Gastrointestinal basidiobolomycosis: an emerging fungal infection causing bowel perforation in a child

Mortada H. F. El-Shabrawi,1 Naglaa Mohamed Kamal,1,2 Riyadh Jouini,3 Abdullah Al-Harbi,2 Kerstin Voigt4,5 and Talal Al-Malki3,6

1Paediatric Hepatology Unit, Faculty of Medicine, Cairo University, Cairo, Egypt
2Paediatric Department, Al Hada Armed Forces Hospital, Taif, Saudi Arabia
3Paediatric Surgery, Al Hada Armed Forces Hospital, Taif, Saudi Arabia
4Jena Microbial Resource Collection, Leibniz Institute for Natural Product Research and Infection Biology, Jena, Germany
5University of Jena, School of Biology and Pharmacy, Institute of Microbiology, Jena, Germany
6Quality and Development, Taif University, Taif, Saudi Arabia

Basidiobolomycosis is an unusual fungal skin infection that rarely involves the gastrointestinal (GI) tract. We report a 10-year-old boy diagnosed as suffering GI basidiobolomycosis after being misdiagnosed first as suffering intestinal malignancy then schistosomiasis. The patient presented with fever, abdominal pain, vomiting, abdominal tenderness and rigidity with marked blood eosinophilia. Abdominal ultrasonographic and computed tomographic scans revealed a large caecal mass. Biopsy of the mass showed transmural granulomatous inflammation interpreted as schistosomal granuloma, ruling out lymphoma. The patient’s condition deteriorated despite anti-schistosomal therapy. Emergency surgery was then performed, and caecal perforation was found. The mass was excised; cultures were negative and histopathological examination was suggestive of schistosomal granuloma. The mass recurred 3 weeks post-operatively. Second-opinion histopathological examination diagnosed Basidiobolus ranarum infection. Treatment with itraconazole produced marked improvement, with diminution of the mass.

Introduction

Basidiobolus species are filamentous fungi that belong to the order Entomophthorales. Unlike other fungi (e.g. Mucorales) classified to the former zygomycetes, they cause subcutaneous zygomycosis in healthy individuals (Singh et al., 2008). Basidiobolus ranarum was first described as an isolate from frogs in 1886. It was cultured from frogs’ intestinal contents and excreta (Ribes et al., 2000). It is commonly found in soil and decaying vegetable matter. It is occasionally found as a commensal in the gastrointestinal (GI) tracts of amphibians, reptiles, fish and mammals such as frogs, toads, turtles, fish, chameleons, horses, dogs and bats (Kaufman et al., 1990; Zahari et al., 1990; Gugnani, 1999). The micro-organism was first isolated in 1955 from decaying plants in the United States. Subsequently it was found in soil and vegetation worldwide (Greer & Friedman, 1966). Basidiobolus is endemic in Uganda and certain other areas of Africa, and in parts of Asia including India (Ribes et al., 2000). In the past, clinical isolates of Basidiobolus were classified as B. ranarum, B. meristosporus and B. haptosporus. However, recent taxonomic studies based on antigenic analysis, isoenzyme banding and restriction enzyme analysis indicate that all human pathogens belong to B. ranarum. In two studies, B. ranarum was commonly isolated from South India (Khan et al., 2001; Sujatha et al., 2003).

Zygomycosis is characterized by tissue invasion with broad, non-septate hyphae of fungal species such as Rhizopus, Rhizomucor, Absidia and Basidiobolus. Fungal elements of B. ranarum include hyphae and zygospores (Hocquet, 1979; Gugnani, 1999). The hyphae are thin-walled, septate, haphazardly branched and surrounded by eosinophilic, amorphous material (Splendore–Hoepli phenomenon). In addition to hyphae, zygospores are present in the infected tissue as spherical bodies that measure up to 30 μm in diameter. They have a thin outer wall, foamy

