, 21, 30, 45, 60 and 70 days. After these durations, the bags were extracted, and a germination test was performed (see section "General germination protocol").

Data analysis – The experiments were repeated twice. The data were pooled due to the similarity between the two experimental runs. The data collected from the pH experiments were tested using an analysis of variance (ANOVA), using SAS 9.1 (SAS institute, Cary, NC, USA). Significant differences among treatments were identified using a least significant difference (LSD) test ($P < 0.05$).

A 3-parameter sigmoidal function (equation 1) was fitted to the data related to the response of seeds to temperature using Sigma Plot 8.0. This equation was also used for the data related to osmotic potential, salt stress, waterlogging and burial depth experiments.

$$G = G_{max} / (1+ \exp (-(x-x_{50})/b)) \quad (1)$$

where G is the total cumulative germination percentage, G_{max} is the maximum cumulative seed germination percentage, x_{50} is the time to 50% of maximum seed germination and b is the slope of the curve.

Table 1
Coefficients of three-parameter sigmoidal model fitted to cumulative germination percentage of sea barley at different temperatures

Temperature (°C)	G_{max}	b	x_{50} (h)	R^2
5	41.53 (0.6)	15.29 (1.3)	123.45 (1.8)	0.99
10	41.97 (0.8)	10.87 (1.5)	44.12 (1.7)	0.97
15	67.66 (0.9)	12.94 (1.2)	30.56 (1.3)	0.97
20	70.84 (0.6)	4.83 (0.5)	10.04 (0.5)	0.99
25	85.85 (0.9)	5.19 (0.6)	8.85 (0.7)	0.96
30	64.14 (0.7)	4.40 (0.7)	9.76 (0.7)	0.96
35	55.82 (0.8)	5.59 (0.9)	13.33 (1.0)	0.95
40	0	–	–	–

G_{max} = maximum germination percentage; x_{50} = time to reach 50% of germination; b = coefficient of the equation. Values in parentheses denote standard error

RESULTS

Response to temperature – Maximum germination percentage of sea barley increased with increasing temperature up to 25 °C and decreased thereafter. This value was 41.53% at 5 °C, which reached 85.85% at 25 °C and decreased to 55.82% at 35 °C. No germination was observed at 40 °C. Ger-

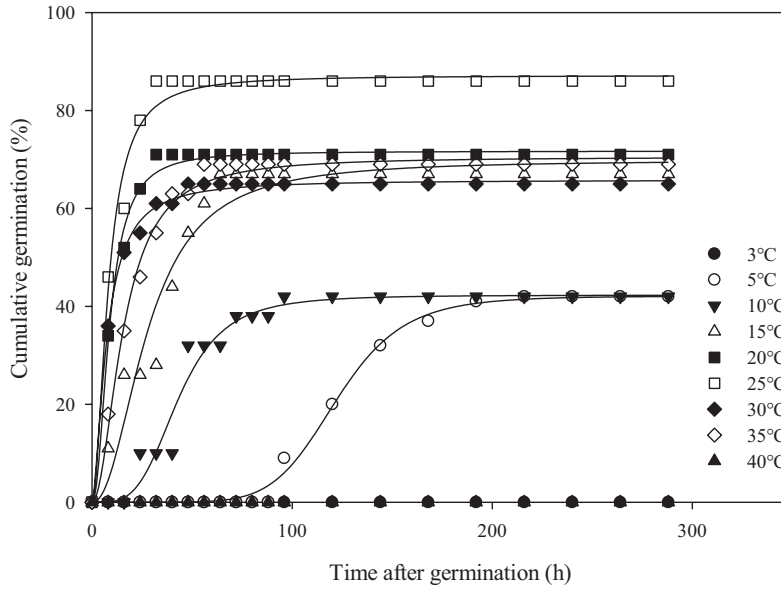

Fig. 1. Effect of temperature on the germination of sea barley

Table 2
Estimated parameters for 3-parameter sigmoidal function at different levels of osmotic potential of sea barley

R^2	b	x_{50} (h)	G_{max}	Osmotic potential (MPa)
0.96	10.80 (1.24)	17.82 (1.35)	81.36 (1.71)	0
0.89	32.97 (9.69)	49.25 (13.88)	52.17 (10.44)	–0.2
0.91	24.47 (5.78)	66.39 (10.02)	42.09 (6.59)	–0.4
0.93	13.88 (2.89)	73.31 (4.27)	40.52 (3.98)	–0.6
0.99	3.57 (0.26)	83.12 (0.24)	23.22 (0.32)	–0.8
0.99	3.21 (0.16)	83.08 (0.16)	13.10 (0.12)	–1
–	–	–	0	–1.2

G_{max} = maximum germination percentage; x_{50} = time to reach 50% of germination; b = coefficient of the equation. Values in parentheses denote standard error

mination rate followed a similar trend, so that x_{50} at 5 °C, 25 °C and 35 °C were 123.45, 8.85, and 13.33 h, respectively (Table 1, Fig. 1). Since the highest germination was observed at 25 °C, further tests were carried out at this temperature.