Abbreviations: CT, computed tomography; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; FFPE, formalin-fixed, paraffin-embedded; GI, gastrointestinal.
cytoplasm, and a nucleus containing a large nucleolus (Choonhakarn & Inthraburan, 2004). *B. ranarum* is opportunistic; it causes infection in immunocompromised hosts and it is considered as an emerging pathogen in immunocompetent hosts (Khan *et al.*, 2001). It is a known cause of subcutaneous zygomycosis (Hocquet, 1979; Gugnani, 1999; Khan *et al.*, 2001). Its aetiological role in GI infections is increasingly recognized (Al Jarie *et al.*, 2003). The presenting symptoms of GI *Basidiobolus* infection include fever, vomiting, abdominal pain and weight loss. *B. ranarum* has been mainly isolated from the extremities, trunk and GI tract, and rarely from other parts of the body (Kaufman *et al.*, 1990; Ribes *et al.*, 2000; Khan *et al.*, 2001; Sujatha *et al.*, 2003; van den Berk *et al.*, 2006). Males are much more frequently affected than females (Gugnani, 1999). Molecular detection of human fungal pathogens, when available, might settle disputed diagnosis in the present era of molecular diagnosis (Rothhardt *et al.*, 2011).

In the present report, we describe a case of GI basidiobolomycosis in a 10-year-old boy from the Southern Region of the Kingdom of Saudi Arabia complicated by bowel perforation.

**Case report**

A 10-year-old boy was admitted to Al-Hada Armed Forces Hospital, Taif, Kingdom of Saudi Arabia, for the evaluation of fever that had started 3 weeks prior to admission. There was severe recurrent pain in the right iliac fossa and recurrent vomiting. His past medical history was unremarkable. Abdominal examination revealed diffuse abdominal tenderness and rigidity, maximally over the right iliac fossa. A tender, firm to hard mass with a smooth surface, fixed to deep structures and measuring 75 × 60 mm, was felt in the right iliac fossa. Initial laboratory tests revealed peripheral blood leukocytosis with a white cell count of 12,200 mm\(^{-3}\) and marked eosinophilia [absolute eosinophilic count of 2070 mm\(^{-3}\) (17%)]. Haemoglobin concentration was 12 g dl\(^{-1}\) and platelet count was 628,000 mm\(^{-3}\), with slight elevation in the erythrocyte sedimentation rate (ESR; 39 at 1 h) and C-reactive protein (CRP; 120 mg dl\(^{-1}\)). Urinalysis, stool analysis, serum electrolytes, total proteins and albumin, biochemical liver function tests, blood urea nitrogen, serum creatinine and immunological profile, as well as cultures from blood, urine and stool for bacteria and fungi, were normal or negative on admission and throughout the hospital course.

Abdominal ultrasonography and computed tomography (CT) revealed an 80 × 60 × 45 mm mass involving the caecum. Surgical exploration was done and revealed a mass 80 × 60 × 45 mm in the caecum, sparing the appendix, with fungation and raised areas outside the caecum and with multiple mesenteric lymph nodes. Biopsy of the mass with one large mesenteric lymph node was performed. Initial histopathological assessment ruled out lymphoma and suggested schistosomal granuloma. Consequently, repeated stool and urine examinations for *Schistosoma* ova were performed but these were all negative. Antischistosomal treatment was commenced with oral administration of praziquantel. The patient’s general condition deteriorated, with high-grade fever of 40°C, toxic appearance, increasing leukocytic count (22,800 mm\(^{-3}\)), marked neutrophilia (13,500 mm\(^{-3}\) or 59.4%), eosinophilia (2730 mm\(^{-3}\) or 12%), thrombocytosis (806,000 mm\(^{-3}\)) and falling haemoglobin (8.9 g dl\(^{-1}\)); ESR reached 72 and CRP rose to 186. Follow-up CT revealed increasing size of the mass, nearly obstructing the whole bowel lumen (Fig. 1). At emergency surgery, the mass was found to be 80 × 70 × 50 mm in size, adherent to the abdominal wall, omentum and mesocolon, and perforating the caecum with minimal splash of stools into the abdominal cavity (Fig. 2a, b). The mass was excised along with the caecum, the ileocaecal valve, the appendix, 70 mm from the terminal ileum, the ascending colon just above the caecum and part of the mesentery with ileo-colic anastomosis (i.e. right hemicolecctomy). The surgical specimen was sent for bacterial and fungal cultures as well as histopathological examination. Cultures were negative. Gross pathological examination of the specimen revealed an 80 × 70 × 50 mm tan–pink, firm mass, with a greyish cut section. The mass invaded and traversed the muscularis propria. The remainder of the mucosa was unremarkable. Five mesenteric lymph nodes were identified in the adjacent adipose tissue. Histological examination was again suggestive of schistosomal granuloma.

The patient showed transient improvement postoperatively for 7 days, then he deteriorated again, despite receiving broad-spectrum antibiotic therapy. Three weeks postoperatively, abdominal CT confirmed huge recurrence of the mass (Fig. 3). The haemoglobin level had dropped to 6 g dl\(^{-1}\) and eosinophils increased to 24.3%. A second histopathological opinion at King Abdul-Aziz Hospital, Taif, Kingdom of Saudi Arabia, suggested GI basidiobolomycosis. Histology showed transmural granulomatous inflammation with prominent eosinophilic component (Fig. 4a and b). Broad, septate, hyphae-like structures surrounded by an eosinophilic sheath (Splendore–Hoepli phenomenon) were identified (Fig. 5a, b). Periodic acid–Schiff staining (Fig. 6a, b) and Grimelius methenamine silver-stained sections (Fig. 6c, d) showed broad fungal hyphae and zygosporos. Antifungal treatment was started with intravenous itraconazole.

![Fig. 1. CT abdomen image showing a mass involving the caecum, nearly obstructing its lumen.](image-url)
4 mg kg$^{-1}$ day$^{-1}$ divided into twice-daily doses for 2 days followed by the same dose orally once daily (the paediatric dose of itraconazole ranges from 3 to 5 mg kg$^{-1}$ day$^{-1}$ administered twice or four times a day). This was followed by dramatic improvement of fever and the abdominal manifestations, along with a decrease of eosinophils to 11 % within 10 days of the start of treatment. The patient was discharged on 100 mg oral itraconazole once daily with regular follow-up every 2 weeks. The mass gradually decreased and had completely resolved at 6 months follow-up. Itraconazole treatment was advised to be continued for another 6 months.

**Molecular diagnosis**

**DNA purification from formalin-fixed and paraffin embedded (FFPE) tissue**

Total genomic DNA was purified from 6-month-old archival FFPE tissue blocks. Tissue sections were deparaffinized with 500 µl 100 % Roti-Histol (article no. 6640; Carl Roth) under a fume hood. Then contents were vortexed for 1 min and incubated overnight. The Roti-Histol solvent was decanted and the procedure repeated one more time. To remove the residual Roti-Histol, the samples were washed five times with ethanol as follows: (1) 5 ml absolute ethanol was added, mixed by vortexing for 10 s and removed after 10 h; (2) 5 ml absolute ethanol was added, mixed by vortexing for 10 s and removed after 10 h; (3) 5 ml 90 % ethanol was added, mixed by vortexing for 10 s and removed after 30 min; (4) 5 ml 70 % ethanol was added, mixed by vortexing for 10 s and removed after 30 min; (5) 5 ml 50 % ethanol was added, mixed by vortexing for 10 s and removed after 30 min.

The tissue sections were then left in a laminar-flow cabinet at room temperature for 30 min to dry the tissues. Tissue lysis was performed by grinding the dried tissue sections in liquid nitrogen to a fine powder and suspension in CTAB extraction buffer containing 2 % hexadecyl trimethylammonium bromide [CTAB; Sigma H-5882], 1.4 M NaCl, 25 mM EDTA and 100 mM Tris/HCl, pH 8.4 in accordance with a modification of the protocol of Doyle & Doyle (1990), followed by heating at 95 °C for 30 min to extract DNA from the cell lysate, chloroform was added, mixed for 2 min and then centrifuged at 12 000 g for DNA precipitation. The tubes were centrifuged at 12 000 g for 15 min and 2-propanol was decanted. The DNA pellet was washed gently twice with cold 70 % ethanol and dried completely in a speed vacuum dryer at 50 °C for 5 min. The dried pellet was dissolved in 50 µl sterile double-distilled water. To complete the solubilization of the DNA the microtubes were put in a 60 °C waterbath for 30 min. Purification of genomic DNA from fungal tester strains was performed likewise.

---

Fig. 2. (a) Intraoperative picture showing the mass involving the caecum. The caecum, appendix, part of the ileum, part of the descending colon and part of the mesentry are to be excised. (b) The excised caecum showing a huge mass involving the whole caecum with caecal perforation.