Drought stress – Increased drought stress led to a decline in seed germination of sea barley. About 30 and 40% reduction in seed germination was found at –0.2 and –0.4 MPa, respectively. Maximum germination (G_{max}) was

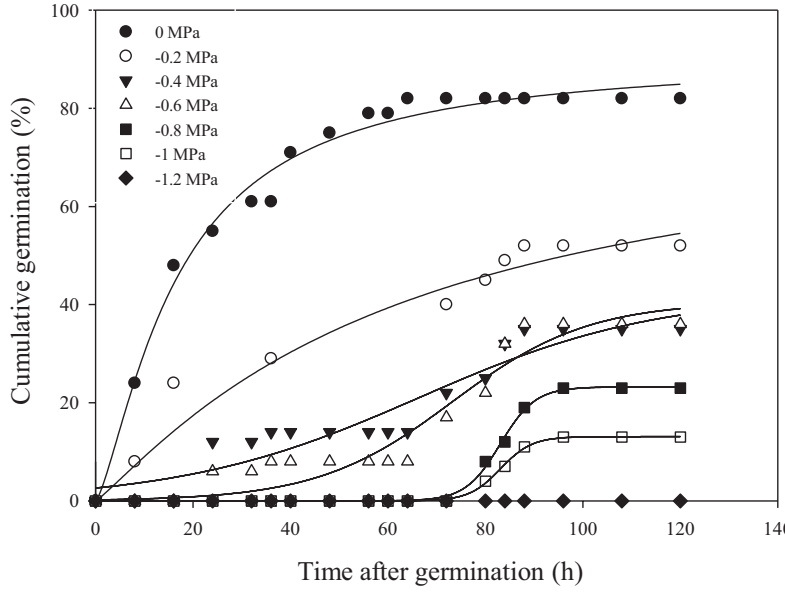

Fig. 2. Effect of drought stress on germination of sea barley

Table 3
Estimated parameters of 3-parameter sigmoidal function for salt stress

R^2	b	x_{50} (h)	G_{max}	Salt stress level (mM)
0.96	(1.42) 10.80	(1.53) 17.82	81.36 (1.71)	0
0.91	(5.14) 24.82	(5.71) 38.32	51.94 (4.17)	40
0.87	(8.63) 31.01	(5.48) 50.64	51.61 (8.25)	80
0.91	(5.73) 25.29	(3.52) 62.71	43.26 (3.21)	120
0.93	(5.50) 27.81	(1.21) 67.86	34.62 (5.10)	160
0.97	(1.20) 6.37	(1.64) 70.87	12.14 (0.58)	200
–	–	–	0	240

G_{max} = maximum germination percentage; x_{50} = time to reach 50% of germination; b = coefficient of the equation. Values in parentheses denote standard error

estimated 81% at 0 MPa and 40% at –0.6 MPa. The maximum and minimum germination occurred at water potentials of 0 and –1 MPa, respectively. Time to 50% maximum germination (x_{50}) increased with increasing osmotic potential and reached from 17.8 h at 0 MPa to 83.1 h at –1 MPa (Table 2, Fig. 2).

Salt stress – The results showed that the maximum germination percentage of sea barley declined as salinity increased from 0 to 240 mM NaCl. The maximum and minimum germination percentages were related to 0 mM NaCl with 80 and 200 mM NaCl with 12%, respectively. No germination was