Fig. 3. CT abdomen image 3 weeks post-operative showing huge recurrence of the mass.
Molecular detection of *B. ranarum*-specific DNA by PCR

Detection was performed using the taxon-specific primer pairs Ba1 (5′-AAAATCTGTAAGGTCAACCTTG-3′)/Ba2 (5′-TGCAGGAGAAGTACATCCGC-3′) (Voigt et al., 1999) and Bs1 (5′-ACTGTTRAMGTAGCTTTGGTAG-3′)/Bs2 (5′-CTTGCAGCCTCCTAACTAG-3′) (Rothhardt et al., 2011). Primer pair Cc1 (5′-CTCTCTAAATTTCTCTTG-3′)/Cc2 (5′-CTTTAATTAAGCTAATCAACATG-3′) (Voigt et al., 1999) specific to *Conidiobolus coronatus* and the universal primer pair NL1 (5′-GCATATCAATAAGCGGAGAAA-3′)/NL4 (5′-GGTCCGTGTTTCAAGACGG-3′) (O’Donnell, 1993) specific to all eukaryotes were used to exclude *Conidiobolus* contamination and to check DNA quality, respectively. Primer pairs Ba1/Ba2, Cc1/Cc2 and NL1/NL4 target the nuclear large subunit (28S) rDNA whereas primer pair Bs1/Bs2 hybridizes to the internal transcribed spacer spanning the ITS1–5.8S–ITS2 region of the nuclear rDNA cluster (Rothhardt et al., 2011). PCR amplification mixtures contained 1 μl genomic DNA (~50–100 ng), 0.2 mM of each deoxynucleotide, 10 pmol of each primer, 3.0 mM magnesium chloride, 1 unit DreamTaq DNA polymerase (MBI Fermentas) and a buffer system based on KCl and (NH₄)₂SO₄ at a ratio optimized for the best performance in PCR according to the manufacturer’s recommendations. PCR products were amplified in a FlexCycler (Analytik Jena) by using the fastest ramp times. The temperature profile of the PCR comprised an initial denaturation step of 5 min at 95 °C; 40 cycles of 0.5 min at 95 °C to denature the template DNA followed by 40 cycles of 1 min at 55 °C for primer annealing; 1 min at 72 °C for primer extension; 0.5 min at 95 °C for denaturation of the DNA double strands; and a final primer extension step of 10 min at 72 °C to ensure the completion of double-stranded amplicons. PCR amplicons were visualized by mixing the PCR mix with 1/4 vol. loading buffer (0.2 g bromphenol blue l⁻¹, 0.2 g xylene cyanol l⁻¹, 10 mM EDTA, 30 % glycerol), and loading on a 1.6 % agarose gel (Seakem LE, Serva) in 1× TAE buffer (40 mM Tris pH 7.8, 20 mM sodium acetate, 1 mM EDTA) followed by electrophoretic separation (5–10 V cm⁻¹ field strength) and staining of the gel in 50 μg ml⁻¹ ethidium bromide solution for 10 min, destaining in tap water for 5 min and photo-documentation on a UV transilluminator (wavelength 321 nm).

Basidiobolus-specific detection by PCR

The results of the *Basidiobolus*-specific detection are shown in Fig. 7. The primer pairs applied to the DNA sample from the tissue block amplified DNA fragments of the expected sizes from the target taxa. Amplification with the primer pair Bs1/Bs2 resulted in a 460 bp PCR fragment specific for both entomophthoralean genera, *Basidiobolus* and *Conidiobolus*, species of which have been reported to be clinically important (for review see Rothhardt et al., 2011). Amplification with primer pair Ba1/Ba2 revealed a 650 bp amplicon specific for the genus *Basidiobolus*, by use of moderately and highly stringent annealing temperatures of 55 °C and 60 °C, respectively. With the exception of the 420 bp amplicons for the positive controls, no bands were obtained for the tissue sample and the negative controls using primer pair Cc1/Cc2 in order to exclude infection with *C. coronatus*. PCR amplification specific for *B. ranarum* as a single species could not be performed due...
to the lack of sequence deviations in the D1/D2 domain of the LSU used as target for PCR amplification (Voigt et al., 1999). However, infection with Basidiobolus species other than B. ranarum (e.g. B. heterosporus and B. meristosporus used in this study) can be excluded due to thermotolerance at 37 °C (human body temperature), which is permissive for B. ranarum, but suppressive for other species of Basidiobolus (Rothhardt et al., 2011). Thus, B. ranarum was unequivocally identified in the FFPE tissue.