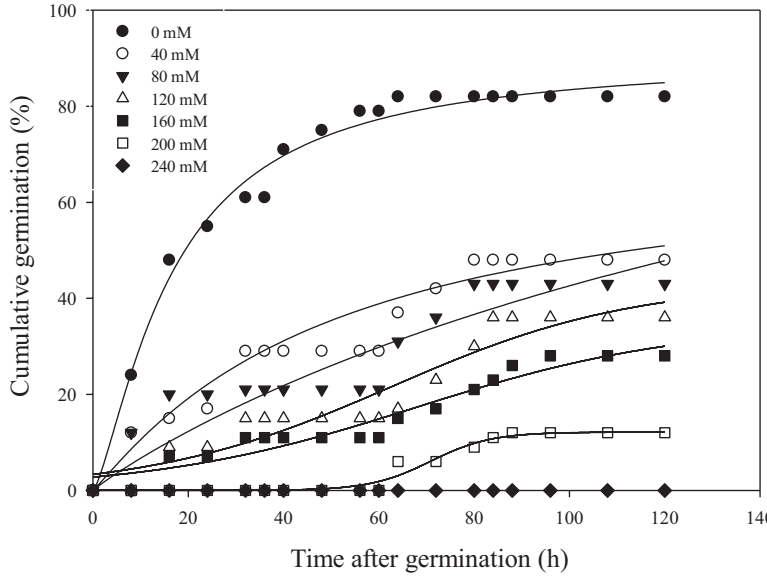

Fig. 3. Effect of salt stress on seed germination of sea barley

Table 4
Effect of pH level on seed germination of sea barley

Germination (%)	pH level
45.33[d]	4
42.67[d]	5
65.33[c]	6
73.33[b]	7
0[f]	8
0[f]	9
82[a]	Distilled water (6.5)

Different letters show significant difference at $p < 0.05$ according to the LSD test

observed in 240 mM NaCl. Also, x_{50} increased from 17.8 h at 0 mM NaCl to 70.8 h at 200 mM NaCl, respectively (Table 3, Fig. 3).

Effect of pH – According to the LSD test, germination was significantly different at varying pH levels. The average sea barley seed germination was 56% in the range of pH 4 to 7, with the highest germination percentage (82%) in pH = 6.5 (distilled water), followed by pH = 7. This weed germinated better under acidic conditions compared with alkaline conditions. Germination was inhibited in the range of pH 8 to 9 (Table 4).

Heating stress – After heating treatment, no germination was observed in the seeds. Most un-germinated seeds tested with TTC were not viable, ex-

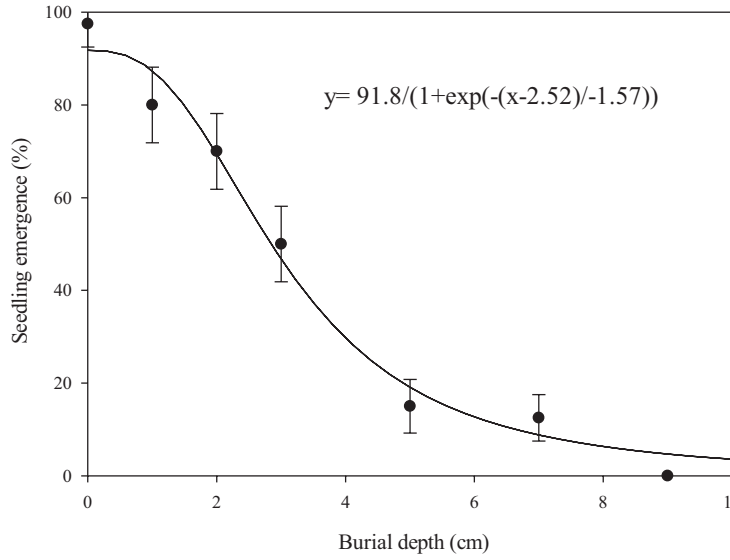

Fig. 4. Effect of burial depth on seedling emergence of sea barley

Table 5
Estimated parameters of 3-parameter sigmoidal function for effect of waterlogging on seed germination of sea barley

Days after waterlogging	b	x_{50} (days)	G_{max}	R^2
0	10.80 (1.42)	17.82 (1.53)	81.36 (1.71)	0.96
7	16.17 (1.48)	45.65 (1.62)	57.53 (1.03)	0.97
14	18.37 (1.39)	113.12 (1.89)	43.02 (1.02)	0.99
21	17.92 (1.21)	117.58 (1.59)	42.99 (0.91)	0.99
30	16.87 (1.06)	112.48 (1.42)	45.28 (0.83)	0.99
45	13.64 (0.63)	118.43 (0.63)	50.33 (0.63)	0.99
60	5.06 (0.77)	69.89 (0.93)	11.91 (0.19)	0.99
75	–	–	0	–

G_{max} = maximum germination percentage; x_{50} = time to reach 50% of germination; b = coefficient of the equation. Numbers in parentheses represent standard error

cept for the seeds exposed to 40 and 80 °C. The viability of seeds placed at 40 and 80 °C was 80% and 70%, respectively. These results indicated that heat shock would have an adverse effect on the survival of the seeds sown on the soil surface (data not shown).

Burial depth – Sea barley seedlings had a greater emergence when seeds were sown in soil depths between 0 and 2 cm. Thereafter, an increase in sowing depth reduced seedling emergence. Seedling emergence was halved at 2.5 cm soil depth and became negligible at 5 cm depth (Fig. 4).

Waterlogging – Germination declined with increasing waterlogging durations. After 45 days of waterlogging, germination was about 50 percent. Also, the time to 50% maximum germination increased over time, with the

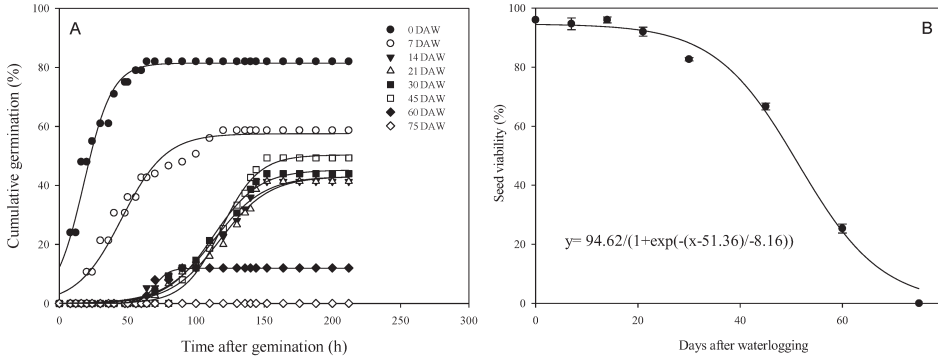

Fig. 5. Sea barley seeds germination (a) and survival (b) at waterlogging condition. DAW = days after waterlogging

exception of day 60. This was due to the small value of G_{max}, which led to a low x_{50} value. The highest rates of increase in germination percentage (b, i.e. slope at x_{50}) were observed 14 and 45 days after waterlogging, respectively (Table 5, Fig. 5A). Waterlogging for 21 days did not affect sea barley seed viability. However, seed viability declined thereafter and reached zero when the seeds were waterlogged for 75 days (Fig. 5B).

DISCUSSION

Response to temperature – Sea barley seeds germinated over a wide range of temperatures from 5 to 35 °C. The study area has a temperate climate and therefore, sea barley seeds can potentially emerge throughout the different times of the year. Also, the wide temperature range may allow this species to infest various areas in the world, provided that other environmental factors are adequate. Similar results were reported in winter wild oat (*Avena ludoviciana* Dur.), in which the species was able to germinate from 5 to 35 °C with the highest germination rate and percentage at 25 °C (Hassanpour-bourkheili *et al*. 2021). Conversely, seeds of curved parapholis (*Parapholis incurva*) and cutleaf geranium (*Geranium dissectum* L.) had a narrower germination range of between 5 to 26 °C (Atabaki *et al*. 2020, Gherekhloo *et al*. 2023).

Drought stress – Sea barley germination remarkably decreased at –0.2 MPa. Similar results about other grasses were obtained by Pereira *et al*. (2012), which reported a reduction of 40.2% in signal grass (*Urochloa decumbens* (Stapf) R. D. Webster) and of 23.7% in Congo grass (*Urochloa ruziziensis* (R. Germ. et C. M. Evrard) Crins) seed germination at –0.2 MPa. Germination of sourgrass (*Digitaria insularis* (L.) Fedde) seeds was about 22% at –0.2 MPa (Martins *et al*. 2017). This value for the serrated tussock (*Nassella trichotoma* (Nees) Hack. et Arechav.) was 46.6% at –0.2 MPa (Humphries *et al*. 2018) and for wild barley (*Hordeum spontaneum* Koch.) was 50% at –0.5 MPa (Hossini *et al*. 2017). Lower osmotic potential slows the metabolic and biochemical processes and consequently, delay or inhibit seeds germination and interrupt the cellular imbibition and elongation of the embryo (Huang *et al*. 1997).