B. ranarum has been detected as aetiological agent in other cases of GI zygomycoses in previous analyses (Khan et al., 2001; Lyon et al., 2001).

Discussion

Basidiobolomycosis is an unusual chronic fungal infection known for dermatological manifestations (Nguyen, 2000). It is caused by Basidiobolus ranarum, a member of the former zygomycetes, subphylum Entomophthoromycotina, order Entomophthorales (Hibbett et al., 2007; Kirk et al., 2008). The fungus is an environmental saprophyte, and is found worldwide (Kwon-Chung & Bennett, 1992; Khan et al., 2001; Lyon et al., 2001; Dromer & McGinnis, 2002). Basidiobolomycosis is usually a subcutaneous infection that affects mostly young males, and it is transmitted through traumatic inoculation (Sugar, 2005). Most cases have been reported from tropical and subtropical regions (Antonelli et al., 1987; Kwon-Chung & Bennett, 1992). The visceral form of infection is rare (Kwon-Chung & Bennett, 1992). GI basidiobolomycosis has been scarcely reported in the medical literature (de León-Bojorge et al., 1988). However, it has recently been increasingly recognized (Khan et al., 2001). The clinical presentation of the subcutaneous disease is quite characteristic, and the disease can be easily diagnosed. The first human case of subcutaneous mycosis was reported in Indonesia in 1956 (Kian Joe et al., 1956). In contrast, GI basidiobolomycosis is very difficult to diagnose, because its clinical presentation is non-specific, with no identifiable risk factors, and all age groups are susceptible (Khan et al., 2001).

In 1978, the first culture proving invasive basidiobolomycosis of the maxillary sinus and palate was reported in the United States (Dworzack et al., 1978). GI basidiobolomycosis is rare (Bittencourt et al., 1979; Khan et al., 1998), with only 14 cases in adults (Khan et al., 2001; Lyon et al., 2001) and 12 cases in children (Al Jarie et al., 2003; Wasim Yusuf et al., 2003) having been reported worldwide. The reported cases included eight cases from the United States; eight from the Kingdom of Saudi Arabia, four from Brazil, two each from Nigeria and Kuwait, one from Iran and one from the Netherlands (de Aguiar et al., 1980; Schmidt et al., 1986; Pasha et al., 1997; Khan et al., 1998; Smilack, 1998; Yousef et al., 1999; Zavasky et al., 1999; Al Jarie et al., 2003). No specific risk factors were identified for GI basidiobolomycosis, except that three patients had a history of diabetes mellitus (Khan et al., 2001). In the above-mentioned cases, intra-abdominal masses were found on imaging studies and during surgical exploration, and were suspected for either malignancy or chronic inflammation as in our case. Histopathological tests revealed predominantly inflammatory changes with rise in eosinophilic count (Wardlaw & Kay, 1995). The presence of the Splendore–Hoeppli phenomenon was confirmatory of the infection (Kwon-Chung & Bennett, 1992; Yousef et al., 1999). Leukocytosis, marked eosinophilia, and elevated ESR and CRP were found in our case as reported previously in other cases (Wardlaw & Kay, 1995; Dromer & McGinnis, 2002). The clinical presentation may

Fig. 6. Fungal zygospores stained with periodic acid–Schiff (a, b), and Grimelius methenamine silver (c, d). Images taken at \( \times400 \) magnification.
simulate lymphoma and inflammatory bowel disease (IBD), e.g. Crohn’s disease or ulcerative colitis, and thus may be misdiagnosed (Khan et al., 2001). The main differential diagnoses of GI basidiobolomycosis are IBD (Crohn’s disease), intestinal tuberculosis, sarcoidosis, schistosomiasis and amoebiasis (Ribes et al., 2000). It was misdiagnosed as schistosomal granuloma in our patient. Because of the non-specific signs and symptoms of this disease, the diagnosis has sometimes been delayed, with increased morbidity (Fahimzad et al., 2006). The mode of acquisition of the disease remains poorly understood. In the cutaneous form, the portal of entry is believed to be through the skin, after insect bites, scratches and minor cuts. It is most common in young children as a disease of the skin and subcutaneous tissues, involving the thighs and buttocks (Bigliazzi et al., 2004). Most cases have been reported in tropical and subtropical climates, mainly in Indonesia and East and West Africa (Antonelli et al., 1987; de León-Bojorge et al., 1988; Bittencourt et al., 1991; Scholtens & Harrison, 1994; Sugar, 2005). GI basidiobolomycosis is exceedingly rare (Centers for Disease Control and Prevention, 1999; Yousef et al., 1999; Zavasky et al., 1999; Khan et al., 2001; Lyon et al., 2001). It is unclear how the fungus is introduced into the host’s GI tract, but this probably occurs through ingestion of contaminated soil, animal faeces or food (Fahimzad et al., 2006). Use of contaminated ‘toilet leaves’ for cleaning of the skin after defecation has also been considered a likely possibility (Ribes et al., 2000). Based on the observations of Lyon et al. (2001), visceral involvement in basidiobolomycosis is being increasingly recognized in industrialized countries, and the reasons for this are unclear. Probably this peculiar geographical distribution is related to a specific style of life and dietary behaviour. Five patients from Arizona in the United States had amphibians or reptiles outside their homes, four patients were reported to have washed vegetables before eating them and three other patients camped near a lake or river during the previous year; however, the patients had a history of diabetes mellitus (in three patients), peptic ulcer disease (in two), or pica (in one) (Pasha et al., 1999).

The history of the patient reported by us gave no information about the method by which he acquired the infection. The common denominator for this case and the series reported from Arizona appears to be involvement of the GI tract. The current experience of treating patients with GI basidiobolomycosis is limited to only a few cases. Surgical resection of the infected tissue and prolonged treatment with itraconazole appear to be the best available clinical options. Patients who were treated with itraconazole showed complete resolution of the disease. It is unclear whether itraconazole alone is sufficient or if simultaneous resection of the affected tissue is necessary to eradicate the infection (Khan et al., 2001). There is some reservation that surgical debridement of the infected area may spread the infection (Cameroon, 1990), a concern which is not shared by others (Davis et al., 1994; Pasha et al., 1997). Among the
azoles, ketoconazole and fluconazole have also been found to be effective in a few reports of subcutaneous basidiobolomycosis (Drouhet & Dupont, 1983; Davis et al., 1994).

Treatment of basidiobolomycosis with amphotericin B has given unsatisfactory results. Among the three patients in whom amphotericin B had been used, one of them died after 2 weeks of therapy and one had a marginal response (after addition of ketoconazole). In the third patient, the isolate of the organism was resistant to amphotericin B, and therefore itraconazole was subsequently used. Yangco et al. (1984) found that amphotericin B was active against only 50% of the isolates of Basidiobolus tested. Potassium iodide (KI) is another compound that has been used successfully for the treatment of subcutaneous basidiobolomycosis. Although KI has no known antifungal activity, its administration (30 mg kg\(^{-1}\) day\(^{-1}\)) to one patient with retrospective basidiobolomycosis resulted in dramatic improvement (Nazir et al., 1997). Within 6 weeks of therapy, the thoraco-lumbar mass and the suprarenal lesion resolved completely. Based on the available limited information, it appears that optimal treatment of GI basidiobolomycosis is combined surgical and prolonged medical treatment (Lyon et al., 2001; Sugar, 2005; Fahimzad et al., 2006). The best choice of antifungal agent is not clear, but itraconazole seems to show higher efficacy (Drouhet & Dupont, 1983; Lyon et al., 2001). Although clinical failure has been described with amphotericin B, KI has been used successfully for treatment of subcutaneous basidiobolomycosis (Nazir et al., 1997).

In conclusion, GI basidiobolomycosis is an emerging infection that leads to diagnostic confusion, morbidity and mortality. It can cause intestinal perforation. Diagnosis requires awareness and consideration of its possibility in the differential diagnosis of patients with abdominal masses and eosinophilia. Special attention in the Kingdom of Saudi Arabia should be for patients coming from the Southern region, because of its subtropical warm, humid climate, which may give optimum conditions for the growth of the fungus, with increased possibility of environmental contamination.

**Acknowledgements**

We thank Moutaz Haiba, MD, FCAP, Consultant Histopathologist, Al Hada Armed Forces Hospital, Taif, KSA. We also wish to express our gratitude to Prof. Dr Lennart Olsson (Institute of Systematic Zoology and Evolutionary Biology, University of Jena, Germany) for recommending and supplying Roti-Histol as an alternative and non-carcinogenic solvent for paraffin in FFPE tissue.

**References**


mimicking inflammatory bowel disease.