Salt stress – According to the results, the tolerance of sea barley to salinity is higher than to drought, which can be detected by the slow reduction of seed germination in response to salt concentration (lower x_{50} and smaller b at 40 mM NaCl). Similar results were obtained for curved parapholis (*Parapholis incurva* (L.) C. E. Hubb.) (Gherekhloo *et al*. 2023), seaside arrowgrass (*Triglochin maritimum*) (Al-Hawija *et al*. 2012) and foxtail barley (*Hordeum jubatum* L.) (Israelsen *et al*. 2011, Kemuel and Irwin 2011). At higher salinities (up to 120 mM), germination rate decreased rapidly, which may be induced by osmotic stress or reduced enzyme activities, etc. Salt has been linked with

reduced α-amylase activity in seeds (Almansouri *et al*. 2001). Amylase is essential in the break-down of starch reserves, which provides sugar for embryo growth and development. Awan *et al*. (2014) reported that soils containing more than 100 mM (≈ 10 ds m^{-1}) NaCl are considered to be highly saline. According to these results, sea ~~barely~~ is a halophyte species (was able to germinate even at 200 mM NaCl) and typically can tolerate saline conditions, and then commence the germination process when salinity stress is reduced or removed (especially with the start of rainy season). It is reported that the salt response mechanisms of *H. marinum* are maintained at low levels of salt ions in the shoot, which indicates the presence of efficient ion exclusion mechanisms at xylem transport level (Garthwaite *et al*. 2005, Islam *et al*. 2007).

pH – This study highlighted that sea ~~barely~~ has a significant preference for a particular pH level (acidic to neutral). Since the pH levels of Iranian fields are between 6 and 8 (Shahbazi and Besharati 2013), the distribution of this species is limited by pH factor. Germination at low pH values has been observed in Egyptian crowfoot (*Dactyloctenium aegyptium* (L.) Willd.) (Burke *et al*. 2003), oregano (*Origanum compactum* Benth.) (Laghmouchi *et al*. 2017), balloon pea (*Lessertia frutescens* subsp. *frutescens*) (Müller 2021) and curved parapholis (Gherekhloo *et al*. 2023).

Heating stress – The results indicated that heating stress affected sea ~~barely~~ seed germination and survival and underline the inability of sea barley to resist heat stress induced by burning of crop residues in the field, which is a common practice in the region (Hassanpour-bourkheili *et al*. 2021). Exposure of weeds to high temperatures disturbs their germination and growth. High temperatures induce damage at the physiological, biochemical and molecular levels (Jemaa *et al*. 2010). Results of some studies illustrated that exposure to a temperature of 130 °C for 5 min completely ceased seed germination of feather fingergrass (*Chloris virgata* Sw.) (Fernando *et al*. 2016). Also, no germination was observed in bamboo piper (*Piper aduncum*) at 80 °C for 30 min (Wen 2015). Seeds of Mexican sunflower seeds showed 20% germination after exposure to heat for 30 min at 80 °C (Wen 2015).

Burial depth – The result verified that the highest values of seedling emergence of the sea barley were found on the surface down to 2 cm soil depth. Emergence was negligible at ≥ 5 cm soil depths. Therefore, tillage may be a good option to bury the seeds. This can be followed by several years of zero-tillage, so the seeds remain buried until they lose their longevity. Also, emergence may decrease in seeds buried in greater depths due to the limited reserves present in the seeds, lower hydration and soil gas permeability at greater depths (Benvenuti 2003, Dinelli *et al*. 2013). Decreases in seed emergence with increased burial depth were reported in other species (Gherekhloo *et al*. 2023, Hugo *et al*. 2014, Khodapanah *et al*. 2023, Rao *et al*. 2008, Sonkoly

et al. 2020, Tang *et al*. 2015). This result can help to predict flushes in seedling emergence, thereby allowing for better timing of control practices.

Waterlogging – According to the result, sea barley can tolerate soil water saturation for a considerable duration. Wetland species such as rice (*Oryza sativa* L.), dallisgrass (*Paspalum dilatatum* Poir.) and sea barley (*Hordeum marinum* Huds.) possess constitutive aerenchyma. Also, the root gas-filled porosity is further increased. Moreover, meta-xylem vessels become thicker when plants are grown under waterlogged or O_2-deficient root-zone conditions (Garthwaite *et al*. 2003, Vasellati *et al*. 2001). This result can indicate the ability of this species to occupy different topographic positions and to resist temporal variations in water and oxygen availability. Also, the introduction of rice in crop rotation may not be a feasible option to control sea barley.

CONCLUSIONS

The results of this study highlight that sea barley seeds germinate over a wide range of temperatures and can resist saline and waterlogging condition, but pH level and water deficiency limited the recruitment and distribution of this species. Heat stress was also observed to have a negative effect on the germination and survival of seeds. Seeds can germinate when buried to a depth of at least 2 cm, and seedling emergence dropped sharply thereafter. The results of this study contributed to our understanding of sea barley germination and emergence of agricultural landscapes and infrastructure habitats, which will help to develop tools and strategies for the long-term management of this weed grass. Tillage to bury the seeds to a depth of 5 cm, burning of crop residues and amendment of soil acidity by applying lime may reduce the emergence of seedlings.

*

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